Cellular Origin, Life in Extreme Habitats and Astrobiology 19

Joseph Seckbach
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The Diatom World

Springer

THE DIATOM WORLD

Cellular Origin, Life in Extreme Habitats and Astrobiology

Volume 19

Series Editor:

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ISSN 1566-0400
ISBN 978-94-007-1326-0 e-ISBN 978-94-007-1327-7
DOI 10.1007/978-94-007-1327-7
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2011932495

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The Diatom World

The impact of diatoms on planet Earth is substantial and far reaching, hence "The Diatom World" is an appropriate title for a book describing the attributes of diatoms, with an emphasis on their ecological roles. The large-scale ecological success of diatoms suggests that they have refined their cellular processes for efficient utilization of nutrients and sunlight to a greater extent than most other unicellular algae. The most visible distinguishing feature of diatoms is their silicified cell walls, and because of its uniqueness, it is reasonable to assume that utilization of silica as a structural material is a valuable adaptation. A rich genomic diversity may also contribute to the diatoms' success; diatom genome sequencing has revealed a roughly equal contribution of plant and animal gene homologs, with a relatively high contribution of bacterial genes.

Diatom research impacts a wide variety of areas. Topics included in this book include morphology, phylogeny/evolution, sexuality/breeding, surface colonization and biofilms, infection and toxicity, bio/nanotechnology, extremophilicity, ecology, and endosymbiosis. Systematics is a major point of emphasis in the book. Two factors contribute to the continuing refinement of diatom systematics: (1) the enormous number of species and (2) distinctions between morphological and genetically based markers. The latter is an especially interesting point because although cell wall structure is ultimately genetically derived, the ability of diatoms to make such a diversity of structures "muddles the message" and there is necessarily no strict correspondence between genetic and structural similarity. Sexuality in diatoms is discussed, which includes an exploration of the concept that classical breeding approaches may be useful for diatom research. Diatoms affect the world as biofouling organisms, both in terms of ecological and economic impacts. The diatom silica cell wall has a universal appeal which encompasses aesthetic beauty coupled with an intellectual fascination about how such structures are made. Several chapters touch upon this subject both in terms of comparisons of the final morphology and the process of structure formation. The value of the silicified cell wall is evident for the diatom, but its usefulness for humans is addressed in terms of a possible source of inexpensive nano-structured materials for nanotechnological applications (with an emphasis on optical properties) and as a bioindicator of water quality. The ability of diatoms to colonize environments with extremes of temperature, pH, and salinity is covered in detail. All is not beautiful in the diatom world, even though they live in protective shells, diatoms can be infected by viruses, and toxin production by diatoms has adverse effects on the ecosystem and human health.

Diatoms impact processes on a wide range of scales, from the nanometer-to-micron range at which their silica structures are formed, through the meters-to-kilometers scale on which diatom communities function, and to the global scale carbon fixation and biogeochemistry. Given their participation in so many processes and at such diverse scales, it is indeed a Diatom World.

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DIATOMS: GENERAL INTRODUCTION

Diatoms are microscopic unicellular or colonial (in the shape of filaments or ribbons, or tube dwelling) eukaryotic algae. Their cell walls are with silica shells, and they are ubiquitously distributed in aqueous habitats.

Their name is derived from the Greek words (dia) = "through" + (temnein) = "to cut," since their cells are divided in two halves or two box-like parts (frustules or valves). They are one of the most common types of phytoplankton. One can detect them in marine as well as in fresh water habitats, at high and low temperatures, at different pH values, in hypersaline environments, and in brackish water. Diatoms especially play an important role in the oceans where they fix large amounts of carbon dioxide and synthesize carbohydrates that serve as a chief source of zooplankton food in the marine food chain. Bacteria adhere to and influence diatoms' growth.

There are over 1,250 genera of diatoms in the Class Bacillariophyceae. Diatoms are very responsive to environmental changes, and analysis of diatom communities can be used to study long-term changes.

Their exoskeleton is made of nanometer-sized particles of SiO_2 (silicon dioxide] obtained from their ability to "metabolize" silicic acid [$Si(OH)_4$] from the environment and form frustules. These silica "shells" of the cell walls are easily preserved and provide a useful tool for fossil research. Fossil evidence suggests that diatoms originated during, or before, the early Jurassic period (~210 to 144 Mya). It is assumed that these unicellular algae arose from the endocytobiosis of a red alga, which penetrated (or was engulfed) into a single-celled heterotrophic host eukaryotic cell. Their chloroplasts do not accumulate storage carbohydrates, as seen in, for example, the green algae.

Diatoms are often visible to the naked eyes as a golden coating growing on vessels, and they commonly form brown films on aquarium glass or rocks. At higher magnification and especially in the scanning electron microscope, their cells appear very attractive and display extremely beautiful designs and patterns, as shown by several photos in this volume.

Recently, the genome sequences of some diatom species as well as compilations of applied research on diatoms have been published, and the taxonomy of the group has been repeatedly revised in recent years. However, general books on their biology and ecology are few. In the current volume, some of the leaders in diatom research present new information and/or summarize recent research efforts on a wide range of topics, including morphology, nanostructure, morphogenesis, motility, ecophysiology with emphasis on their wide range of habitats and ecological niches, biogeography, taxonomy, molecular evolution and phylogeny,

cryptic and endosymbiontic species, toxic species, viruses of diatoms, and more. However, aesthetical aspects are not forgotten.

It is our hope that the *The Diatom World* will foster greater appreciation and research contributions on this incredibly diverse and fascinating group of organisms. We thank all our authors for their contributions and their patience. A special appreciation is due to the anonymous reviewers who evaluated the chapters of this book.

Acknowledgments

We thank Professors Aharon Oren, Richard Gordon, and David J. Chapman for revising and improving the above introduction to *The Diatom World*.

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ACKNOWLEDGMENTS

We thank our authors for the contributions and patience and are grateful to the reviewers for their careful review of the chapters. We wish to acknowledge all of our colleagues who assisted us with their good advice for this volume, among them are: Aharon Oren, Assaf Vardi, David Chapman, Helga Stan-Lotter, Mark Hildebrand, Maud M. Walsh, Richard Gordon, Russell Chapman, and Scott Balogh. If any other name has been omitted, we apologize for the oversight. Special appreciation is due to Melanie van OverBeek and Maryse Walsh (Springer, Dordrecht, NL) for their cooperation with all the COLE books. Last but not least, the senior author (JS) is grateful to his wife, Fern Seckbach, for her patience, understanding, and assistance during the compiling of this book (and other volumes in the series).

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 1–18
DOI 10.1007/978-94-007-1327-7_1, © Springer Science+Business Media B.V. 2011

COLLECTING, CLEANING, MOUNTING, AND PHOTOGRAPHING DIATOMS

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1. Introduction

Our beautiful world surrounds us with diatoms, but because they are at or below the limit of resolution of the naked eye, we can literally swim through them and never know that they are there. When people first learn about diatoms, they are amazed that diatoms exist, and then fascinated to find out how to locate and collect them. How can one collect something that one cannot see? One clue to the presence of diatoms is the color of the photosynthetic pigments in live diatoms, which ranges from a golden brown through a brownish olive drab to dark brown, and the typically mucoid appearance of a colony of diatoms. In life, one is essentially looking for golden-brown slime.

The places most collectors look are in freshwater, such as streams or lakes, in brackish or marine waters, and in fossil sites. A problem in collecting is that particles in soils, and silts in marine settings, can approximate the size of diatoms, so that the goal in collecting is to acquire a clean sample without significant contaminants that are difficult to separate. As a consequence, one searches as much as possible for diatoms that are not in contact with mud or silt. Each of these sample types requires a slightly different approach.

2. Collecting in Freshwater

In freshwater settings, the simplest approach to collecting diatoms is to obtain samples of stalked multicellular green algae (or aquatic weeds) that grow on the bottom of the stream or lake. These freshwater green algae are regularly covered with a forest of diatoms, and if the green algae are placed into a zip lock plastic bag and shaken, pummeled, and agitated, the water in the bag will rapidly become a cloudy golden-brown or olive-drab color, as the diatoms are released from the weed and become suspended in the solution. The water is poured into a vertical glass cylinder through a coarse sieve to hold back the green algae, and the diatoms are allowed to settle to the bottom of the cylinder over hours or overnight. This process can be repeated about four or five times to extract additional diatoms

from the same sample of green algae with little or no loss of production each time, and there is no damage to the diatom frustules.

There are some other simple collection techniques for freshwater diatoms. Diatoms tend to prefer cold water that is not in direct sunlight, so that the undersides of lily pads, or the damp concrete in the shadow of a bridge, are more likely to have interesting samples than specimens resulting from a search in the sunlight. Some diatoms grow in a sheath around plant stems of rushes, and these diatoms can be stripped off by hand. Other species may grow as a shiny or glossy coating on rocks or on wood exposed to splashing water, appearing like a mucus growth with the characteristic color. Still others may grow in microscopic, essentially colorless tubes, or on the end of tubes, and the whole colony appears like a dirty off-white cotton mop attached to rocks in the river. The most notorious of these is *Didymosphenia geminata* Lyngbye (Schmidt) which was once thought to be rare and limited to high alpine settings with very clean waters. But this beautiful diatom (Fig. 1) has now been renamed by the public as "Rock Snot" as it has been transported by the felt soles of fly fishermen to streams around the world; it has been found to be a highly invasive species that can grow to cover the bottom of a stream and kill normal insect and plant life. *Didymosphenia* grows at the end of a colorless stalk, and the colony can have the appearance of a dirty sheep's fleece on rocks under water. Other *Cymbella* species grow within tiny tubes, and can

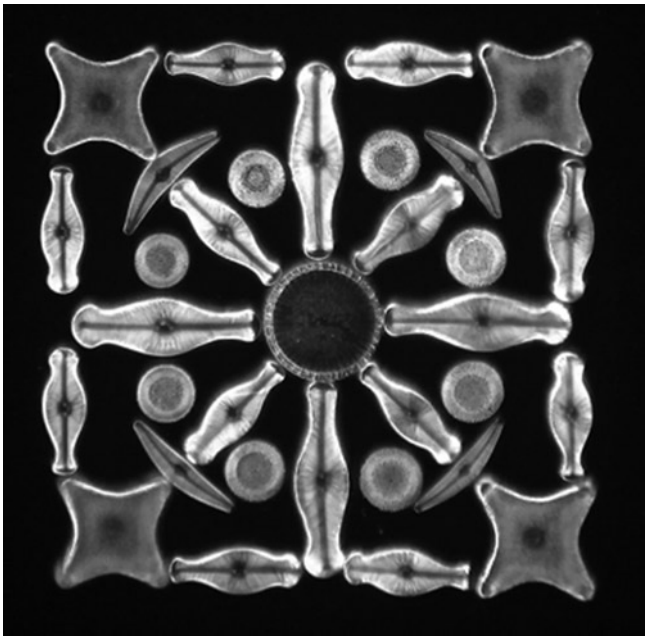

Figure 1. An ornate square of arranged diatoms by the author, which includes freshwater and marine diatoms. Didymosphenia forms the main spokes of the arrangement and has a shape similar to a classic bottle of Coca-Cola (Photo copyright retained by author).

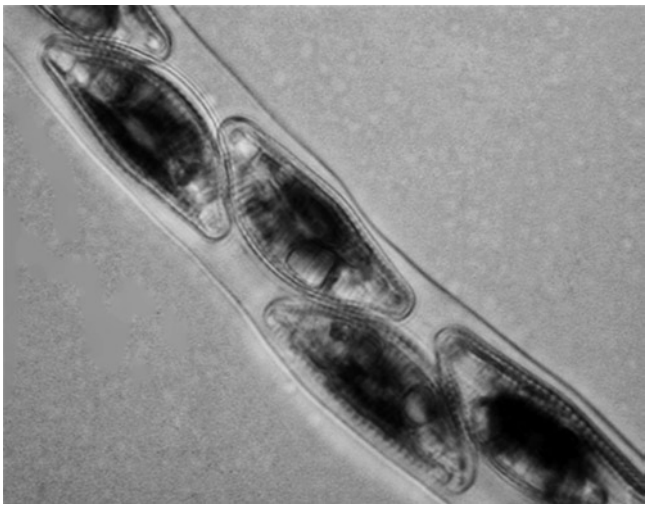

Figure 2. Cymbella prostrata var auerswaldii (Rabenhorst) Reimer growing in a tube (Photo copyright retained by author).

have a similar appearance to the naked eye, that of an off-white or gray mass that appears like a bunch of soft, depigmented green algae (Fig. 2). Under the microscope in a wet mount, the living diatoms line up within these tubes like beans in a pod. Other freshwater diatoms grow as single cells, and can be found between the pebbles on the bottom of slow-moving waters as a fine brown or golden-brown dust. If there is a river with a constant stream flow, there can be a layer of sediment on the tops or upstream surfaces of underwater rocks, which includes accumulations of single diatoms, and this material can be collected with a turkey baster or other suction device, and allowed to settle in a cylinder as above.

3. Collecting in Brackish and Marine Waters

Diatoms in the ocean are both colonial and free living, and are somewhat more challenging to collect in any quantity than diatoms in freshwater settings. Growth of marine algae that do not have a shiny or smooth surface may be sources of diatoms, similar to the freshwater technique described above. Often these will grow on buoys or on anchor lines, or on the surfaces of wood or plastic that are in contact with the sea for a prolonged period of time. A traditional source of exotic diatoms is washings from the surfaces of marine shells, such as the Conch, and a newly harvested shell can produce extraordinary specimens by simply brushing the surface of the shell with a toothbrush swished in water, and repeatedly rinsed in a collecting bottle as the brush color changes to brown, stained with living diatoms. Free-living marine diatoms can settle onto the surface of rocks or the sand under water, and a collection of sediment or “dust” from the upper surface

of rocks in an area where the water is quiet can provide a lovely marine sample. A sample of sand from the surface of a flat as the tide has receded can include a collection of larger sand granules and smaller diatoms, which need to be rinsed and released from the sand grains. Sediment adhering to a marine anchor can be treated in the same way. Of course, the traditional technique for collecting planktonic species is to tow a very fine funnel-shaped net behind a boat at slow speeds, which is beyond the realm of possibility for most individuals with a casual interest in diatoms. There are other diatoms that grow between the grains of mud or sand, such as *Pleurosigma angulatum* (Queckett) W. Smith, which finds its way to the surface of the sand at low tide, appearing as a brownish color on moist sand when compared to adjacent areas. These diatoms and some substrate can be gently scraped off of the material underneath using a credit card or other small piece of plastic. The diatoms and the sand can be placed in a shallow dish, moistened with seawater, and covered with a muslin handkerchief, and at the next low tide the *Pleurosigma* will find their way through the handkerchief to the upper surface of the handkerchief, where they can be removed easily with a sable brush.

4. Collecting Fossil Diatoms

There are sites around the world where diatoms fell as sediment out of marine or freshwater bodies of water over time, and formed deep concretions on the bottom. Over time, the organic material decomposed and the diatom frustules were pressed together, resulting in diatomaceous earth, or diatomite. Perhaps, the most famous location to diatomists are the deposits at Oamaru, New Zealand, a marine deposit with extinct and exotically unique forms unlike those found anywhere else in the world. Additional sites of some notoriety include: the freshwater deposits at Terrebonne, Oregon, on the eastern slope of the Cascade Mountains north of Bend, Oregon on the banks of the Deschutes River. These deposits are quite loosely packed, appear as white layers in road cuts near the Deschutes River, and appear to be composed of about 97% unbroken frustules. There are freshwater fossil deposits in Klamath Falls, Oregon, well known to Victorian diatomists as the source of varied freshwater species which form brilliant white, hard chalky deposits throughout the Klamath basin, the site of an ancient lake which preceded the formation of the Cascade Mountains. This diatomite or diatomaceous earth is much more densely compressed and is actually used as chalk by children growing up there to draw hopscotch courts on the pavement of driveways.

Marine deposits at Lompoc, California have long been the site of commercial extraction and production for industrial use for many years. A much smaller deposit of marine diatoms near Dunkirk, Maryland was once a source of diatomite on the US East Coast, featuring exotic extinct diatoms and some diatoms which are still living, perhaps most famous for the large numbers of the beautiful centric form, *Actinoptychus heliopelta* Grunow. Outside of the USA, sites of notoriety include freshwater deposits from Toome Bridge, Ireland, and marine deposits from Szent Peter, Hungary, the exact location of which appears to have

Figure 3. The light band is fossil freshwater diatomaceous earth next to Highway 97 at Klamath Falls, Oregon. This diatomite is estimated to be 1.5 million years old. The columnar basalt is thought to have appeared at the time that the Cascade Mountains were formed, ending the life of the lake that produced the diatoms (Photo copyright retained by author).

been lost as national boundaries changed during the Second World War, and which may have been nearly completely extracted, used in the preparation of dynamite during this conflict. Additional marine fossil deposits are located at Mors, Jutland, Denmark, which is so heavily compressed that many valves are fractured, and at additional sites in France and in Russia.

The diatomite in these fossil deposits can range from white to tan-colored to green deposits, which may be found at road cuts as exposed strata, ranging in texture from friable, easily crumbled material to specimens that appear to be quite dense and hard. They are typically compressed to break and form fragments of diatoms, interspersed with whole forms and with mineral deposits that approximate the size of the frustules of the diatoms. If very dense, the diatoms may be cemented together with calcium carbonate and other minerals leached and condensed around the diatoms. It is typically impossible to determine if a deposit is silt or diatoms without looking at a bit of the specimen through the microscope (Fig. 3).

5. Cleaning Diatoms

The goal of cleaning diatom samples is to have as a final product a sparkling white suspension of diatom frustules, valves, and girdle bands in distilled water, free of diatom fragments and contaminating sediments, so that a single diatom valve can be examined accurately. This suspension can be made into a strew slide, placing a drop

onto a glass coverslip or glass slide, which is then allowed to dry without heat, which will tend to make the diatoms clump or form lines. When the drop dries, if the diatoms are clean and without any chemical residue remaining in the solution, they will not be adherent to the glass and may be lifted off to make selected slides. Or, once the strew is made, the slide can be heated to glowing red in order to sinter these small bits of nature's glass onto the surface of the coverslip and then covered with a tiny drop of high refractive-index mountant (Naphrax, Zrax, Styrax, or Hyrax) and coverslip for subsequent examination. In practice, it is sometimes simple and at other times quite difficult to obtain a specimen of this degree of cleanliness, depending upon the extent of contaminants present in the sample.

All fossil deposits must be broken up without damaging the frustules, which precludes a mechanical approach of crushing. The simplest technique is to repeat multiple cycles of freezing and thawing in fresh water until the sample breaks into dust. This may take as few as 3 cycles or as many as 50 or more freeze–thaw cycles.

The next step in cleaning both recent and fossil forms involves the neutralization and removal of carbonates, which is best done with the addition of hydrochloric acid or nitric acid and gentle heat. (Diatoms are remarkably stable in strong acids, but are dissolved by alkalis.) In some cases, such as in cleaning the tubular forms (Cymbella and Didymosphenia) mentioned above, the mass of tubular material simply disappears in contact with this acid with a dramatic "whoosh!" However, in cases of fossil material steeped in carbonates, the diatom suspension may show little if any reaction to the acid, and may require prolonged low heat to extract the carbonates, which typically leach out creating a yellow or tan color in the acid solution. Some fossil samples that I have worked on have required treatment with multiple changes of hydrochloric acid, and heat applied for days before the carbonates are removed adequately. This acid must be washed free and removed completely before any next step can occur.

There have been cold or warm processing techniques described involving solutions of hydrogen peroxide, bleach, or a mixture of dilute sulfuric acid and crystalline potassium permanganate, but each of these has drawbacks. High-concentration hydrogen peroxide can damage the delicate sieve plates present in diatoms (K.A. Kemp, 2005, Scanning electron microscope observation by Frank E. Round of sieve plates cleaned with peroxide, Personal communication), and these techniques are significantly less effective, but also less dangerous, than hot processing with concentrated sulfuric acid using added potassium chlorate.

In the hot-acid technique, the sample is heated in a Pyrex beaker over a burner with concentrated sulfuric acid added to a moist solution of diatoms and water, which will boil until all water is driven from the specimen, leaving the sample in fuming sulfuric acid. The addition of this acid will convert recent samples, and some fossil samples, to a black color, reflecting the oxidation of organic elements to carbon. Then, very carefully, small spatulas of crystalline potassium chlorate, a strong oxidizing agent, are added to the sample under continuing intense heat until the color changes to a dark brown, then tan, and finally to a white or pale yellow color, at which point heat may be discontinued and the sample allowed to cool. When the beaker is no longer hot, a very small amount of distilled water from a

wash bottle is added by allowing a few drops at a time to run down the side of the beaker, and the beaker is swirled. This will result in vigorous boiling and spattering at first, until the acid concentration is reduced.

As anyone trained in inorganic chemistry knows, this is not a technique recommended by chemists ("always add acid to water"), as the reaction releases a great deal of heat, and the concentrated acid can literally erupt from the beaker if too much water is added too quickly, potentially causing severe thermal and chemical burns or destroying equipment. However, if this addition is done with great caution, slowly and gradually, swirling the mixture between additions of the drops of water, the acid solution can be diluted and then decanted off when the diatoms settle. The diatoms are then further rinsed multiple times until all traces of acid are removed.

This will result in a sparkling clean specimen composed of a mixture of whole frustules, valves, girdle bands, fragments of frustules, and possibly tiny amounts of quartz or silica as a contaminant. If there are whole frustules remaining in large numbers or if examination reveals that the diatoms taken from fossil samples have adherent bits still stuck to them or if valves are stuck to one another, the sample can again be brought to a boil in distilled water, then "shocked" by the addition of a dilute solution of sodium hydroxide, allowed to boil for 15–30 s, and then reacidified by the addition of excess hydrochloric acid. A longer period of time in an alkaline environment can, of course, dissolve the diatoms completely. Stubbornly adherent samples may require several shock treatments.

The sample can be further separated by running it through very fine sieves, in the range of 140–600 mesh per inch, allowing the removal of small contaminants while the diatoms are held back. The sample is aggressively squirted with water from a laboratory wash bottle while in contact with the sieve, and the smaller pieces pass through. Both the retained and the sieved material should be kept and examined microscopically, as there may be very small diatoms that pass through the mesh that are worthy of study.

Finally, the diatoms may be stored in glass vials with a drop or two of phenol added to prevent fungal growth. Prior to making a strew slide, the water should be removed with a pipette, and fresh distilled water added, as the phenol will cause the diatoms to stick to the glass slide when placed in a strew.

These techniques are described in only a very general way here, and further information may be found in a series of articles originally published in The Microscope (Meakin, 1939; Swatman, 1937). There is no more succinct summary of cleaning and mounting techniques available than this series of papers.

6. Notes on Mountants

Microscope slides of diatoms are made by placing a sample between a microscope slide and thin coverslip of glass, typically with a "mountant" between the two which stabilizes the subject matter and causes the coverslip to adhere to the glass slide. Historically, diatoms were originally mounted dry, in liquid, or in a resinous

mountant. Since diatoms are made of glass, the usual mountant, Canada balsam, was not well suited, since it has a similar tendency to bend light that glass does, or a similar index of refraction. Consequently the diatoms became extremely difficult to see or to photograph, especially since Phase Contrast and Differential Interference Contrast techniques were not available. Microscopists searched for mountants which were of a higher refractive index than the diatoms to make them visible. Dr. Henri Van Heurck proposed in 1885 that a mountant made from the resinous gum of a shrub, *Styrax liquidambar*, could be used (van Heurck, 1885). Subsequent discussion among diatomists as recorded in *The Journal of the Quekett Microscopical Club* in England included concerns about the difficulty of getting this liquid resin to harden, the problem of crystals appearing at a later date in completed mounts, and the observation that material sold as "Styrax" included resins from America which came from a different species of tree, *Styrax officianalis*, the resins from which tended to have different solubilities in organic solvents. While no Styrax diatom mountant is marketed commercially at present, either liquid gum or chunks of hardened resin may be obtained from commercial sources now and processed into a mounting medium. The problem of crystals appearing in subsequent mounts can be minimized by boiling the gum or hardened material in several changes of water, which extracts water-soluble elements which will crystallize when dried, and the problem of lack of hardening is solved by exposing a thin film of the resin to full sunshine in open air for a whole summer season or longer, resulting in polymerization into a dried and hard material (at the time referred to as "horny") which is contaminated with insects and dust. This hardened resin can be removed and dissolved in acetone, which is then filtered, removing the foreign objects. The acetone is then allowed to evaporate, and the resin is dissolved in toluene or toluene/chloroform for final use. These resins have components which are less soluble in toluene, and a mixture of the two solvents may be needed or one can simply remove the fraction which goes into solution in the toluene. It appears that this mountant is permanent and much less likely to decompose over time when prepared in this manner. Klaus Kemp believes that Styrax is the most stable mountant now available. Styrax has been mixed with polychlorinated biphenyls (PCBs) to increase the refractive index of the mountant. This mixture is known as Styraclor, and obviously has its own added potential for toxicity to the diatomist. It also tends to shrink more aggressively over time than plain Styrax, sometimes resulting in shattered valves over weeks or months.

Although several other high refractive-index mountants have been used historically, at this time only two are available commercially: Naphrax and Zrax. Both are available through Internet sources. Naphrax is commonly used by diatomists, a synthetic resin which can be made relatively simply by an organic chemist, but it is not an archival material, and over time this may develop small bubbles. It is a condensation product of naphthalene and formaldehyde. Organic Chemistry Professor Dr. Bill Dailey at the University of Pennsylvania developed a new product, Zrax, which has similarities to Hyrax and which promises to be more stable over time than Naphrax.

Original articles about several high refractive-index mountants (Naphrax, Pleurax, and Hyrax) can be found in the following references (Hanna et al., 1929; Hanna, 1930, 1949; Flemming, 1943, 1954; Von Stosch, 1974).

7. Mounting Selected Diatoms

The first viewing of a selected diatom mount is a moment that most people remember, seeing from 20 to 50 or several hundred diatoms mounted in a geometric arrangement that approximates the size of the head of a pin (Figs. 1 and 4). The originator of this art form was Johann Diedrich Möller in Germany. These mounts are fascinating, and nothing quite compares to the sense of wonder about how all of these diatoms can be arranged in such a tiny space with order and symmetry.

Although a number of diatom mounters from the Victorian era are famous among diatomists and microscopists, the art was passed down in an oral tradition, and no records were kept of exactly how diatom mounts were done. Klaus Dieter Kemp of Microlife Services in Somerset, U.K. reinvented the art form to a refinement that made it a teachable skill. He also devised an inexpensive *micromanipulator* which can be constructed with simple machine tools. Details regarding the design and construction of this device can be found online at Leszek Wolnik's *Diatoms Ireland* Web site. There are detailed construction directions available here: http://www.diatomsireland.com/micromanipulator-intro/. Although the design on the Web site involves drilling a hole through the stage of an optical microscope, a clamp can be placed on the side of a plain stage with the shaft

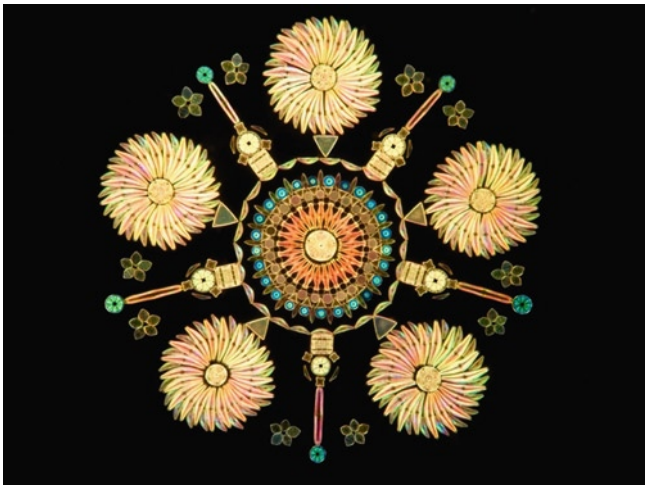

Figure 4. Exhibition arrangement of 471 diatom valves by Klaus Dieter Kemp, photographed in polarized light, digitally reversed. The diameter of the arrangement is 2.57 mm (Photo copyright retained by author).

running through the clamp, thus making this attachment nondestructive to expensive equipment.

The micromanipulator lifts and lowers a pulled glass needle directly on the optical axis of the microscope, and is typically used with an Achromatic 10× objective which is a compromise between acquiring the magnification needed to see the diatoms, and having sufficient working space between the front of the objective and the microscope slide to lift diatoms and move them. Other types of needles have been used in the past, but pulled glass from a capillary tube has stood the test of time, since it is both flexible and easily replaceable. The slide is simply manipulated by hand, pushed over the surface as needed, without the use of a mechanical stage, centering a dry uncovered well-cleaned strew slide with a diatom on it that will be moved to the final slide for mounting. If the cleaning has been done as described, the needle may be lowered to touch the surface of the strew slide adjacent to the diatom, the slide pushed gently so that the needle inserts itself under the diatom, and then the screw on the micromanipulator is engaged, lifting the diatom off of the strew slide, and then lowered onto a temporary slide for safekeeping.

In addition to the micromanipulator, a *slide ringing table* is another necessary piece of equipment. This is a heavy brass disc that turns on several ball bearings, with concentric circles and clips on the surface that allow a glass slide to be centered and then rotated around a vertical axis. A good ringing table should be constructed with a massive brass disc that will turn smoothly for at least 20 s when spun; this allows the diatomist to apply concentric rings to the surface of the slide or a ring around the edge of the round coverslip. This table is best used with a low-power stereomicroscope for exact centration.

In brief, the technique for making a selected slide is as follows. The final slide for mounting is first cleaned thoroughly, and then centered on the ringing table upside down. Two or three concentric rings are placed on the reverse of the slide, one about 0.5 mm in diameter to mark the exact center of the slide, and others at about 3 mm and at 10 mm to help orient the coverslip when it is finally attached. The slide is inverted and placed to the side for a moment.

A second slide is prepared in a similar fashion, and the round coverslip temporarily attached with Canada balsam, centered with the help of the two circles. Then, a liquid adhesive is rubbed onto the upper exposed surface of the coverslip in a very thin film, which will cause the diatom to adhere where it is placed. A diatom, or several, are placed onto this film of adhesive, and delicately pushed with the needle into the desired position on the slip. When the orientation of these diatoms is correct, the slide is heated to dry the adhesive, and then a ring is placed around the diatoms using a fine sable brush and India Ink, or a very fine marking pen, on the ringing table. A drop of Toluene or Chloroform, the solvent for the mountant, is placed on the coverslip to see that it fills all of the spaces under the diatom valves cemented to the coverslip. If there is a trapped air bubble, it may be that with the passage of time the solvent will find its way into the space under the valve, or a hole may need to be punched in the edge of the valve to allow the solvent to enter the space. If there is solvent inside of every valve, the mountant will mix with this and yield a final mount without any trapped air bubbles.

The temporary slide and slip are reheated and the coverslip removed, and another drop of solvent is placed to fill all potential air spaces. Seeing that the valves are all filled with solvent, a tiny drop of mountant is placed on the center point of the surface of the final slide for mounting, the slide is inverted, and this is then carefully lowered onto the coverslip, mating the slide to the slip. The slide is then turned right side up, the coverslip is centered using the guide circles on the reverse of the slide (Fig. 5). The coverslip may be kept from moving while the mountant is still liquid by the use of three glazier's points placed equidistantly around the edge of the coverslip on the surface of the slide as it is set aside to dry over low heat for a prolonged period of time. This will avoid the experience of returning to find the coverslip skated away towards an edge of the slide. This technique centers the diatom on the microscope slide, and keeps the diatom from being separated from the coverslip by a mass of mountant, which makes examination impossible with high-magnification oil-immersion objectives that have a very short working distance.

I must comment about how many delicate steps are involved in this process, any of which can go wrong and ruin the slide before it is completed. Oddly, after drying solvent from the mountant for an extended period of time, it is not rare to find that a single diatom valve in an arrangement has moved away from its neighbors, ruining the symmetry of the mount and leaving the observer wondering what motive force could possibly move only one valve through the resin, leaving all of the others in their places? Thus, a successful arrangement is both a creation of great beauty and a source of joy.

To my knowledge, electron microscopists have not utilized any of these manipulative techniques to mount an individual diatom valve on a coverslip for examination, but this technique could allow an electron microscopist to isolate and examine a specimen without a lengthy search through a strew.

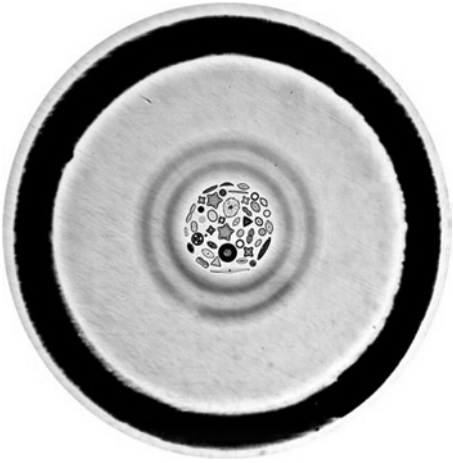

Figure 5. *Light-colored guide rings* on the rear of the slide and *dark India ink ring* on the mounting glass to help locate the diatom arrangement. The guide rings are removed when the slide is finished (Photo copyright retained by author).

8. Photographing Diatoms

Before one can obtain good-quality images of one's slides, one must have adequate equipment and also know how to use it. Unfortunately, there are more microscopes than individuals who take the time to learn how to use them correctly, paraphrasing a comment made by the President of the South African Association for the Advancement of Science (Pijper, 1939). In a classic textbook Charles Shillaber said, "Of the four outstanding laboratory optical instruments – the camera, the telescope, the spectroscope, and the microscope – the microscope with its accessories is by far the least understood, the most inefficiently operated, and the most abused." (Shillaber, 1944). Many who use microscopes casually are frankly unfamiliar with the need to fill the rear element of the objective fully with light to obtain maximum resolution from the lens, and with the need to align, focus, and set the aperture of the condenser correctly for each objective for the additional purpose of maximizing contrast. A basic understanding of how to get optimal performance from the equipment should be the foundation of work with this instrument, yet how many microscope users ever immerse themselves in a textbook of microscopy? Several classics bear reading and rereading (Shillaber, 1944; Hartley, 1962; Loveland, 1970; Needham, 1977). I also recommend a series of books that offer practical tips about maximizing the functionality of the equipment that one has, some of them unexpected (Jackson, 2005–2010).

The great shift from film to digital imaging has been a significant help to the challenge of photographing diatoms. In the past, options included processing black and white negative film, or shooting slides. Color negative film was prohibitively expensive and required careful temperature controls in the chemical baths required for processing. In black and white photomicrography one had the ability to manipulate the final image in the darkroom, but color slide images were not editable for exposure, contrast, or color saturation. Now one can find either fantastically expensive dedicated digital cameras for photomicrography from the microscope manufacturers, or the option of using a point-and-shoot camera with an adapter for the microscope. Assuming that one has a good optical link between the microscope and the digital camera, a task harder than it might seem, additional training and experience is needed to understand the camera and how best to use its variable settings. This typically involves setting up the camera for exposures with a fixed, fully open aperture, with the focus set to infinity. Although one may attempt to adjust the link to make the camera parfocal with the visual image seen through the oculars, it is prudent to focus each image at the camera. Running a larger flat-screen monitor off the camera has been helpful in fine focusing, as is using additional magnification to examine the small flat screen in the camera with great care. In this set-up, the camera may adjust its exposure duration, but the microscope optics set the focus and aperture of the final image.

While postexposure processing does not make up for gross errors of technique, the ability to manipulate color balance and saturation, contrast, exposure, and other variables can help to make an image really stand out. The interested

reader is referred to perhaps the best recent educational title regarding techniques of imaging and digital recording (Murphy, 2001).

Thus, the challenge to the photomicroscopist is first to have a diatom slide of good technical quality, second to have adequate microscopic equipment with a good digital camera and optical linkage, and third for the photographer to have a practical grasp of the principles of optical microscopy, digital imaging, and the understanding of a postexposure software package, minimally Helicon Filter or Photoshop Elements, or better still, Photoshop CS5 to allow further refinement of the image. Image-stacking software, such as Helicon Focus or newer editions of Photoshop, allow one to take a series of photographs at different focal planes through a subject too deep to be in focus with a single exposure, then to extract the areas of each photo that are in focus automatically and to blend them seamlessly to create an image with an apparent depth of focus which is optically impossible. As magnification increases, the depth of focus diminishes, so that this technique allows the creation of a photograph which shows the subject in a way that it has never been seen before. In addition, this software can seamlessly blend overlapping images to create a much larger field of view than is optically possible, allowing one to preserve the increased resolution of an objective of larger magnification, while creating a wide field of view which defies normal optical limits.

Having a clear tabular record which documents settings of the microscope and camera for all images, will allow one to review what one did and to learn from this process, hopefully leading to improvement over time.

There are several illumination techniques which seem well suited to imaging diatoms. These include Darkfield, Oblique Illumination, Circular Oblique Illumination, Hoffman Modulation Contrast, Differential Interference Contrast, and Jamin–Lebedeff Interference Contrast. Phase contrast is less effective than might be imagined since this technique creates either light or dark-colored artifacts in the images surrounding objects of interest. Darkfield imaging may be produced with a simple stop in the light path to the condenser on lower powers or with digital image manipulation, by inverting the values of a brightfield image. This latter approach can create interesting, if artifactual, colors in the image, and avoids a significant problem of darkfield photomicrography, namely, that every tiny speck in the mountant will show up like a firefly in the image, necessitating additional work to eradicate the spots. This also allows one to create acceptable low-power pseudo-darkfield images, since the area covered by a standard darkfield condenser is quite small. Oblique illumination involves adjusting the light path through the condenser so that only a sector of the glass is used. This can cause an interesting three-dimensional effect that looks somewhat similar to the images produced by Differential Interference Contrast after Nomarski. Hoffman Modulation Contrast may be regarded as a type of oblique illumination, and cannot be used with objectives of a higher degree of correction than Planachromats, thus limiting the resolving power of the optical system. Some quite exceptional images have been created through the use of Circular Oblique illumination, which reduces the intensity of light passing directly through a diatom. It is thus a type

of interference contrast without expensive equipment, but also without much ability to adjust the illumination, either.

In my opinion, the techniques of illumination which involve interference are the most effective for photographing diatoms. Nomarski Differential Interference Contrast may be adjusted to provide a pseudo-darkfield image, or adjusted so that any of the interference bands may serve as a brilliant color background to one's image. Because the most highly corrected microscope objectives can be used with it, Differential Interference Contrast is a technique which produces a sharp image with a shallow depth of focus, which is no longer a limitation now that image- stacking software is available. While five to ten exposures at different focal planes may be needed to create the final composite image, the results speak for themselves. Jamin–Lebedeff Interference Contrast provides images which are significantly more dramatic, as the system converts differences in optical path length to differences of color in the final image. Hence, a colorless diatom may appear to have a spectrum of color across apparently planar surfaces as a consequence of the use of this system (Fig. 6). The limitations are that finding this equipment is extremely difficult. It was rare when new, made by Carl Zeiss Oberkochen in the 1960s and 1970s. When located, the equipment may not work, and adjustments or repairs are not possible either through Zeiss or through independent individuals servicing microscope equipment. Jamin–Lebedeff illumination involves the use of a paired condenser and Achromatic objective which must be carefully aligned on the optical axis with the other optical components of the microscope, with the condenser and objective placed between crossed polarizing and analyzing filters. Light coming from the condenser is split into two rays by use of a calcite plate in the upper surface of the condenser. One ray travels through the diatom, and the other ray travels adjacent to it. Another calcite plate in the objective recombines these two rays, and as the ray passing through the diatom is slowed, the interference of the waves produces the change in visible light. Zeiss provided three sets of paired condenser and objective, in 10×, 40×, and 100× objective magnifications. The 40× pair is ideally suited to imaging individual diatom valves.

The system is designed to be used quantitatively with monochromatic light, but when used with full-spectrum light, the interference bands can be painted across the image by simply turning a knob, with brilliant changes in both background

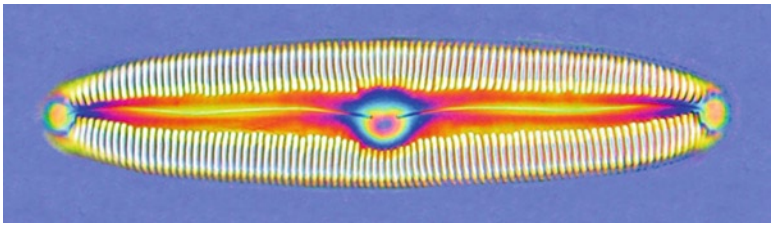

Figure 6. *Pinnularia major* (Kützing) Rabenhorst with Jamin–Lebedeff Interference Contrast. Note the color changes along the raphe which reflects optical path length differences through the diatom valve (Photo copyright retained by author).

and diatom colors. The 40× Achromat has a numerical aperture of 0.65, and the raw images have low contrast and tend to look milky, with inadequate color saturation and clarity. Here is where the judicious application of corrections possible through Photoshop can increase saturation and contrast and apparent sharpness, resulting in brilliant-colored images of individual diatoms which are impossible to create with any other illumination technique (Figs. 6 and 7).

It is unfortunate that interference techniques have not remained available and evolved, since they can be used with living specimens in biology. At the time that this equipment was commercially available, there was a surge of interest toward electron microscopy, and now the fashionable imaging techniques are Fluorescence and Confocal Laser Scanning techniques. Perhaps, microscope manufacturers will consider reissuing this equipment in the future as its usefulness is reconsidered. The reader is referred to the following reference for more information about interference techniques (Tolansky, 1968).

Finally, a combination of several techniques may produce unexpectedly impressive visual results, such as Darkfield with crossed polarizing filters or Hoffman Modulation Contrast with crossed polarizing filters. Modern Planapochromats are constructed to be strain free, and if one is lucky, an Achromat fitted for Hoffman Modulation Contrast may be manufactured sufficiently without strain to allow polarized light techniques. This combination is especially pleasing when used with lower-power objectives.

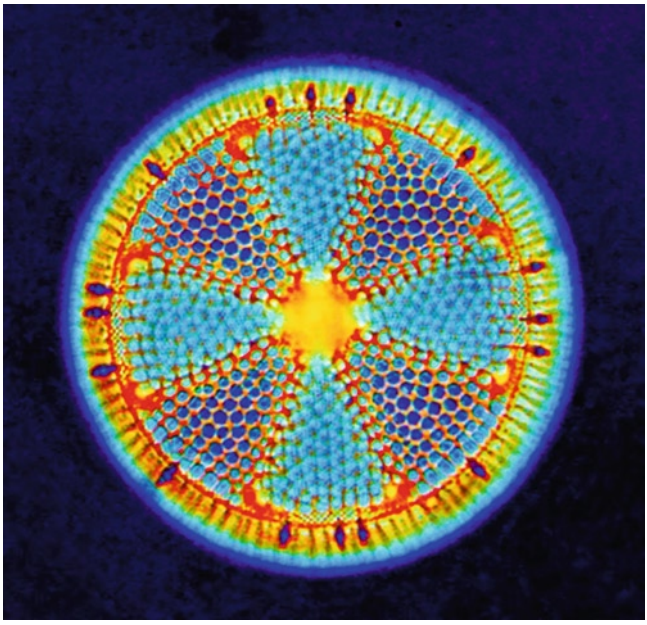

Figure 7. Jamin–Lebedeff Interference Contrast image of *Actinoptychus heliopelta* Grunow from fossil material at Dunkirk, Maryland. Single exposure with no image stacking (Photo copyright retained by author).

9. Summary and Appreciation

It is my hope that this brief summary of the techniques used to collect, clean, mount, and photograph diatoms will provide the reader with an overview of these processes. I further hope that the review will evoke interest rather than confusion (or, worse: boredom). I would like to express my heartfelt appreciation for the time and effort expended by Klaus D. Kemp to teach me the arts of cleaning and mounting diatoms and also teaching me patience, necessary in a diatomist, over many years time. I extend appreciation and thanks to my wife, C.J. Puotinen, for her compassion, and to my dear children, Peter, Karen, and William for their many contributions to my life. This chapter is dedicated to my first grandchild, Layla Lynn Nagy, who was born in Eugene, Oregon on November 9, 2010, while this manuscript was also in its final stages of being birthed. Welcome to this wonderful planet with all of its mysteries, dear little one!

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PART 1:
TAXONOMY, SYSTEMATICS AND PHYLOGENY

Cox
Williams
Kociolek
Medlin
Theriot
Ruck
Ashworth
Nakov
Jansen

Biodata of **Eileen J. Cox** author of "***Morphology: Cell Wall, Cytology, Ultrastructural and Morphogenetic Studies***."

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 21–45
DOI 10.1007/978-94-007-1327-7_2, © Springer Science+Business Media B.V. 2011

MORPHOLOGY, CELL WALL, CYTOLOGY, ULTRASTRUCTURE AND MORPHOGENETIC STUDIES

Overview and Specific Observations

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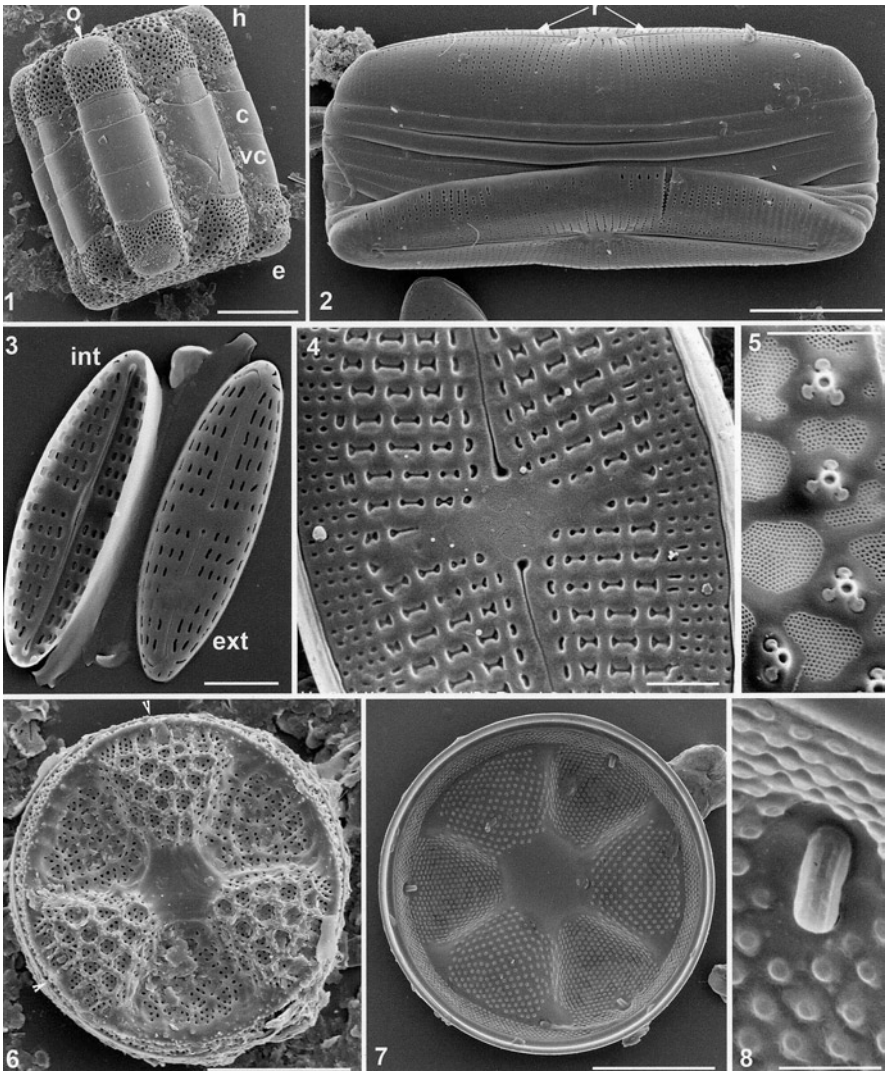
1. Introduction

With a few exceptions, e.g., *Phaeodactylum tricornutum* Bohlin and endosymbiotic species, all diatoms are recognized by the possession of distinctive, essentially bipartite, variously perforated and ornamented, silica cell walls (frustules) that enclose the eukaryotic protoplast. The silica wall constrains and protects the protoplast, but must also provide routes for nutrient uptake, gaseous exchange, and the secretion of cellular products, e.g., polysaccharides. Thus, particular structural features can be associated with particular functions, e.g., raphe slits with motility, fultoportulae with the excretion of chitan fibers, ocelli, pseudocelli, and apical pore fields with mucilage secretion for attachment, peripheral spines with cell linkage to form filaments. However, whether every structural feature has a discrete function remains an open question.

A complete frustule comprises two (usually) relatively larger components (valves), each with an associated series of bands (the girdle or cingulum), the older valve and its cingulum being termed the epitheca, the younger valve and cingulum, the hypotheca (Figs. 1 and 2). Unlike most algae (but cf. desmids), wall formation in diatoms is therefore semiconservative. When a cell divides in two, each offspring inherits one half of the parent wall but must also synthesize a new half wall before separating from its sibling.

Silica deposition to form new valves and cingulum elements occurs within membrane-bound vesicles (silica deposition vesicle, SDV) within the protoplast, being released to the exterior when complete by fusion between the silicalemma and plasmalemma (Pickett-Heaps et al., 1990). This mode of formation, with the often close proximity of forming valves, allows for interactive growth of new valves and potential linkage to form chains and filaments. That new valves are formed within the parent frustule also constrains the shape and size of the hypovalves, often producing the well-known size reduction phenomenon (MacDonald, 1869; Pfitzer, 1869; Crawford, 1981; Mann, 1984), although some diatoms do not undergo size reduction, presumably because there is sufficient flexibility in the girdle region to allow cell size to be maintained.



Figures 1–8. Scanning electron micrographs. Figure 1 Girdle view of *Hydrosera* showing epitheca (e) with valvocopula (v) and one copula (c) and hypotheca (h) with its valvocopula. Valves have ocelli (o) at their corners. Figure 2 Girdle view of *Parlibellus*, showing raphid valves (raphe slits indicated by r) and series of split ring girdle bands, opening at opposite poles. Figure 3 Valves of *Navicula incerta*, one showing external face (ext), the other the internal face (int), with girdle bands lying inbetween. Note lack of pores in the bands. Figure 4 External central area of *Aneumastus*, showing transition from uniseriate to biseriate striae. Figure 5 Internal detail of *Thalassiosira* valve showing fultoportulae with three satellite pores (Courtesy of Pat Sims). Figure 6 External valve surface of *Actinoptychus* with alternately raised and depressed areas. Arrowheads indicate two (of three) external rimoportula openings. Figure 7 Internal view of *Actinoptychus* showing three rimoportulae, positioned in the middle of the periphery of the internally depressed (externally raised) areas. Figure 8 Internal detail of rimoportula of *Actinoptychus*. Scale bars represent 20 μm (Fig. 1), 10 μm (Figs. 2, 6 and 7), 2 μm (Figs. 3–5), 1 μm (Fig. 8).

While all diatoms share the same basic components, the enormous number of morphologically defined genera and species is witness to the variation in shape, symmetry, types of wall structure, e.g., pores, processes, spines, and in the arrangement of those structures. Similarly, diatoms share many cytological features, but may differ in their number and configuration. This chapter will review the common features of wall and cell structure, but will also indicate how these may vary between species, with life history and/or environment, and will discuss some of the underlying cytological processes.

2. Frustule Morphology, Shape, and Symmetry (Light Microscopy)

For over a century, light microscopy (LM) was the primary tool for the study of diatoms, and with the discovery of the intricacies, symmetry, and apparent stability of their wall patterns, frustule morphology formed the basis of their identification and classification. However, early interpretations of diatom morphology were limited by the available technology, thus raphe slits were first described as lines interrupted by a nodule along the center of the valve, and the marginal raphe of *Nitzschia* Hassall, *Surirella* Turpin, *Cymatopleura* W. Smith, etc., was not recognized as such until Otto Müller (1896) demonstrated its presence and its role in movement.

H.L. Smith (1872) devised an artificial key to the identification of diatoms, using valve shape, symmetry, presence or absence of raphe and nodules, as diagnostic characters for tribes and genera, which, apart from its treatment of *Nitzschia* and other diatoms with a canal raphe, largely remained the basis of the accepted diatom classification until the late twentieth century. Thus, three main groups were recognized, raphid (with a raphe on one or both valves), pseudoraphid (usually with a long axis of symmetry but without a true raphe) and crypto-raphid (generally circular, subcircular, or angular, without a true raphe), generic distinctions usually resting on the possession of particular features (or groups of features) of the valve and/or cingulum. A contrasting classification, based on chloroplast type had been devised by Pfitzer (1871), although it was recognized (Schütt, 1896) that Smith's Cryptoraphideae and Pfitzer's group 1A (Coccochromaticae) included more or less the same genera. Incorporating Müller's (1896) findings on the presence of a raphe in *Nitzschia*, *Surirella*, etc., Schütt (1896) presented a new classification, which incorporated the now-traditional split into centric and pennate diatoms, the latter subdivided into the araphid and raphid pennates, according to the absence or presence of a raphe, respectively. However, because LM only provided an approximation of the 3D structure and the siliceous ultrastructure remained obscure, some of these taxonomic groupings are now being dismantled as relationships are reevaluated. Nevertheless, traditional LM morphological characters continue to be used for identification purposes, and the terms centric, pennate, raphid, and araphid are useful shorthand when discussing morphology, symmetry, and pattern, although they do not refer to monophyletic groups (except the raphid diatoms).

2.1. FRUSTULE CONSTRUCTION

The diatom frustule is often thought of as a pillbox, and its morphology is usually described from two views, valve and girdle, looking, as it were, at the top or the side of the box, respectively, valve views usually showing greater morphological variability than girdle views. Whereas valve outline can be circular to oval or variously angled, a variety of elongate shapes (linear, lanceolate, rhombic, panduriform) to sigmoid, the degree of symmetry about different axes can vary, outlines becoming variously heteropolar or dorsiventral, occasionally both. Useful illustrations of shape variation and its description can be found in Hendey (1964) and Barber and Haworth (1981). Valves also vary in topography: flat, convex, variously undulate, with or without clear demarcation between a valve face and mantle. Valve margins may be stepped, e.g., *Orthoseira* Thwaites and *Ellerbeckia* Crawford; may be thickened, e.g., *Ellerbeckia* and *Aulacoseira* Thwaites; or flanged to form pseudosepta, e.g., at the apices of pennate diatoms such as *Rhoicosphenia* Grunow, *Stauroneis* Ehrenberg, and *Gomphoneis* Cleve. Girdle views tend to be simpler in outline, often approximately square or rectangular, occasionally cuneate, but change in proportion as bands are added and/or slide apart during growth, before the next mitosis.

2.2. PATTERNS AND PORE ARRANGEMENTS

The patterning seen on most diatom valves is the result of regular small perforations through the silica (Figs. 1–7). Diatoms with circular, triangular, or multi-angular valve faces almost invariably having radiating patterns on the valve (centric), whereas elongate valves usually have a sternum or long axis of symmetry (pennate), with or without a raphe system (paired slits through the valve). However, there are some bipolar diatoms with a radiating pore arrangement, e.g., *Biddulphia* Gray, *Odontella* C.A. Agardh, and others with elongate valves without obviously radiating patterns, yet also without a sternum, e.g., *Cymatosira* Grunow, etc. (Hasle et al., 1983), which are more closely related to the centric diatoms (Hasle et al., 1983). Several authors have explored the relationships of elongate and centric diatoms (Kooistra et al., 2003, 2004; Medlin and Kaczmarska, 2004; Alverson et al., 2006), and further taxonomic revisions are to be expected in the light of molecular and developmental studies.

The visibility of individual pores varies according to their size and spacing; some are so small that their presence can only be inferred from linear patterns (striae) on the valve surface, whereas others are large and easily resolved with LM. In some cases, single rows of pores give rise to double rows near the valve margin, e.g., *Aneumastus* Mann & Stickle (Fig. 4), or areolae are arranged in single rows at the valve center, but in fascicles at the margin, e.g., *Cyclotella* Kützing, *Cyclostephanos* Round. It is also sometimes apparent that there are

different types of perforations through the valves, e.g., slightly larger, more conspicuous, pores that may be isolated or more sparsely distributed over the valve, or groups of more closely spaced pores that may or may not be bordered by thicker portions of the walls. These have been variously named, often depending upon position or presumed function, but electron microscopy (EM) is usually required to confirm identity and structure (see below). Diatom terminology is highly specialized, but a number of papers provide useful descriptions and definitions (Ross and Sims, 1972; Anonymous, 1975; Ross et al., 1979; Cox and Ross, 1981; Mann, 1981a; Cox, 2004).

Whereas some diatom valves are relatively thin and more or less the same thickness throughout, e.g., *Fistulifera* Lange-Bertalot, *Mayamaea* Lange-Bertalot, *Cymatosira*, and some *Thalassiosira* Cleve spp., in some the virgae (solid silica between striae) are thicker than the vimines (cross connections between virgae), e.g., *Navicula* Bory, *Gomphonema* C.A. Agardh, *Cymbella* C.A. Agardh, and *Encyonema* Kützing. Other diatoms are chambered (loculate), the inner and outer pore openings differing in size and/or shape, e.g., *Coscinodiscus* Ehrenberg, *Diploneis* Ehrenberg, and *Gyrosigma* Hassall, or have partial occlusion of the inner openings between pairs of virgae to create alveoli, e.g., *Pinnularia* Ehrenberg and *Gomphoneis*. Virgae may be of more or less the same thickness throughout the valve, or be variously thickened to form more conspicuous ribs (costae), either at regular intervals, generating apparently bi- or tri-seriate striae, e.g., *Achnanthes* Bory, or irregularly when they may also partially obscure the pores of the striae, e.g., *Diatoma* Bory, *Denticula* Kützing, and *Gomphotheca* Hendey & Sims. Occasionally, costae project into the cell lumen as thin plates, e.g., *Anaulus* Ehrenberg, *Eunotogramma* Weisse, and have then been termed pseudosepta, although this term should be restricted to marginal flanges (see above, 2.1).

With light microscopy, hyaline (solid, nonporous) areas are also variously present. These may be along the long axis (e.g., sternum) or perpendicular to it, on one or both sides of the valves, particularly at the center of the valve, e.g., *Hannaea* Patrick, *Synedra* Ehrenberg, *Stauroneis*. Transverse hyaline areas may be no thicker than the virgae (*Hannaea* and *Synedra*), but they can be distinctly thicker (*Stauroneis*) (Cox, 2001). Hyaline areas may interrupt the rows of pores (striae), forming a regular shape, e.g., lyre-shaped areas around the raphe sternum, e.g., *Lyrella* Karayeva, or be more irregular over the valve face, e.g., interrupting the striae in *Brachysira* Kützing and *Stenoneis* Cleve.

Striae may also be variously interrupted by longitudinal canals, e.g., *Neidium* Pfitzer, *Scoliopleura* Grunow, *Diploneis* Ehrenberg, and *Fallacia* Stickle & Mann. In some genera, more complex chambering is seen, with few simple openings to the exterior, but larger areas of small pores to the interior, e.g., *Scoliotropis* Cleve, *Biremis* Mann & Cox, and *Progonoia* Schrader. The striae adjacent to the raphe system may also be covered externally by a nonporous flap, the conopeum, e.g., *Sellaphora* Mereschkowsky, *Proschkinia* Karayeva, and *Amphora* Ehrenberg.

2.3. RAPHE SYSTEM

With light microscopy, the raphe system is usually seen as a pair of longitudinal lines, interrupted at the center by a slightly thicker area of solid silica often referred to as the central nodule. In naviculoid diatoms, the raphe is more or less central along the long axis of the valve, although it is laterally displaced in dorsiventral diatoms, such as *Cymbella* and *Encyonema*, usually extending more or less the full length of the valve, with a few exceptions, e.g., *Amphipleura* Kützing and *Berkeleya* Greville. However, in the nitzschioid diatoms, the raphe is usually strongly lateral and subtended by struts (fibulae), variously visible as dots or bars, while in the surirelloid diatoms, the raphe slits run along each long side of the valve, one end of the valve representing the position of the central nodule, the other of two fused polar nodules. A simpler raphe system is present in the eunotioid diatoms, usually short and strongly lateral and sometimes difficult to see. It is still uncertain whether the raphe slits of *Eunotia* Ehrenberg and its allies are homologous to those in the other raphid groups (Cox and Kennaway, 2004), the Eunotiales being unusual in possessing both raphe slits and rimoportulae, the putative ancestral state of true raphe slits.

2.4. CINGULUM

The cingulum (girdle) usually comprises two to many, slightly overlapping, bands that may be entire, split or partial rings, and which allow the cell to expand in a pervalvar direction. In a few instances, the cingulum comprises many, scale-like bands that are each less than half the cell circumference, e.g., *Rhizosolenia* Brightwell (von Stosch, 1975). Bands are frequently perforated, but some lack pores (*Navicula*, *Haslea* Simonsen, *Gyrosigma*, and *Pleurosigma* W. Smith), while others are partially bridged by thin plates (septa), that may be straight (*Tabellaria* Ehrenberg and *Striatella* C.A. Agardh) or undulate (*Grammatophora* Ehrenberg). Septa or craticular bars that grow out from the girdle bands on each side of the frustule, interdigitating or linking in the middle are seen in *Climacosphenia* Ehrenberg, *Climaconeis* Grunow, and *Diatomella* Greville. Bands may also be ligulate, i.e., with a tongue-like extension on one side, which fills the gap between the ends of the adjacent band.

Although girdle bands are often thinner and more simply constructed than the valves, in some genera they are robust and complex, particularly some marine, epiphytic diatoms, e.g., *Rhabdonema* Kützing (Pocock and Cox, 1982). There may also be considerable variation in band morphology, from the valvocopula to the most distal band. Girdle bands may be of fixed or indeterminate number, being added over a period of time, the last hypothecal band sometimes being formed just before cytokinesis (Pickett-Heaps et al., 1990 and references therein).

3. Vegetative Cell Wall Structure (Electron Microscopy)

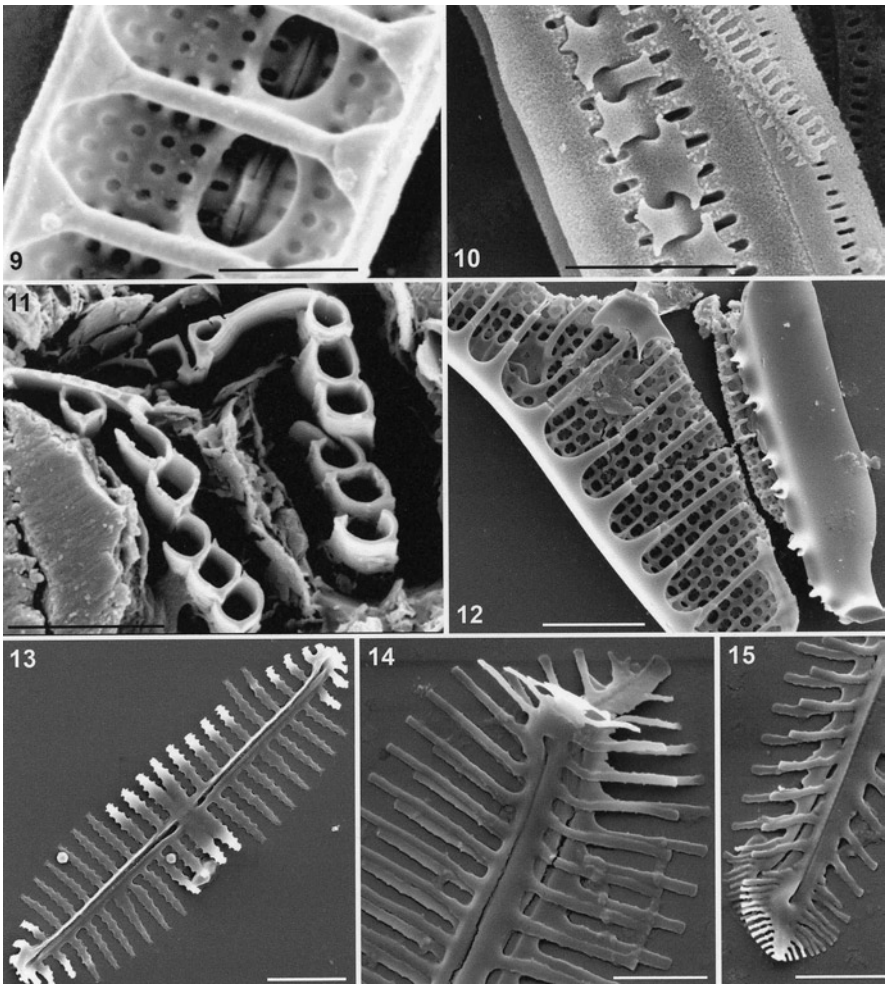
3.1. PORE STRUCTURE AND FUNCTION

While LM can reveal the pattern of pores in diatom valves, electron microscopy is usually required to elucidate their substructure (Figs. 1–12), which varies across orders and families. Round et al. (1990) provides an excellent overview of structural variation across the diatoms although many new taxa have since been recognized. Rarely are the stria pores that are visible in LM simple holes, except for those forming ocelli or apical pore fields. Usually, there is some sort of finer occlusion across the inner or outer opening, or both, although TEM may be required to resolve the finest occlusions (Cox, 1975, 2004). Different types of occlusion have been recognized and named (Ross and Sims, 1972; Mann, 1981a; Cox, 2004), some of which seem to be distributed across the major divisions, e.g., cribra, while others appear more restricted in their occurrence, e.g., hymenes seem to be found only in raphid taxa, rotae only in some centric and araphid pennate diatoms. However, the application of pore occlusion terminology has been less than rigorous, e.g., vola has been used for at least two different types of occlusion (Ross and Sims, 1972; Mann, 1981a), therefore care should be taken when interpreting published accounts.

Particularly in the bi- and multipolar centrics, and many araphid and raphid pennates, contrasting areas of pores are found associated with attachment and/or colony development. Thus, so-called ocelli (Fig. 1) and pseudocelli in centric diatoms (e.g., *Hydrosera* Wallich, *Auliscus* Ehrenberg, *Amphitetras* Ehrenberg, and *Odontella*) and apical pore fields (APFs) in pennate diatoms (e.g., *Rhoicosphenia*, *Gomphonema*, *Glyphodesmis* Greville, and *Synedra*) comprise simple pores or slits (e.g., *Licmophora* C.A. Agardh and *Neosynedra* Williams & Round) through which mucilage can be secreted, attaching the cell by a pad (or stalk) to the substratum and/or to a sibling cell. In the case of attachment to another cell, different types of colony form can be developed, stellate, zigzag, or straight, depending on which poles of adjacent and more distant cells are involved. Isopolar diatoms have such pore fields at both poles, although mucilage secretion is usually restricted to one end at any particular time, e.g., *Synedra* and *Cymbella*. Heteropolar diatoms usually have APFs at one end only (Fig. 15), typically the narrower end, which is then often referred to as the foot pole, because that is where the cell will attach.

3.2. SPINES AND COLONY FORMATION

Cell linkage can also be achieved by mucilage secreted between adjacent valve faces, e.g., *Meridion* C.A. Agardh, *Melosira* C.A. Agardh, and/or by the development of linking spines and projections. Such spines/projections may be found on valve faces, e.g., radiating ridges on *Orthoseira* and *Paralia* Heiberg, or on raised



Figures 9–15 Scanning electron micrographs. Figure 9 Internal view of center of *Denticula*, showing central raphe endings, fibulae spanning the raphe canal, and costae (above the fibulae) spanning the entire valve (Courtesy of Andrew Carr). Figure 10 External view of two sibling valves of *Diadesmis* linked by interlocking spines. Figure 11 Section through one valve of *Proschkinia* and its associated bands, showing the channel-like structure of the girdle bands (Courtesy of Andrew Carr). Figure 12 Internal view of part of an *Epithemia* showing the edges of the valvocopula extending over the transapical costae. Broken portion shows how the valvocopula edges curve over the costae. Figure 13 Internal view of developing valve of *Navicula incerta*, showing virgae and incipient vimines. Arrowheads indicate Voigt discontinuities. Figure 14 Head pole of developing sibling valves of *Gomphonema*. Figure 15 Foot pole of same developing sibling valves of *Gomphonema*, showing the initiation of the apical pore field. Scale bars represent 5 μm (Fig. 12), 2 μm (Figs. 10, 11 and 13–15), 1 μm (Fig. 9).

elevations of bipolar diatoms like *Briggera* Ross & Sims, but more typically linking spines develop around the valve face margins (Crawford and Sims, 2008). They can be so tightly interlocked (Fig. 10) that the sibling valves cannot separate without the spines being broken, e.g., *Fragilaria* Lyngbye, *Neofragilaria* Williams & Round, and *Pseudostaurosira* Williams & Round. As a result, in the absence of cell death and dissociation of the frustule elements, filament/colony length would extend indefinitely. However, some taxa are able to regulate filament length by changing the form of spines developed on adjacent valves such that the spines are straight rather than splayed, e.g., *Aulacoseira*, allowing the valves to slide apart (Davey and Crawford, 1986). The cellular process by which this is regulated is unknown, but a switch between interlocking and straight spines in *Aulacoseira* was simulated using a computer model (Bentley, 2006; Bentley et al., in press). In this model, a change in growth rate was sufficient to switch between the two modes of spine production. In the field and in culture, separation valves occurred less frequently under low nutrient conditions (Davey, 1987). Centric diatoms, e.g., *Skeletonema* Greville, can also link via external extensions of their marginal fultoportulae (see below, 3.3); fusion of the tips of extensions on intercalary cells holds valves together, but extensions on terminal (separation) cells are usually flared and not fused (Round et al., 1990; Sarno et al., 2005). An unusual example of the ability to switch between individual motile cells and interlocked filaments is shown by *Diadesmis gallica* W. Smith (Granetti, 1977; Cox, 2006), putatively due to growth in liquid rather than subaerially.

3.3. PROCESSES

Two distinctive types of opening have been recognized, the rimoportula (labiate process) and the fultoportula (strutted process). While the latter is restricted to members of the Thalassiosirales, rimportulae occur in most centric diatoms, many araphid pennates and the Eunotiales. Rimoportulae consist of a tube through the valve, opening internally by a slit and externally by a simple aperture or open tube (Figs. 6–8). They have been associated with mucilage secretion for attachment and with limited motility in some non-raphid diatoms, e.g., *Actinocyclus* Ehrenberg (Medlin et al., 1986), *Odontella* (Pickett-Heaps et al., 1986), and *Licmophora* (Sato and Medlin, 2006), but in many taxa their function remains unclear. Fultoportulae are more complex in structure (Fig. 5), the tube being flanked by a number of satellite pores, which connect with the central tube (see Round et al., 1990 for a more detailed description). Fultoportulae seem to be involved primarily with β-chitin fiber secretion, but may perform other, as yet unidentified functions. Both rimoportulae and fultoportulae vary in number, structure, and position between species and genera and such variation has been used diagnostically. Various scenarios have been suggested to explain the evolution of the fultoportula (Kaczmarska et al., 2006), which are discussed by Theriot (2008). The restriction of fultoportulae to the Thalassiosirales would suggest that they are homologous, but whether all rimoportulae are homologous remains largely unexplored.

3.4. CINGULUM STRUCTURE

Although it has been given less attention than the valves (but see Hoagland and Rosowski, 1978; Roermer and Rosowski, 1980; Phipps and Rosowski, 1983; Rosowski et al., 1983; Brogan and Rosowski, 1988; Johnson and Rosowski, 1992), cingulum structure varies across the diatoms, being relatively simple and uniform in some taxa, robust and variously differentiated in others. A suggested terminology for many cingulum elements was produced by von Stosch (1975). That paper also illustrates some of the variation in cingulum organization, particularly within the centric diatoms. As mentioned above (2.4), bands may be similar throughout a cingulum, e.g., split rings with two rows of hymenate pores in *Berkeleya*, *Parlibellus* E.J. Cox (Fig. 2), or may vary, with the valvocopula usually being more complex than more distal bands. For example, the valvocopula of *Mastogloia* Thwaites is chambered with a few large pores to the exterior and many fine pores on the interior face of the chambers (Novarino, 1987, 1990), whereas the other bands are simpler, perforated strips. Meanwhile in *Rhabdonema*, the valvocopula and copulae are chambered rings, giving way to one simple, closed pleura and several simple, half bands and terminal plates (Pocock and Cox, 1982). In many diatoms, the valvocopula is modified where it lies against the interior of the valve mantle, e.g., having finger-like extensions that lie over the marginal virgae, e.g., *Epithemia* Kützing (Sims, 1983), *Cocconeis* Ehrenberg (De Stefano et al., 2000, 2003) some *Navicula* (Cox, 1999a), such extensions presumably facilitating linkage with the valve (Fig. 12) (De Stefano and De Stefano, 2005). In the majority of diatoms, girdle bands are essentially perforated strips of silica, but they may also be folded to form channel-like structures, e.g., *Proschkinia* (Fig. 11) (Brogan and Rosowski, 1988; Carr et al., 2008).

Conspicuous septa are characteristically found in some araphid pennate diatoms, e.g., *Tabellaria* Ehrenberg, *Tetracyclus* Ralfs, *Striatella* C.A. Agardh, and *Grammatophora*, partially occluding the cell interior, but also probably providing structural stability to frustules which have narrowly elongate valves and deep girdles. In other cases, e.g., *Licmophora*, septa are less well developed and restricted to the apical regions. Whereas septa in all these genera are thin plates of bridging silica, in *Climacosphenia* and *Climaconeis*, outgrowths from the two sides of the valvocopula project, meet and fuse along the apical axis of the cell and are referred to as craticular bars (Cox, 1982).

Pores through girdle bands may be similar or dissimilar in their structure and occlusion to those through the valves. Thus, while in *Berkeleya* both the valves and girdle bands have poroid areolae with hymenate occlusions, in *Amphipleura* and *Frustulia* Rabenhorst, valves have loculate areolae with round, hymenate occlusions to the interior, the poroid areolae in their girdle bands are transversely elongate (Cox, 1975). Other taxa have valves with loculate areolae or alveoli, but their girdle bands are perforated by simple elongate areolae, e.g., *Pinnularia* (Cox, 1999b), or the bands lack pores entirely, e.g., *Navicula*, *Gyrosigma*, *Pleurosigma* (Fig. 3) (Cox, 1999a).

4. Resting Stages

Some diatoms are able to survive unfavorable growing conditions by forming physiologically resting stages (Sicko-Goad, 1986; Sicko-Goad et al., 1986, 1989) or morphologically distinct resting spores (Hargraves and French, 1983; Hargraves, 1986). Whereas resting stages undergo cytological change but retain their vegetative cell walls, resting spores have characteristically heavily silicified walls, with relatively few pores, and often lack girdle bands. Different types of resting spores (exogenous, semiendogenous, and endogenous) have been described (Ross et al., 1979) based on their relationship to the parent vegetative cell. Some resting spores show morphological similarities to their vegetative stages, e.g., *Detonula* Schütt, *Stephanopyxis* (Ehrenberg) Ehrenberg, but others exhibit contrasting wall morphology, e.g., *Bacteriastrum* Shadbolt, *Leptocylindrus* Cleve (Hargraves, 1976), requiring the observation of encystment or germination to link the different phases. For example, *Eucampia balaustium* Castracane represents the resting spore of *Hemiaulus antarcticus* Ehrenberg, while vegetative cells and resting spores of the same species have been given different form names (see Hoban et al., 1980). Similarly, some of the many resting spores known from the fossil record are probably resting spores of other genera (Hargraves, 1986), although nomenclatural revisions can only be made if this can be demonstrated unequivocally.

Factors controlling the development and germination of both resting stages and spores are poorly known. Physiological resting stages are initiated in some *Aulacoseira* spp. when the light regime falls below a minimum (Gibson and Fitzsimons, 1990; Aslamov and Jewson, 2009), a response that, based on culture studies, is enhanced by a fall in temperature (Sicko-Goad, 1986). In other species, e.g., *Aulacoseira skvortzowii* Edlund, Stoermer & Taylor, low phosphate can induce resting stage formation (Jewson et al., 2008). The formation of resting spores, which are produced predominantly by neritic, marine planktonic taxa in temperate and boreal regions, may be a response to fluctuation in a variety of stress factors, including nutrients (Hargraves and French, 1983). While some resting stages survive long periods in the dark, and resting spores survival times of around 2 years have been recorded, they can also excyst after a few days and may therefore not represent a truly dormant phase (Hargraves and French, 1983).

5. Auxospores and Initial Cells

Because of the tendency of vegetative cell division in diatoms to lead to a reduction in cell size (see chapter by Mann), some mechanism is required whereby maximum cell size can be restored. This occurs in two ways: occasionally by expansion of a vegetative cell (von Stosch, 1965) but more usually by the formation of an auxospore after plasmogamy, whether or not nuclear fusion has occurred to form a zygote (Round et al., 1990). Unlike vegetative cells, the auxospore is not constrained by rigid siliceous valves and can expand to restore

maximum cell size, after which so-called initial valves are laid down and normal vegetative cell division follows.

5.1. AUXOSPORE STRUCTURE

Auxospore walls comprise both organic and siliceous elements, but the latter are either in the form of scales, or bands and hoops, which allow the enclosed cell to expand, although there may be constraints on the way in which the cell can expand. Radially symmetrical centric diatoms, such as *Melosira*, *Ellerbeckia*, *Orthoseira*, *Actinoptychus*, and *Stephanopyxis* form isodiametric auxospores with a primary organic wall and small siliceous scales (Crawford, 1974; von Stosch, 1982), and the initial valves are hemispherical. On the other hand, although they start as isodiametric auxospores, bi- or multipolar centric diatoms such as *Lithodesmium* Ehrenberg, *Chaetoceros* Ehrenberg, and *Odontella*, possess a properizonium composed of siliceous bands and hoops, which helps to generate their different shapes (von Stosch, 1982). The properizonium is continuous with the primary auxospore wall, unlike the perizonium of pennate diatoms. The latter structure also comprises numerous transverse and/or longitudinal bands (Mann, 1994; Mann and Stickle, 1989), but forms beside and just underneath the organic polar caps as the auxospore expands. Unlike the centric diatoms, the initial auxospore wall of most pennate diatoms does not usually contain siliceous scales, although some species do, e.g., *Rhabdonema* (von Stosch, 1962, 1982), *Gephyria media* Arnott (Sato et al., 2004), *Grammatophora marina* (Lyngbye) Kützing (Sato et al., 2008), and scales have been reported at one end of expanding auxospores of *Pseudo-nitzschia* H. Peragallo (Kaczmarska, 2000). Many pennate diatoms are also surrounded by mucilage during auxospore formation and expansion, but recently irregular strip-like elements (incunabula) have been found surrounding the perizonium of *Nitzschia fonticola* Grunow (Trobajo et al., 2006) and in *Pinnularia* cf. *gibba* (Poulíčková et al., 2007).

5.2. INITIAL CELLS

Once the auxospore has expanded to its maximum size, two initial valves are laid down (beneath the organic wall, the properizonium or perizonium depending on the taxon), after which the cells produce normal vegetative valves after mitosis. Initial valves almost invariably differ from vegetative valves in surface topography and may also have rather more irregular striation or other peculiarities, in some cases being described as discrete taxa, e.g., the initial cells of *Caloneis* were mistakenly described as a new genus *Amphiraphia* Chen & Zhu (Mann, 1989). Hemispherical valves in radial centrics or convexly curved valve surfaces in pennate diatoms (Cohn et al., 1989; Passy-Tolar and Lowe, 1995; Sato et al., 2008) are a consequence of the spherical or cylindrical shape of their respective auxospores (Crawford, 1974), and the fact that the initial valves are not formed back-to-back with a sibling, where opposing forces from developing sibling valves will produce

flatter valve faces. Apical pore fields may be lacking or reduced in initial valves (Mann, 1984; Sato et al., 2008), stria spacing may differ from that in vegetative valves (Cohn et al., 1989) and septa are absent from the valvocopula of the first initial valve of *Grammatophora* (Sato et al., 2008).

6. Diatom Cytology

As typical photosynthetic eukaryotes, diatom protoplasts contain a nucleus, chloroplast(s) with or without pyrenoid(s), mitochondria, endoplasmic reticulum, golgi body, and other inclusions, surrounding one or two, often large, vacuoles. The plasmalemma usually lies just below the internal valve and girdle band surfaces, although an additional layer, the diatotepum, may be present between the frustule and the plasmalemma in some diatoms. The diatotepum may lie in close proximity to, and be closely associated with the frustule, or may only be attached to the frustule in certain places, creating spaces between the frustule and the protoplast, e.g., *Attheya* T. West (Schnepf and Drebes, 1977; von Stosch, 1977) and *Craspedostauros* E.J. Cox (Kennaway and Cox, unpubl.).

6.1. NUCLEUS POSITION AND BEHAVIOR

The position of the nucleus varies with taxon, against the girdle in many centric species, at the center of one of the valves in some genera and suspended in the center of the vacuole in *Ditylum*, or in a central cytoplasmic bridge in some araphid pennate diatoms and the majority of raphid diatoms (Pickett-Heaps et al., 1990). In the latter case, it is either always associated with one side of the cell or oscillates across the cell with subsequent mitoses (Mann, 1983). Because the position of the nucleus during valve morphogenesis in raphid diatoms determines the location of the primary side of forming valves, its behavior is associated with the potential to develop valve and cell dorsiventrality. All dorsiventral raphid diatoms have cis symmetry, i.e., the primary sides of both valves are on the same side of the cell and trans valves (primary sides diagonally opposite) do not occur. Depending on the taxon, either the primary side of the valve is wider than the secondary, or vice versa, and there will be a consistent relationship between the shape of the cell and the positions of the nucleus and chloroplast. The position of the nucleus will reveal the primary side of the valves in live cells. Although asymmetry is subtler in centric diatoms, it is invariably linked to nuclear movements during the cell cycle.

6.2. CHLOROPLAST NUMBER, SHAPE, AND ARRANGEMENT

One or more, usually brownish chloroplasts are present per cell, although color varies with taxon and can even be greenish in some species. Chloroplasts have four bounding membranes, evidence of their presumed origin via a secondary

endosymbiosis and an encircling lamella. The lamellae contain three thylakoid stacks and there may be one or more pyrenoids within each chloroplast (Round et al., 1990; Mann, 1996; Bedoshvili et al., 2009). Pyrenoids are refractive proteinaceous bodies that are the site of the Rubisco pathway and may vary in shape and size according to taxon (see below, 7.3). In the majority of non-raphid diatoms, there are many, small, lobed, or irregular chloroplasts per cell, arranged around the cell in the peripheral cytoplasm, whereas raphid diatoms have a determinate number of proportionately larger chloroplasts per cell (Cox, 1996).

Chloroplasts in centric diatoms divide after telophase or during interphase but may also move within the cell, either in relation to diurnal cycles or the environment, e.g., illumination (Pickett-Heaps et al., 1990 and references therein). When there are only one or a few chloroplasts per cell (as in most raphid diatoms), they are usually larger, often plate-like, lobed or H-shaped, and usually consistently positioned in the interphase cell, e.g., under the valve face or against the girdle, along the length of the cell or in pairs fore and aft (Cox, 1996; Round et al., 1990). Their behavior is linked to the cell cycle, chloroplasts moving in relation to the reorganization associated with, and usually dividing prior to mitosis, although some divide as a result of cytokinesis, i.e., the invaginating plasmalemma cuts through the chloroplast or completes chloroplast division as in *Amphora arcus* Gregory, some *Achnanthes* and *Nitzschia* spp (Mann, 1996). Chloroplasts may also divide shortly after cytokinesis, as in *Donkinia* (Cox, 1981), which has four chloroplasts per cell.

6.3. PYRENOIDS

As with chloroplasts, there is also variation in pyrenoids across the major groups (Mann, 1996; Schmid, 2001). Centric diatoms (excluding the Thalassiosiraceae, but including bi-, tri-, and multipolar taxa) usually have single, membrane-bound, inter-thylakoidal or embedded, rectangular or oval pyrenoids that are more or less lenticular in section, with intra-pyrenoidal membranes that are not continuous with the thylakoids. Where there is a tendency to form fewer large plastids per cell, these may contain several pyrenoids each. Members of the Thalassiosiraceae also have single, membrane-bound embedded pyrenoids, usually lacking traversing membranes. Within the pennate diatoms, pyrenoids can be embedded, peripheral, and protruding (occupying the space between the thylakoids and the chloroplast envelope to the cell interior), or intermediate (peripheral but surrounded by thylakoids), although a few seem to lack pyrenoids altogether, e.g., some *Pinnularia* and *Eunotia* species. Pyrenoid shape varies from spherical to dumbbell shaped, roundish to angular or rod-like, or even tetrahedral, and one to many pyrenoids can be present in a chloroplast according to species. When only one pyrenoid is present, it is usually more or less central; higher numbers of pyrenoids are more or less evenly spaced along the chloroplast (Mann, 1996; Cox, 1996).

6.4. GOLGI, MITOCHONDRIA, AND CYTOSKELETAL ELEMENTS

Golgi bodies are usually numerous, may be visible in living cells (Pickett-Heaps et al., 1984) and are probably highly active throughout the cell cycle, being surrounded by many small vesicles. In some centric diatoms (Schmid, 1988), they are closely associated with endoplasmic reticulum and mitochondria, forming the so-called G-ER-M complex, whereas in other centrics and in raphid diatoms, they are perinuclear (Medlin et al., 2000). Golgi bodies are often involved with mucilage secretion, for locomotion and for attachment (Pickett-Heaps et al., 1990). Mitochondria can be dispersed throughout the cytoplasm, but may also lie just under the valve surface, e.g., in *Pinnularia* they can occupy the alveoli (Pickett-Heaps et al., 1979).

Like other eukaryotic cells, diatoms possess microtubules and microfilaments (actin) as components of their cytoskeleton, but compared to naked cells, the diatom cytoskeleton is poor (Schmid, 1994). It is however active in wall morphogenesis, mitosis, and the movement of cytoplasmic components within the cell. A microtubule center (MC) is closely associated with the nucleus in most diatoms, often in an indentation on the nuclear surface (Pickett-Heaps et al., 1990). As well as being involved with the formation of the mitotic spindle, the MC is a focus for microtubules and associated with the movement of cytoplasmic components, such as mitochondria and granules. It is also involved in valve morphogenesis, and disruption of microtubules by the addition of colchicine in raphid pennates results in abnormal raphe systems (Pickett-Heaps et al., 1990). Microfilaments (actin fibers) are also closely associated with forming valves and girdle bands (Hildebrand et al., 2009) and may be crucial to the patterning process (Cox, 2002 and refs therein), as well as being involved with cell cleavage, motility, and mucilage secretion for attachment (Pickett-Heaps and Spurck, 1982; Edgar and Pickett-Heaps, 1984; Cox and Kennaway, 2004).

7. Morphogenesis

As mentioned in the introduction, new valves are formed within a membrane-bound vesicle, the silica deposition vesicle (SDV), within the protoplast, being released to the exterior of the cell when complete. Valve formation is invariably preceded by mitosis (Geitler, 1963), although in some situations, e.g., resting spore and initial valve formation, one of the resulting nuclei is aborted. Girdle band formation (presumably also within an SDV), occurs over a more extended time period, allowing the cell to expand in a pervalvar direction, formation of the last band sometimes occurring immediately before the next cytokinesis (Pickett-Heaps et al., 1990). Valve and band morphogenesis is a highly ordered, taxon specific, process (Fig. 13), often more or less synchronous in sibling valves (Figs. 14 and 15), but susceptible to modification by environmental factors and disruption by cytoskeletal inhibitors and other chemicals. The extent to which final structure is

a function of silica chemistry, the internal SDV chemistry, or cytoskeletal activity modulated by the genotype, or interplay between them, remains unresolved, different approaches being required to investigate development at the biochemical, nano- and ultrastructural scales.

7.1. BIOCHEMISTRY OF SILICA DEPOSITION

Silicic acid uptake into diatom cells from the external medium is an active mechanism mediated by silicon transporters (Hildebrand et al., 1997), which, given its intracellular concentrations, presumably involves an organic silicon complex that maintains silicon in solution (Martin-Jézéquel et al., 2000). Polymerization occurs within the SDV and is believed to be accelerated by cationic polypeptides, known as silaffins (Kröger et al., 2000). In conjunction with long-chain polyamines, silaffins induce rapid precipitation of silica *in vitro* from silicic acid solution, silica nanosphere size being linked to the length of the polyamine chains (Kröger et al., 2000). Acidic phosphoproteins have also been implicated (Wenzl et al., 2008). The similarity of the nanospheres to those formed in some diatoms has been highlighted (Kröger et al., 1999, 2000), and a phase separation model has also been suggested (Sumper, 2002) to explain nanopatterning in diatoms. However, others argue against a purely chemical–physical model of silicification (Schmid et al., 1996; Cox, 2002).

7.2. PATTERN CENTERS, DEVELOPMENTAL SEQUENCES, AND PORE STRUCTURE

Valve morphogenesis within the SDV is initiated either as a circular (centric diatoms) or elongated (araphid taxa) silicified annulus (Pickett-Heaps et al., 1990; Round et al., 1990; Sato et al., 2008, Mayama, pers. comm.), or as a π-shaped siliceous structure in raphid diatoms (Vartanian et al., 2009), from which ribs of silica variously extend and cross connect to define the taxon-specific arrangement of pores. Growth from a circular annulus generates the radial symmetry of many centric diatoms, although this may be difficult to discern in some bipolar taxa, e.g., Cymatosiraceae, *Toxarium* Bailey, *Odontella*, *Biddulphia*. Elongated annuli generate the central axial rib of many araphid diatoms (Mayama pers. comm.), and the virgae are usually more or less perpendicular to the sternum and parallel to each other (Cox and Kennaway, 2004). The sternum of raphid diatoms is however intrinsically asymmetrical, the top of the π-shaped initial structure extending to form the primary side of the raphe sternum, which is deflected back at the apices to meet the growing secondary side, the point of fusion (Voigt discontinuity) often being detectable as an irregularity in the stria spacing or orientation (Fig. 13) (Mann, 1981b).

As mentioned above, pores may be relatively simple perforations through the valve, or they may be variously chambered, loculate, or alveolate. Whereas loculate valves are usually built up from the inside out, e.g., *Thalassiosira* and *Coscinodiscus* (Schmid et al., 1981; Schmid and Volcani, 1983; Pickett-Heaps et al., 1990), alveoli are usually defined later in morphogenesis by internal thickening of the virgae and the development of axial and marginal laminae (Cox, 1999b). In *Pleurosigma*, the inner perforated siliceous layer is formed first, on which rows of struts are positioned and then overlaid by a layer of silica with slit-like openings above the inner hymenate openings (Sterrenburg et al., 2005). The finest pore occlusions are usually completed last during morphogenesis (Cox, 1999b).

Pore substructure has been used as a diagnostic taxonomic character at the generic level, e.g., the presence or absence of cribra, volae, hymenes (Round et al., 1990), but has been prone to misinterpretation or limited investigation, e.g., the presence of hymenes inferred from scanning electron microscopy (SEM), whereas transmission electron microscopy (TEM) is required to confirm their presence. Similarly, distinctions have often made on the final appearance of flap-like occlusions in the Cymbellales (foriculae *sensu* Cox, 2006), but without consideration of their ontogeny.

Various authors have documented aspects of valve morphogenesis, elucidating the ontogeny of some of the characteristic features of diatom valves, including rimoportulae, fultoportulae, spines and processes, fibulae, solid and variously thickened valvar regions, raphe infilling, etc. (Pickett-Heaps et al., 1990 and references therein, Pickett-Heaps, 1998; Cox, 1999b, 2001, 2004, 2006; Tiffany, 2002; van de Meene and Pickett-Heaps, 2002, 2004; Cox and Kennaway, 2004; Sterrenburg et al., 2005; Sato et al., 2008), but much more comparative work is required to document morphogenesis across major groups, as well as establishing the extent of shared or divergent developmental pathways.

7.3. MODIFICATION OF STRUCTURE IN A SINGLE GENOTYPE

Although diatoms are renowned for the fidelity with which valve morphology and structure are replicated, in addition to morphological changes associated with size reduction, valve morphology may be modified in response to environmental conditions. This is evidenced by the occurrence of Janus (McBride and Edgar, 1998) or unusual heterovalvate cells (Stoermer, 1967; Teubner, 1995; Klee and Houk, 1996) in natural samples, and by the results of experimental studies (Schultz and Trainor, 1970; Syvertsen, 1977; Kling, 1992; Trobajo et al., 2004). All reveal that different names have been applied to contrasting phenotypic expressions of single genotypes. More recently, it has been shown that the degree of silicification can increase in response to grazing (Pondaven et al., 2007), although this study did not indicate whether there were any distinguishable morphological differences. Careful experimental work is required to determine the full extent of phenotypic plasticity in diatoms and to identify its causes. Such information will not only

provide insights into the controls on diatom morphogenesis and structure, but is also essential to the appropriate delimitation and description of diatom taxa, as well as the interpretation of their distribution and occurrence in an ecological context.

8. Conclusions

Despite the long history of diatom studies, much remains to be discovered about their structure, development, and the controls on morphology. In particular, more experimental approaches are required to explore the process of valve formation and the interplay between genotype and environment.

9. Acknowledgments

Thanks are due to Pat Sims and Andrew Carr for some of the SEM images and to Pat Sims and Jonathan Tyler for their useful comments on the manuscript during its preparation.

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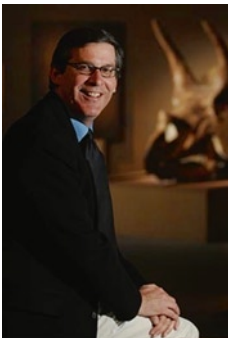

J. Patrick Kociolek

J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 47–91
DOI 10.1007/978-94-007-1327-7_3, © Springer Science+Business Media B.V. 2011

AN OVERVIEW OF DIATOM CLASSIFICATION WITH SOME PROSPECTS FOR THE FUTURE

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1. Introduction

Diatoms were apparently first recorded by someone known only as Mr. C., a fellow of the Royal Society, in the November–December issue of *Philosophical Transactions* for 1703 (Anonymous, 1703). It was penned in response to a letter from the noted Dutch microscopist Antonie van Leeuwenhoek (1632–1723), who appears to have recorded the existence of diatoms a little earlier (Leeuwenhoek, 1703). The organism Leeuwenhoek noted moved around and thus he assumed it to be an animal rather than a plant. He applied the name animalcule, meaning 'little animal'. Linnaeus is not often discussed in the context of diatom classification, but it is of note that in the 12th edition of *Systema Naturae* (1767), the few diatoms that had by then been described were listed under Vermes, in the Zoophyta (*zoon*, animal, *fyton*, plant), which was a mix of many diverse organisms, of which Linnaeus is supposed to have said: 'Mysterious living molecules, to be understood by our descendants' (see Williams, 2009 for further discussion).

Our focus in this chapter is classification. There is a variety of ways of defining classification systems. One way may be to divide them into artificial and natural classifications, prompting two questions: What is a natural classification? What is an artificial classification? Although, strictly speaking, an artificial classification is not always the same as an identification scheme, it is a useful way of viewing it. A natural classification is usually understood as an attempt to represent relationships among taxa and is (as much as can be expected) natural if it reflects *real* relationships. Whereas, because an identification scheme is designed purely to facilitate recognition, it can be considered an 'artificial classification' or system (there are differences between classifications and systems, which need not detain us here; see Williams et al., 2010); artificial classifications do indeed have their uses, a fact known for many years:

> For their unique purpose and their unique result, artificial systems have, as we have seen, to make it possible to learn with more or less ease, the names of the species to which the system are applied. (DeCandolle, 1844, p. 44; DeCandolle, 1813, 1819)

Artificial classifications are useful but they are not the same as, nor can they substitute for, a natural classification. Today, because we recognise evolution as the unifying principle in biology, a natural classification can be thought of as an information retrieval system that reflects as best as we can the evolutionary history of any particular group of organisms: This makes it both predictive (we expect that sampling further characters will conform to relationships in the classification) and explanatory (the characters can be explained by their phylogenetic relations). A natural classification is based on the concept of monophyly, the guiding principle of all phylogenetic studies. A monophyletic taxon can be defined as a group that includes *all* taxa hypothesised to share among themselves only the same common ancestor. While this functions perfectly well as a definition, monophyletic groups are *discovered* by nested sets of synapomorphies, which are hypothesised as shared derived characters. That is, character data (of whatever kind or source) provide the evidence for making hypotheses concerning monophyletic groups. Some characters may be discovered to be plesiomorphic, meaning they are primitive. These characters are useless for discovering taxon relationships as they are more widely distributed among the organisms in question. To suggest feathers as a character of eagles (Accipitriforme birds) is pointless as all birds (Aves) have feathers.

Taxonomic groups other than monophyletic are possible. Paraphyletic groups are composed of only some taxa that share a common ancestor, omitting at least one group derived from the same common ancestor. Reptiles are paraphyletic as they exclude birds. More significantly, there is no evidence to support paraphyletic groups as they are taxa defined by a constellation of derived and primitive characters and are thus artificial. A simple example is given in Fig. 1. A and B together form a monophyletic group as there is evidence they are related – a synapomorphy, or shared derived character, possessed by both taxon A and B has been hypothesised and they both presumably shared a common ancestor (indicated by the black square in Fig. 1). C and D also share a common ancestor (indicted by the black circle in Fig. 1) but that hypothesised ancestor also includes

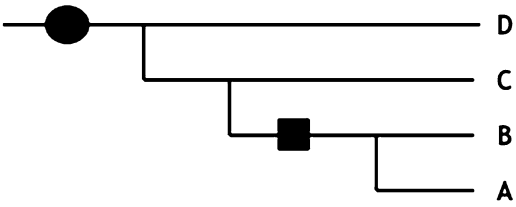

Figure 1. Relationships among four taxa A–D. A and B form a monophyletic group as they share a common ancestor, the black square. C and D also share a common ancestor, the black circle. That ancestor includes A and B as well. If C and D are grouped together separately from A and B, they are paraphyletic. Evidence that corresponds to the node at the black circle corresponds to A–D. If the black circle is interpreted as a character of which the black square is derived, the black circle is plesiomorphic (primitive), the black square is derived (synapomorphic).

A and B as they have been derived from it as well. Thus, if C and D are grouped together separately from A and B, they are paraphyletic. Any evidence that corresponds to the black circle will correspond to all included taxa, A–D, even if in A and B the character is in a modified form. These few principles outline what is known as the cladistic approach to taxonomy (a simple account can be found in Kociolek et al., 1989).

A natural classification is neither a key (an identification system) nor a system of convenience based on tradition or taxonomic 'judgement'; it is based on the evaluation of all available evidence in terms of monophyly and synapomorphy. In this chapter, we are interested in natural classification. First, we will provide a very brief overview of the 'kinds' of data that can be applied to diatom classification. There are very good summaries of these data already and we need not repeat the details. We follow this with a simplified review of diatom classification from Agardh to the present (further details can be found in Williams, 2007a, b), a summary of recent classifications derived from molecular data and palaeontology, and then discuss taxon numbers in diatoms. We finish with a review of methods in classification, offer brief interpretations of characters that apply to discovering diatom higher taxa and close with a few recommendations for future study of diatom classification.

2. 'Kinds' of Data

Development of diatom interrelationships and their classification across the many levels of the taxonomic hierarchy has used a variety of different kinds of data. Here we review some of the main categories of data used to construct the classification systems discussed below. These data have been applied both independently and in combination. We have not dealt with these data in any detail but offer a summary to assist the understanding of the reasons behind the various classifications that follow and our subsequent interpretation of them. Our primary concern is higher level relationships.

2.1. FRUSTULE MORPHOLOGY

2.1.1. Valves and Girdle Structure

The organisation, symmetry and special structures found in the diatom cell wall have received greatest attention in the last few decades, largely because of developments and advances in scanning electron microscopy (SEM) (these data are collated in Gaul et al., 1993; Henderson and Reimer, 2003). These data have been significant in generating various classifications (e.g. Nikolaev, 1984a, b, 1988, for 'centric' diatoms; Round et al., 1990). Examination of the valves and girdle with the SEM revealed many special features extending from the surface or as part of the surface structure itself. Definitions for many of these structures

were first complied in Anonymous (1975; von Stosch, 1975, for girdle band features), developed in Ross et al. (1979) and commented on and enhanced in various comprehensive texts (Round et al., 1990; Krammer and Lange-Bertalot, 1986–2004). The two terminology guides, and subsequent commentary, are useful for identifying and discriminating particular kinds of structures – the parts of the valve and girdle – and for establishing their use in taxonomic investigations, particularly identification. Rather than summarise details concerning the parts of the diatom valve and girdle, we offer a few suggestions for future study of taxonomic characters relevant to deriving a natural classification.

Following the two guides (Anonymous, 1975; Ross et al., 1979), many characters of the valve were defined somewhat simplistically. For example, a standard description of the valve surface will refer to the striae, the major component of the basal siliceous layer. The striae are defined as '… a row of areolae or alveoli …, or a single alveolus where this is not part of the row' (Ross et al., 1979, p. 526) and the basal siliceous layer is defined as '… the layer that forms the basic structure of the various components of the frustule …' (Ross et al., 1979, p. 525; the word 'frustule' was probably used in error as it should refer to the basic structure of the *valve*). A variety of 'kinds' of striae have been described. A more precise description of a stria would involve its two separate components, the transverse bars (virgae) and the transapical bars (vimines) (Cox and Ross, 1980).

At the valve margin, spines are often found emerging from the surface. The definition of a spine is straightforward: 'a closed or solid structure projecting out from the [wall] surface of the frustule' (Anonymous, 1975, p. 328, the word 'wall' was deleted in Ross et al., 1979, p. 522). Although the definition of spine was subdivided to account for linking spines (linking being a functional attribute) and spinules (indicating size: small spines), no consideration was given to their position in relation to the components of the striae. The terms virgae and vimines were not available at the time the most recent terminological guide was written but no reference was made to the valve components except to simply note that spines projected from the surface. Under this general definition, 'spines' is a character that is distributed widely among diatoms, occurring in almost every major group. Given this fact, their significance has been downplayed, as if the widespread distribution makes them phylogenetically (systematically) uninformative. However, it is the modifications of the valve surface that forms a 'spine' that presents the phylogenetically informative data.

Given that a stria is composed of both transverse (virgae) and transapical bars (vimines), if spines emerges from the virgae then that structure is better understood as a modified virga that *functions* as a spine. Whereas if a spine emerges from *between* virgae (as opposed to from them), it is really a modified vimine that *functions* as a spine. In the first example, virgae are homologous to the spine; in the second example, vimines are homologous to the spine. 'Functions as a spine' means simply that the structure is found 'projecting out from the surface of the frustule'. Of course, a spine, in this general sense, may have a particular function, such as interlocking adjacent valves. Therefore, the term 'spine' might

be seen as a functional attribute rather than a structural one, contrasting modified virga or modified vimine (both structural) with a spine (functional). Thus, while definitions may help add precision to the understanding of the parts of the valve and girdle, it does not necessarily act as a guide to what are or are not homologous attributes (see below and Williams and Reid, 2006).

The same rationale may apply to the various pore fields such as the pseudocelli and ocelli, to other structures such as pseudosepta and pseudonodulus (Ross et al., 1979, pp. 520–521), the different types (kinds) of raphe structures (Ross et al., 1979, pp. 522–525) and the various parts of the girdle (Ross et al., 1979, pp. 524–525).

To date, there are a number of large and detailed compendia of valve morphological features, such as the early light microscope-based Schmidt's Atlas (1874–1959) and the electron microscope collections such as Helmcke & Kreiger (in Helmcke, 1954–1977; Round et al., 1990). Along with these are two detailed bibliographic compilations of electron microscope studies on diatom valve and girdles (Gaul et al., 1993; Henderson and Reimer, 2003), none of which have been fully exploited. While studies of diatoms using SEM are around half a century old, many taxa, including entire families and genera (and many, if not most, species), have never been observed using this approach; most of the data gathered so far remains unanalysed, and its relevance is yet to be determined in any meaningful way. We explore some of these issues further below.

2.2. CELL MORPHOLOGY

1. *Sexual reproduction*: Though the number of species for which data are available remains small relative to the total number of known taxa, observations for diatoms in terms of the processes of sexual reproduction suggest diversity in strategies adopted, patterns of reduction division, pairing, and the resulting number of products of sexual reproduction. Overviews and summaries began with Merezhkowsky (1903a), continued with Geitler (see Geitler, 1932), Cholnoky (see Kiss, 1999–2000), Drebes (1977) and von Stosch (Drebes, 1987). The most recent reviews are Edlund and Stoermer (1997, who provide a summary of relevant data and a cladistic interpretation of diatom phylogeny) and Chepurnov et al. (2004, who provide a summary of relevant data and various explanatory scenarios). The latter authors suggest six 'rules' for diatom sexual reproduction: 'Rule 1 is that the life cycle is diplontic. Diatoms are almost unique among algae in having a diplontic life cycle…' (Chepurnov et al., 2004, p. 94); 'Rule 2 is that vegetative multiplication is accompanied by gradual cell size reduction' (Chepurnov et al., 2004, p. 95); 'Rule 3 is that cell size is restored through development of a specialized cell called an auxospore, and formation of auxospores results from sexual reproduction' (Chepurnov et al., 2004, p. 96); 'Rule 4 is that cells that fail to undergo sexual reproduction and auxosporulation continue dividing mitotically until they become critically small and finally

die' (Chepurnov et al., 2004, p. 96); 'Rule 5 states that the capacity of cells to become sexualized is size dependent: Only comparatively small cells can be triggered to switch from mitotic cycles to meiosis' (Chepurnov et al., 2004, p. 96); 'Rule 6 states that the immediate products of sexual reproduction, the auxospore, and initial cell, are not dormant stages' (Chepurnov et al., 2004, p. 97).

The six rules are useful in focusing on potential problems in determining any particular characters' variability, as size change can cause significant effects on the siliceous parts (valves, girdle) of the cell. In addition, distinguishing the auxospore stage from the vegetative valves provides an additional suite of characters for study in a phylogenetic sense (Edlund and Stoermer, 1997; Medlin and Kaczmarska, 2004).

There is little point in repeating the main points in the review by Chepurnov et al. (2004). Our focus is classification and how data can be bought to bear on those kinds of problems. Here, we merely mention the supposed correlation between certain taxonomic groups ('centric', 'araphid' and raphid diatoms) and the processes identified as oogamy, isogamy and anisogamy, and auxospore characters (scales, properizonial bands, transverse perizonial bands, and longitudinal perizonial bands, as outlined Edlund and Stoermer, 1997; Medlin and Kaczmarska, 2004, p. 257, fig. 12; Sato, 2008, and summarised in Medlin and Sato, 2009, fig. 1) a subject we discuss more fully below in Methods and Classification: Character Conflict and Cladistics.

2. *Plastid morphology*: There is now an extensive literature on diatom plastid morphology (a useful survey from the point of view of identification can be found in Cox, 1996). Study of chloroplast morphology in relation to diatom systematics goes back to the very beginning as the living cell was first observed unprocessed (Agardh, 1824, for details see below). The first comprehensive review was Pfitzer (1871, for details see below) followed by Lauterborn (1869–1952) (1896, for a partial translation into English, see Pickett-Heaps et al., 1984; for biographical information on Lauterborn, see Melkonian and Mollenhauer, 2005) and Merezhkowsky (1902–1903), a useful summary table appears in Bedoshvili et al. (2009). Classifications based on the combination of plastid characters and 'hard' part characters have been extensive (reviewed in Williams, 2007b) and is briefly reviewed below. Clearly, there is considerable variation with much remaining unstudied. Again, as our purpose is to deal with classification, we outline below the relation of the major groups of diatoms relative to what has been called the placochrome and coccochrome condition (Pfitzer, 1871), with the former defined as a '… lamellate endochrome [plastid] …' (Petit, 1877b, p. 65, translated from Petit, 1877a, p. 64) and the latter as '… granular endochrome [plastid] …' (Petit, 1877b, p. 71, translated from Petit, 1877a, p. 67). That is, one group – the coccochromes – with many small plastids, another group – the placochromes – with few large plastids. This relation too is discussed more fully in Methods and Classification: Character Conflict and Cladistics.

In summary, there is a vast range of characters available for study; their relevance in diatom classification has never really been explored in any great depth.

3. Diatom Classification

3.1. IN THE BEGINNING …

Rather than explore the many early accounts of diatom classification (a succinct early history is given by Kitton, 1880–1882, a later one by Taylor, 1921 and 1928 and, more recently, some classifications were discussed in Williams, 2007b), a useful if not compulsory place to start is with Carl Adolph Agardh (1785–1859) and his *Systema Algarum* (1824), an early comprehensive treatment of all 'algae' (Stafleu, 1966). Agardh classified diatoms as one order ('Ordo I') of algae (Diatomeae), its total number of species did not exceed 50 (48) and were distributed among nine genera: *Achnanthes*, *Frustulia*, *Meridion*, *Diatoma*, *Fragilaria*, *Meloseira*, *Desmidium*, *Schizonema* and *Gomphonema* (*Desmidium* was later recognised to not be a diatom genus). It is worth noting that while Agardh named only a few taxa (i.e. assigned a taxonomic name to a particular level in the hierarchy), he divided the genus *Diatoma* into five subgroups (Table 1).

A few years later, Agardh published *Conspectus Criticus Diatomacearum*, a more detailed study on diatoms (Agardh, 1830–1832). Agardh arranged the genera into three families, each based on the shape of the siliceous valves: Styllarieae included genera with cuneate (wedge-shaped) valves, Cymbelleae included genera

Table 1. Classification of diatoms in Agardh's *Systema Algarum* (Agardh, 1824).

Genus	Species no.	Subgroups	Species no.
1. *Achnanthes*	2		
2. *Frustulia*	6		
3. *Meridion*	3		
4. *Diatoma*	16	a. Flabelliformes	2
		b. Genuinae	7
		c. Notatae	3
		d. Articulis inaequalibus & inordinate ruptis	1
		e. Articulis rotundatis	3
5. *Fragilaria*	3		
6. *Meloseira*	5		
7. *Desmidium*	2		
8. *Schizonema*	9		
9. *Gomphonema*	2		
Total	48		

with cymbelloid (more or less crescent-shaped) valves and Fragilarieae included genera with rectangular valves. These three families were compared with four different colony 'types': those with no obvious colony formation ('libera'), those attached by a stalk ('Stipitata'), those attached in chains ('In frondem composita') and those in 'cymbelloid' chains ('Fila cymbellarum frondem formantia'). Agardh's conclusions were presented as a table, which contrasted frustule shape with colony structure. While the table is of interest in its contrasting characters, Agardh's classification reflected the frustule shape of the organism, which he believed supported a natural classification of these organisms (Williams, 2007b). In the *Conspectus Criticus Diatomacearum*, Agardh arranged the species (now twice as many, with a total of 111) in 20 genera (2 genera – *Desmidum* and *Hydrurus* – are now not recognised as diatoms), some of which are subdivided (Table 2). Depending on how one counts the divisions and subdivisions, there are at minimum three distinct hierarchical levels, indicating some degree of understanding of relationships (the fewer subdivisions, the less information, real or otherwise).

At the same time Agardh was working, Friedrich Traugott Kützing (1807–1893) published his *Synopsis Diatomearum* in 1834. He organised diatoms into two major groups, Diatomaceae Liberae ('*Frustula non inclusa*', Kutzing, 1834a, p. 535; Kutzing, 1834b, p. 7) and Diatomaceae Inclusae ('*Frustula tubulis inclusa*', Kutzing, 1834a, p. 589; Kutzing, 1834b, p. 64) and recognised 16 genera, 10 placed in the first group, the remaining 6 in the second. Two of the ten genera – *Frustulia* and *Gomphonema* – were further subdivided: *Frustulia* into seven groups of species; *Gomphonema* into three groups of species. Again, as in Agardh, there is a minimum of three hierarchical levels even though some were not given formal names. Interestingly, of the 16 genera, 10 were first described by Agardh, 4 from others and only 2 added by Kützing. Comparison between the classifications of Agardh and Kützing is instructive as Kützing's Diatomaceae Inclusae is roughly equivalent to Agardh's Cymbellae and his Diatomaceae Liberae roughly equivalent to Agardh's Styllariae plus Fragilarieae (Table 3).

In the more comprehensive *Die kieselschaligen Bacillarien oder Diatomeen*, Kützing (1844; an English translation of part of the introduction to Kützing's *Die kieselschaligen Bacillarien oder Diatomeen* can be found in the *American Journal of Microscopy*, see [Smith] 1877–1881) arranged diatoms into three informally named tribes: *Status singularis*, *status congregationis* and *status involucratus*, divided to reflect their colony structure, which was either solitary (no colony), gathered in large numbers (with a colony) and 'wrapped in a soft gelatinous substance'. The first two Tribes were subdivided into two orders, 'Astomaticae' and 'Stomaticae'. The whole classification has many subdivisions, displaying much greater complexity than all previous classifications.

In summary, the beginnings of diatom classification were attempts at capturing the details of the living organism relative to the few microscopic details known at that time – but dealing with *all* the data.

Table 2. Agardh's classification in *Conspectus Criticus Diatomacearum* (Agardh, 1830–1832).

Cymbellae		**Styllariae**	**Fragilariae**		
			Seriella prima: Diatomeae		
I. *Cymbella*	1. Frustulis simplisibus (6) 2. Frustulis coadunatus (9) 3. Frustulis sigmoideis (2)	X. *Stylaria* (2)	XIII. *Frustulia* (6)[1]		
II. *Schizonema* (13)		XI. *Meridion* (2)	XIV. *Diatoma*[1]	I. Bacilliformis…	a. Receptaculo haemisphaerico (5) b. Receptaculo stipiformi (1)
				II. Bacillis vel filis liberis… (2)	
				III. Bacilis transversaliter…(6)	
				IV. Inquirendae (2)	
III. *Micromega* (6)		XII. *Licmophora* (4)	XV. *Isthmia* (2)		
IV. *Berkeleya* (1)			XVI. *Odontella* (1)		
V. *Homeocladia* (2)			XVII. *Desmidum** (3)[1]		
			Seriella secunda: Fragilarinae		
VI. *Gloiodictyon* (1)			XVIII. *Achnanthes* (5)[1]		
VII. *Hydrurus** (4)			XIX. *Striatella* (2)		
VIII. *Gloionema* (4)			XX. *Fragilaria* (3)[1]		
IX. *Gomphonema*[1]			XXI. *Grammomena* (2)		
	a. Simplicia (4) b. Ramosa (7)				
			XXII. *Meloseira* (4)[1]		

[1] Genera that appeared in Agardh's 1824 classification.
* not diatom genera.

Table 3. Kützing's classification in *Synopsis Diatomearum* (Kutzing, 1834a, b).

Diatomaceae Liberae ('*Frustula non inclusa*' Kutzing, 1834a, p. 535; Kutzing, 1834b, p. 7)	**Diatomaceae Inclusae ('Frustula tubulis inclusa' Kutzing, 1834a, p. 589; Kutzing, 1834b, p. 64)**
I. *Frustulia* C.A. Agardh	XI. *Encyonema* Kutzing (1)
a. *Cyclotella* (1)	[XII. *Schizonema* C.A. Agardh]
b. *Aplotella* (7)	XIII. *Berkeleya* Greville (1)
c. *Cymbella* (18)	XIV. *Homoeocladia* C.A. Agardh (2)
d. *Paltonella* (19)	XV. *Gloeodictyon* C.A. Agardh (1)
e. *Pandurella* (1)	[XVI. *Micromega* C.A. Agardh]
f. *Sigmatella* (5)	
g. *Sphenella* (4)	
II. *Meridion* C.A. Agardh (2)	
III. *Exilaria* Greville ('*Psygmatella* Ktz. in litt. 1831') (6)	
IV. *Aristella* Kutzing (1)	
V. *Gomphonema* C.A. Agardh	
a. *Cymbophora* (2)	
b. *Paltonophora* (2)	
c. *Sphenophora* (17)	
VI. *Achnanthes* Bory (10)	
VII. *Isthmia* C.A. Agardh (1)	
VIII. *Diatoma* C.A. Agardh (12)	
IX. *Fragilaria* Lyngbye (5)	
X. *Melosira* C.A. Agardh (7)	

3.2. PFITZER (1871), H.L. SMITH (1872) AND SCHÜTT (1896): FORGING THE NINETEENTH AND TWENTIETH CENTURY PERSPECTIVE

Ernst Pfitzer (1846–1906) proposed a novel diatom classification based on plastid structure. He created two primary divisions: Coccochromaticae and Placochromaticae (Pfitzer, 1871), with Coccochromaticae described with 'Endochrom an zahlreiche Körner gebunden …' (Pfitzer, 1871, p. 152, loosely translated as 'endochromes [plastids] numerous') and the Placochromaticae with 'Endochromoplatten, wenn zu zweien vorhanden, stets den beiden, wennn [sic] einzeln, fast stets … einem Gürtelband mit den oder der Mediane anliegend' (Pfitzer, 1871, p. 151, roughly meaning 'Endochromes [plastids] two or one …'). Thus, plastids were thought to be of prime significance in determining a major subdivision within diatoms. Coccochromaticae had two subdivisions of its own ('Bilaterale Formen' and 'Centrische Formen') as did Placochromaticae ('Mit Knoten' and 'Ohne Knoten'), both related to the structure of the valve rather than the contents of the frustule – the cellular part (see Table 4 for a summary). Pfitzer also presented a diagram that related the taxa in addition to his classification (reproduced as fig. 1 in Williams and Kociolek, 2010b).

Table 4. The primary divisions of Pfitzer's (1871) classification, and in the greater part, Petit (1877a, b).

Coccochromaticae	
I. Bilaterale Formen	a. Nach der Querebene symmetrisch
	b. Nach der Querebene asymmetrisch
II. Centrische Formen	a. Schalen mit theilweise zygomorpher Gestaltung
	b. Schalen rein centrisch
Placochromaticae	
a. Mit Knoten	α. asymmetrische Formen
	β. symmetrische oder diagonal gebaute Formen
b. Ohne Knoten	

Paul Petit (1834–1913) followed Pfitzer's scheme by dividing diatoms into two subfamilies, Placochromaticeae ('… lamellate endochrome …' Petit, 1877b, p. 65 translated from Petit, 1877a, p. 64) and Coccochromaticeae ('… granular endochrome …' Petit, 1877b, p. 71, translated from Petit, 1877a, p. 67). Coccochromaticeae consisted of seven tribes, Placochromaticeae of nine. One commentator, Clarence Elmore, suggested that 'Of all existing systems, that of Paul Petit [1877a] seems to approach most nearly to a natural one because it is based on characters having physiological significance' (Elmore, 1896, pp. 532–533), thus suggesting that 'naturalness' could be detected in the kind of character used, in Elmore's view 'physiological significance'.

A significant contribution to diatom classification was published at around the same time as Pfitzer's. Hamilton L. Smith (1818–1903) focused on the silica parts of the organism, using the presence of a raphe on the valve as *the* feature with which to subdivide diatoms. Smith defined a raphe as '… a true cleft, generally on the valve …' (Smith, 1872, p. 2), which he contrasted with a pseudo-raphe, defined as '… a simple line, or blank space, without nodules …' (Smith, 1872, p. 3); 'crypto-raphid' species accounted for those with neither a 'true' raphe nor pseudo-raphe, although Smith wrote that 'Probably all diatoms have a more or less perfect raphe' (Smith, 1872, p. 3). He continued: 'In Tribe I. it [the raphe] is quite evident; in Tribes II. and III. it is obscure; most of the valves, however, of Tribe II. have a pseudoraphe' (Smith, 1872, p. 3). On the next two pages of his account, Smith presented a tabulation of these tribes, which he refers to as a 'Synoptical Arrangement'. The three tribes were named as: Tribe I. Raphidieae; Tribe II. Pseudo-raphidieae; Tribe III. Crypto-raphidieae. The three divisions are now known by more colloquial names: raphid diatoms (Raphidieae), 'araphid' (Pseudo-raphidieae) and 'centric'[1] (Crypto-raphidieae), the first two – raphid and

[1]'Araphid' and 'centric' diatoms appear in single quotes as they are not now recognised as natural groups of diatoms – they are artificial assemblages of taxa.

'araphid' – were combined as the pennate diatoms. Thus, Smith divided diatoms into the 3 tribes, with 15 families (Raphidieae with 5, Pseudo-raphidieae with 3, Crypto-raphidieae with 7) and 110 genera (the total number of species was by now quite large).

Smith's classification was enthusiastically adopted by Henri Van Heurck (1838–1909), who included it in the 3rd edition of his book *Le Microscope* (1878), the first edition to have a separate chapter on diatoms (Frison, 1959, p. 27). That chapter would form the basis of all Van Heurck's subsequent work, retaining Smith's classification, modified only in relatively minor details (Van Heurck, 1880–1885, 1896). De Toni's *Sylloge Algarum* (1891–1894), another significant contribution to diatom taxonomy, was also based on Smith's classification.

The next classification of any importance was provided by Franz Schütt (1859–1921) in his contribution to Engler and Prantl's *Die natürlichen Pflanzenfamilien* (Schütt, 1896). Schütt noted inconsistencies between Smith's and Pfitzer's classification (of which more later). Schütt understood Pfitzer's two Coccochromaticae subgroups, the 'Schalen centrisch gebaut' group and the 'Schalen nach Umriss und Structur bilateral gebaut' group, to have closer relationships to taxa other than each other (Schütt, 1896, pp. 54–55). That is, only part of Coccochromaticae was equivalent to Smith's Crypto-raphideae (the 'Schalen centrisch gebaut' group), with the remaining part (the 'Schalen nach Umriss und Structur bilateral gebaut' group) plus Placochromaticae being equivalent to Smith's Raphideae plus Pseudo-raphideae and hence deserving of a name. Schütt's solution was to propose two groups, Centricae and Pennatae, a system that became the standard, appearing virtually unchanged in the second edition of *Die natürlichen Pflanzenfamilien* published over 30 years later, the latter a classification that, according to Hendey, 'completely dominating the literature' (Hendey, 1937, p. 199).

Schütt's classification is subdivided into four named taxonomic levels. He made his system rather complicated, and added some redundancy, by denoting further subdivisions indicated by a series of numbers and letters (both Greek and Roman), as well as some symbols, such as a star (*) (Table 5). For the most part, George Karsten (1863–1937), who wrote the diatom chapter for the 2nd edition of *Die natürlichen Pflanzenfamilien* (Karsten, 1928), followed Schütt differing only in the position of the Eunotoid diatoms, retained in Fragilariodeae (=Araphideae) by Schütt, but moved to Raphioideae (Eunotiaceae) by Karsten, and by the internal arrangement of some groups within Pennales. Interestingly, both authors (Schütt and Karsten) subdivided Centrales, into Eucyclicae and Hemicyclicae, the former more or less equivalent to the radially symmetrical 'centric' diatoms, the latter to the bilaterally symmetrical 'centric' diatoms (a summary is given in Table 5).

Schütt offered no explicit phylogenetic discussion or interpretation of his classification. However, the botanist Charles Bessey (1845–1915) did (Bessey, 1900; for commentary on Bessey see Pool, 1915 and Overfield, 1993). Bessey adopted Schütt's classification and presented an illustration of a phylogenetic tree

Table 5. Schütt's (1896) classification.

A. Centricae [Centrales]			
{Eucyclicae}			
	a I. Discoideae	α1. Coscinodisceae	I. a. Melosirinae
			II. b. Sceletoneminae
			III. c. Coscinodiscinae
		β 2. Actinodisceae	I. 1. a. Stictodiscineae
			I. 2. b. Planktoniellinae
			II. 1. c. Actinoptychinae
			II. 2. d. Asterolamprinae
			[II. 3. e. Actinoclavineae]
		γ 3. Eupodisceae	I. 1. a. Pyrgodidcinae
			I. 2. b. Aulacodiscinae
			II. 1. c. Eupodiscinae
			II. 2. d. Tabulininae
	b II. Solenoideae	4. Solenieae	I. a. Lauderiinae
			II. b. Rhizosoleniinae
{Hemicyclicae}			
	c III. Biddulphioideae		
		I. 5. Chaetocereae	
		II. 6. Biddulphieae	1. a. Eucampiinae
			1. b. Triceratiinae
			1. c. Biddulphiinae
			1. d. Isthmiinae
			2. e. Hemiaulinae
		III. 7. Anauleae	
		IV. 8. Euodieae	
	d IV. Rutilarieae	9. Rutilarieae	
B. Pennatae [Pennales]			
[B. I. Araphideae]			
	a V. Fragilariodeae		
		I. 10. Tabellariae	
			1. a. Tabellariinae
			2. b. Entopylinae
		II. 11. 1. Meridioneae	
		II. 12. 2. Fragilarieae	
			* a. Diatominae
			* b. Fragilariinae
			** c. Eunotinae
			[c. Amphicampieae]
[B. II. Raphioideae]			
	[B. VI. Eunotiaceae]		
			[13. Peronioideae]
			[14. Eunotiodeae]

(continued)

Table 5. (continued)

B. Pennatae [Pennales]			
[B.111.Monoraphideae]			
	b VI. Achnanthoideae		
	[B. VII Achnanthaceae]		
		α 13. Achnantheae	
		[1. 15. Achnanthaceae]	
		β 14. Cocconeideae	
		[2. 16. Cocconeioideae]	
[B. 1V. Biraphideae]			
	c α VII. Naviculoideae		
	[B VIII Naviculaceae]		
		I. 15. Naviculeae	
		[α 1. Naviculoideae]	
			1. a. Naviculinae
		[α 2. a. Gomphonemoideae]	2. b. Gomphoneminae
		[α 2. b. Cymbelloideae]	3. c. Cymbellinae
		[β B. IX Epithemiaceae]	[1. 20. Epithemioideae]
			[2. 21. Rhopalodioideae]
		II. 16. Nitzschieae	[1. 22. Nitzschiioideae]
		[2. B. X. Nitzschiiaceae]	
	c β VIII. Surirelloideae		
		17. Surirelleae	[2. 23. Surirelloideae]

There are four named taxonomic levels (excluding the names in column 2 in curly brackets; these are extra divisions included in the text but not in the classification). Schütt made his system more complicated by denoting further subdivisions without naming them. These were indicated by a series of numbers and letters (both Greek and Roman) and some symbols, such as the star (*). There is some redundancy to his scheme. Taxon names in square brackets are additions or changes made by Karsten (1928), who, for the most part, followed Schütt. The major differences between Karsten and Schütt is the position of the Eunotoid diatoms, in the Fragilariodeae (=Araphideae) for Schutt, in the Raphioideae, Eunotiaceae for Karsten, and the internal arrangements in the Pennales

derived from it (although Bessey writes: 'I have accepted Schütt's interpretation with a slight modification … introducing, however, some changes … which I fear he [Schütt] may not accept', Bessey, 1900, p. 61, see Table 6 for a summary of Bessey's classification and fig. 1 in Williams and Kociolek, 2010b, for a reproduction of Bessey's tree).

Bessey offered the hypothesis that diatoms originated as colonial organisms with the more derived forms evolving into free-living individuals. Bessey's diagrams illustrate an interpretation of certain 'trends' in each of his two major groups, Centricae and Penatae, related to his general hypothesis. Thus, Bessey writes that '… I have regarded them [the two sub-families] as constituting two separate but somewhat parallel genetic lines, in which the Coscinodisceae and Fragilariaeae are approximately primitive, the former having given rise to the Centricae and the latter to the Pennatae' (Bessey, 1900, p. 63). His diagram is a

Table 6. Bessey's major groups, those related to his phylogenetic tree (compare with Table 5).

Centricae
Coscinodisceae
Actinodisceae
Eupodisceae
Solenieae
Chaetocereae
Biddulphieae
Euodieae
Anauleae
Rutliarieae
Pennatae
Tabellarieae
Meridioneae
Fragilarieae
Naviculaeae
Bacillarieae
Surirelleae

little difficult to render into a more conventional two-dimensional bifurcating tree but the two 'parallel genetic lines' clearly indicate each sub-family following a 'trend' towards both bilateral symmetry as well as free living. Interestingly, Bessey seemed to care little for what the fossil record offered with Rutilariaceae appearing as the most derived of the Centricae branch. A similar diagram appeared many years later in Chin (Jin Dexiang) (1978), with the genera *Rutilaria*, *Chaetoceros* and *Rhizosolenia* as the most derived of the 'centric' lineage (Chin, 1991, p. 93; see also Chin, 1978, pp. 2–3).

3.3. MEREZCHKOWSKY, FORTI AND THE PERAGALLO FRÈRES: FUNCTIONALITY AND PHYLOGENY

Merezhkowsky (1855–1921) considered 'the presence or absence of movement' the most important characteristic of diatoms, noting that it is 'merely dependent on the presence or absence of a slit in the walls of the frustule [the raphe]; this character should be taken into consideration before any other' (Merezhkowsky, 1902, p. 65, adapted from Merezhkowsky, 1901). In this sense, H.L. Smith's classification is 'explained' by functionality: movement. As a consequence, Merezhkowsky named 'two great groups', the 'mobilées' (Mobiles) and 'immobilées' (Immobiles), with the 'mobilées' subdivided into Raphideae and Carinatae and the 'immobilées' subdivided into Bacilloideae and Anaraphideae.

Merezhkowsky used a number of diagrams to portray his understanding of the evolutionary relationships among diatoms (Merezhkowsky, 1903a, b, opposite

p. 204, reviewed in Williams, 2007b). Alongside taxa named for real organisms, Merezhkowsky's 'phylogenetic trees' also included hypothetical ancestral taxa: the taxon *Archaideae* was considered ancestor to the raphid diatoms ('mobilées'), *Protonées* was considered ancestor to *Archaideae*, *Copuloneis* was considered ancestor to *Protonées* plus Tabellarioideae and *Urococcus* was considered ancestor to all diatoms – a trail of imaginary ancestors, from *Uroccus* to *Archaideae*. Merezhkowsky revised his classification adding *Archaideae* to the 'mobilées' (instead of it being an ancestral group) and the Anaraphideae were renamed Centrales (Merezhkowsky, 1903b, pp. 203–204). Merezhkowsky's genealogy differed from his classification as both Bacilloideae and Immobiles are rendered paraphyletic on his diagram. As noted above, by paraphyletic we mean that one of the group's descendants is missing. Thus, for Merezhkowsky, only one group – the raphid diatoms, the 'mobilées' – are monophyletic; all the others are artificial to one degree or another.

Hippolyte Peragallo (1851–1921) and Achilli Forti (1878–1937) both used Merezhkowsky's classification as a basis for their discussion. Hippolyte Peragallo wrote on diatom classification in 1897, prior to the publication of the comprehensive *Diatomées Marines de France* (Peragallo and Peragallo, 1897–1908; Peragallo, 1897), in which the Peragallo brothers adopted a tripartite division of anaraphid, pseudo-raphid and raphid diatom groups. In his preliminary 1897 paper, Hippolyte added a genealogical table with 'centric' diatoms at the base and raphid pennate diatoms at the tips. Various 'araphid' genera linked them (Peragallo, 1897; later, Hippolyte wrote on the evolution of diatoms, wedding a scheme roughly based on that of Merezhkowsky but, in Peragallo's views, suggested a diphyletic origin for diatoms, 'Centriques' being related to 'Peridinées' and 'Bilatérales' derived from Chromomonades; see Peragallo, 1906, his diagram on p. 121). Achilli Forti also embraced Merezhkowsky's classification, and provided some complex genealogical diagrams in an attempt to offer explanations for the classification (Forti, 1911). Like Peragallo, Forti's diagram depicting diatom genealogy placed various 'centric' diatom genera at the base, raphid pennate diatom genera at the tips, with 'araphid' diatom genera linking the two.

In short, phylogenetic interpretation of diatoms was converging on a 'centric' – 'araphid' – raphid lineage, whereas regardless of motivation for constructing the classification (characters, functionality, phylogeny), a bipartite system (centric–pennate) dominated and as Hendey wrote in 1937, 'all authors of the last 40 years have been influenced by Schütt' (Hendey, 1937, p. 199; cf. Hustedt, 1930a, b). One might argue, with some justification, that Hendey's 1937 classification for the *Plankton Diatoms of the Southern Seas* represents another turning point, marking out what we call the 'Modern' Era (Hendey, 1937, and it is interesting to note Hendey's later comments on Merezhkowsky, who '… put forward the only system of classification for the diatoms that had any semblance whatever of being "natural" … Here for the first time the system related structure to function …' Hendey, 1974, p. 280). This stands neatly in contrast to Elmore's idea (cited above) of what constituted natural.

3.4. THE 'MODERN' ERA

The 'Modern' era is so named to coincide with the up and coming decades of electron microscope investigations that started around the mid-1930s with the investigation of diatom hard parts, the first account being that of Krause in 1936, published a year before Hendey's *Southern Seas* flora (Krause, 1936, see Helmcke and Krieger 1953 in Helmcke, 1954–1977; Gaul et al., 1993).
In his *Plankton Diatoms of the Southern Seas*, Hendey proposed a simpler classification than that of Schütt, motivated by his dissatisfaction with the bipartite 'centric'–pennate division:

> For some considerable time I have felt dissatisfied with Schütt's method of classification and with every modification of it that insists upon the two sub-orders based upon either radial and concentric structure on the principal axis or bilateral structure upon the polar axis of the valve, for a large number of genera that have been included in Centricae possess neither radial nor concentric structure, and their construction can in no way be referred to a point. (Hendey, 1937, p. 200)

Many other accounts of diatom classification expressed dissatisfaction with valve symmetry being the principle guide – it simply didn't work. Hendey's solution was to eliminate Schütt's two major groups (Centricae and Pennatae) and classify diatoms in one class, with one order (Bacillariales), subdivided into ten suborders, where the last five 'correspond to Schütt's Pennatae' (Hendey, 1937, p. 201) and the classification 'differs in no material respect from that of Schütt's with the exception that it does not recognize the initial division into two groups …' (Hendey, 1937, p. 202). Hendey's comment is interesting, as it implies that Hendey was not dissatisfied with the notion of a Pennatae group but only with the corresponding Centricae. In any case, Hendey's (1937) classification was more or less retained *in toto* in his contribution to the *An Introductory Account of the Smaller Algae of British Coastal Waters* series, published 27 years after his *Southern Seas* monograph (Hendey, 1964; see also Hendey, 1954).

One might compare Hendey's classification with a few others published during the same period. Hustedt's classification (1930a, b), for example, was a more or less faithful recreation of Schütt's, whereas Patrick (in Patrick and Reimer, 1966) reflected the uniformity of Hendey's sub-orders (but as orders); Simonsen more or less recreates Schütt's classification but via Hustedt in resurrecting the primary divisions of Centrales and Pennales (Simonsen, 1979; see Table 7 for comparison – admittedly, the significant detailed differences in Simonsen's classification and others reside at the family and sub-family level); later Krammer and Lange-Bertalot (1986) follow Simonsen, with a few minor alterations.

Simonsen suggested two reasons for retaining the Centrales–Pennales division. The first was that in his view, symmetry had proved useful – but he then goes on to provide examples of taxa that violate that division, species in the '*Odontella sinensis* group', for example (Simonsen, 1979, p. 10). Second is that all 'centric' diatom species are oogamous, although Simonsen notes that 'exceptions are to be expected [sic] in the araphids as well as in the Eupodiscaceae' (Simonsen, 1979, p. 10). More will be said on this below.

Table 7. Comparison of five classifications covering the period of 1930–1979: Hustedt (1930a), Hendey (1937), Hendey (1964), Patrick (in Patrick and Reimer, 1966) and Simonsen (1979).

Hustedt (1930a)		**Hendey (1937)**	**Hendey (1964)**	**Patrick and Reimer (1966)**	**Simonsen (1979)**	
Centrales					Centrales	
		Suborders		[Orders] Eupodiscales		
	Discineae	Discineae	Coscinodiscineae			Coscinodiscineae
		Aulacodiscineae	Aulacodiscineae			
		Auliscineae	Auliscineae			
	Biddulphineae	Biddulphineae	Biddulphineae	Biddulphiales		Biddulphineae
	Soleniineae	Soleniineae	Rhizosoleniineae	Rhizosoleniales		Rhizosoleniineae
	[Rutilarioideae]					
Pennales					Pennales	
	Araphidineae	Araphidineae	Fragilariineae	Fragilariales		Araphidineae
	Raphidioidineae	Raphidioidineae	Eunotiineae	Eunotiales		Raphidioidineae
	Monoraphidineae	Monoraphidineae	Achnanthineae	Achnanthiales		
	Biraphidineae	Biraphidineae	Naviculaineae	Naviculales		
		Surirellineae	Surirellineae			

Simonsen provided a phylogenetic tree to accompany his classification (Simonsen, 1979, p. 44, fig. 3). His tree differed from the traditional 'centric' – 'araphid' – raphid lineage by placing a number of the 'centric' groups on separate lineages (indicating they did not all share a common ancestor, and, therefore, in today's vernacular, are paraphyletic), uniting the single 'araphid' group with the recently described Protoraphidaceae (Simonsen, 1970) and Eunotiaceae, and leaving the remaining raphid pennate diatom lineages at the apex of the tree (Simonsen, 1979). Earlier, he presented a different diagram that suggested the Centrales *were* monophyletic but composed of same three suborders as outlined in 1979: Coscinodiscineae, Rhizosoleniinae and Biddulphiineae (Simonsen, 1972, pp. 50–51; see Fig. 2 for diagram from Simonsen, 1972 and Fig. 3 for diagram from Simonsen, 1979). The composition of the suborders differs from 1972 to 1979, with the removal of the obvious non-centric 'centric' diatoms (Eupodiscaceae, Rutilariaceae) and Biddulphiineae.

The most notable revision in diatom classification after Simonsen is that of Round et al. (1990). Here the tripartite division, captured in the supposed 'centric' – 'araphid' – raphid lineage, was solidified into yet another formal classification. In part, the 'centric' – 'araphid' – raphid lineage arose because it was a straightforward way to classify diatoms into three neat groups – groups derived from an understanding that a classification could be synonymous with some sort of identification scheme and an assumed phylogenetic lineage of 'centric' – 'araphid' – raphid. This particular tripartite division was derived from the notion that any diatom might easily be identified by first noting its symmetry. That is, dividing it on the basis of whether its valves are radially or bilaterally symmetrical. Second,

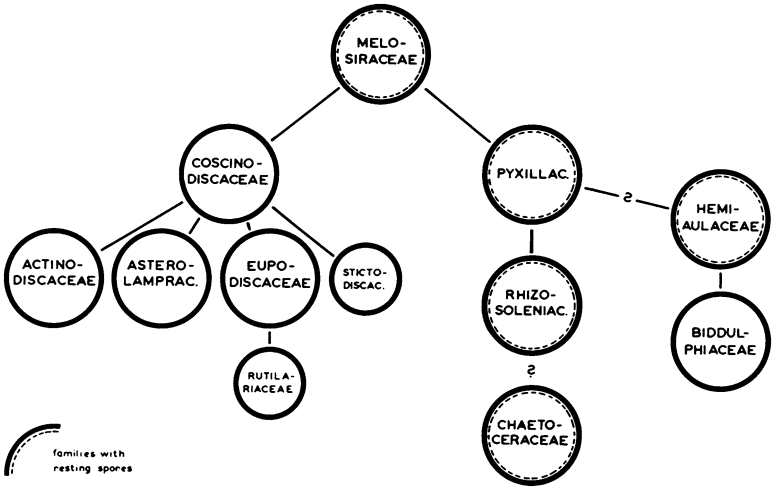

Figure 2. After Simonsen (1972, pp. 50–51), relationships among the 'centric' diatoms.

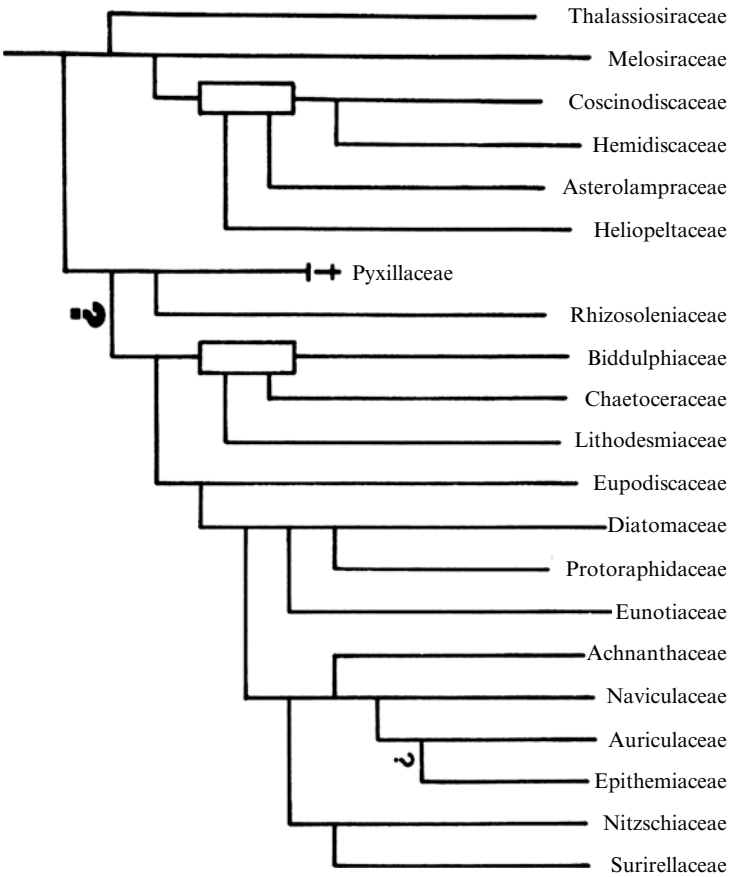

Figure 3. After Simonsen (1979), relationships among all diatoms.

the bilaterally symmetrical diatoms – the pennate diatoms – could themselves be subdivided on the basis of the presence or absence of a raphe. This view has its origins, once again, with H.L. Smith, who suggested that 'These form the three Tribes of the Synopsis, and very seldom will any difficulty arise as to which tribe a diatom may belong to…' (H.L. Smith, 1872, pp. 4–5; Kociolek, 1991). While the accuracy of that statement might be doubted, if one gives serious consideration to Schütt's division of 'centric' diatoms into Eucyclicae and Hemicyclicae, and Hendey's reluctance to use symmetry and shape as the major indicators of difference, it is a surprise that Round et al. adopted so readily their tripartite division. They did so formally, where they recognised three classes, Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae, and 11 sub-classes (divided unequally among the three classes, see Table 8) and a great many other subdivisions. Of the 3 classes and 11 sub-classes, all except Bacillariophyceae, were described as new (Round et al., 1990) – an exceptional number and possibly an excellent example

Table 8. Classification of Round et al. (1990) for classes and subclasses (there are 45 Orders and numerous families, not included in this table).

Coscinodiscophyceae	Thalassiosirophycidae
	Coscinodiscophycidae
	Biddulphiophycidae
	Lithodesmiophycidae
	Corethophycidae
	Cymatosirophycidae
	Rhizosoleniophycidae
	Chaetocerotophycidae
Fragilariophyceae	Fragilariophycidae
Bacillariophyceae	Eunotiophycidae
	Bacillariophycidae

of redundancy in 'over classifying'. For example, the genus *Rhabdonema* was placed in its own Order (Rhabonematales) and its own family (Rhabdonemataceae), as was *Corethron*, *Ardissonea*, *Toxarium* and many genera in Naviculales (the problem of monotypic taxa is discussed in Williams, 2009; the general problem has sometimes been known as Gregg's paradox, of which there is a large literature. Buck and Hull, 1966, provides commentary, Ebach and Williams, 2010, a general solution).

We suggest that two very separate approaches were now being confused: (1) the simplicity of a diatom identification scheme when assigning specimens to one of three 'centric', 'araphid' or raphid diatom groups, a system that still works reasonably well today for identification but clearly an artificial classification and (2) equating this simple (artificial) system with a supposed phylogenetic lineage, as if the 'centric' – 'araphid' – raphid lineage had some reality.

One might, then, suggest that the 'modern' era came to a close with Round et al. (1990). By this, we mean that after 1979 (the date of Simonsen's classification), not only did new classifications appear but different approaches to diatom systematics and classification were changing. There were three reasons: (1) the focus of the primary taxonomic group for investigation shifted from species to genus, (2) the beginning of the era of molecular data, and (3) an explosion of new and puzzling fossil diatoms. The first item relates to methodology, the remaining two to sources of data.

4. Classification and the Molecular Dimension

Molecular studies on diatom systematics are now over 20 years old, having humble beginnings in 1988 with the sequencing of the 18S rRNA gene for *Skeletonema costatum* (Medlin et al., 1988) to the most recent analysis that included 673 diatoms sequences (Theriot et al., 2009). During this period, a new classification was

proposed by Medlin and Kaczmarska (2004; see also Mann in Adl et al., 2005 – for an explanation of their approach, see Adl et al., 2007; Sims et al., 2006; Medlin et al. 2008a, b; for commentary see Sorhannus, 2004; Williams and Kociolek, 2007). Medlin and Kaczmarska's classification disposed of the old tripartite division – 'centric' – 'araphid' – raphid diatom groups – made popular by Round et al. (1990) and proposed another, recently named the CMB hypothesis (Theriot et al., 2009). Using evidence derived primarily from molecular data, Medlin and Kaczmarska (2004) divided diatoms into three major groups. The first were two reformulated subdivisions, Coscinodiscophytina and Bacillariophytina, with Bacillariophytina further subdivided into the redefined class Mediophyceae and the emended class Bacillariophyceae. That is, Bacillariophyceae and Mediophyceae are considered to be more closely related to each other than either is to Coscinodiscophytina, which can be represented as follows: Coscinodiscophytina, (Mediophyceae, Bacillariophyceae), hence the CMB hypothesis (Theriot et al., 2009, p. 277). The revolutionary nature of the classification was the composition of the three groups, especially Mediophyceae, which consisted of '… "bi (multi) polar" and some radial centric[s] [diatoms]' including Chaetocerotales, Biddulphiales, Cymatosirales, Thalassiosirales, Triceratiales, Hemiauliales, Lithodesmiales, Toxariales 'and a suspected bipolar centric (+Ardissoneales)' (Medlin and Kaczmarska, 2004, p. 267). That is, the non-centric 'centric' diatoms, referred to above (a group roughly equivalent to Schütt's Eucyclicae), were now separated into a group of their own (along with a few other taxa) and the radially symmetrical 'centric' diatoms (excepting Thalassiosirales) were retained in the basal Coscinodiscophytina. Bacillariophyceae now included many (but not all) 'araphid' diatoms and all raphid diatom families (Medlin and Kaczmarska, 2004, p. 267). The consequences of this proposal meant that both the 'centric' and 'araphid' diatoms, as traditionally conceived and as represented in the tripartite division of Round et al. (1990), are paraphyletic, '… the diatom equivalent of "invertebrate"' (Alverson and Theriot, 2005). Evidence supporting the CMB hypothesis has not been without its critics (Sorhannus, 2004; Williams and Kociolek, 2007; Theriot et al., 2009).

It is beyond the scope of this chapter to discuss the data and the results in detail (the reader is referred to Theriot et al., 2009) and we have previously offered some comments on the groups within the CMB classification (Williams and Kociolek, 2007). To summarise, there appear to be three main conclusions, the first two negative. The negative conclusions are: (1) detailed study of the available sequence data do not support the CMB hypothesis unequivocally (Theriot et al., 2009); indeed many of the phylogenetic trees published using molecular data differ with respect to the monophyly of Coscinodiscophytina or Mediophyceae (Kooistra et al., 2003b, fig. 3; Medlin and Kaczmarska, 2004, figs. 2–4; Sorhannus, 2004, figs. 1–3; Damsté et al., 2004, fig. 1; Sims et al., 2006; Kooistra et al., 2006, fig. 1; Chepurnov et al., 2008, fig. 1; among others); thus (2) it is highly unlikely that either the Coscinodiscophytina or Mediophyceae are monophyletic in their present form. Given these conclusions, any evolutionary interpretation of this

arrangement is entirely spurious (e.g. Kooistra et al., 2003b, 2006; Sims et al., 2006; Chepurnov et al., 2008, among others).

The positive conclusion is that within the remodelled Coscinodiscophytina, Mediophyceae and the old heterogenous 'araphid' diatoms, some subgroups are gaining support as well as a certain amount of stability. There is growing evidence (from all sources of data) to support a group including all (or most) non-centric 'centric' diatoms (the 'bi-, multi-polar' diatoms listed above) and certain subgroups within the 'araphid' diatoms, whose relationships form several groups some basal to the raphid diatoms (the 'basal araphid' diatoms of Sato, 2008; Medlin and Sato, 2009), others more directly with the raphid, pennate diatoms (the 'core araphid' diatoms of Sato, 2008; Medlin and Sato, 2009). The translation of these various groups into a written classification is premature but, in general, it is congruent with the simple reassessment of morphological characters undertaken below (and Williams and Kociolek, 2010b). What is clear is that statements such as 'Studies of morphology, the fossil record, and molecular data leave little doubt that the centric group is the most ancient and that it is ancestral to the pennates' (Chepurnov et al., 2004, p. 98), or titles such as '*Leudugeria thermalis* sp. nov. (Bacillariophyta): a new centric diatom from shallow hydrothermal venting systems in Saba, Dutch Antilles' (Stancheva and Deheyn, 2010) make no sense at all: 'Centric' diatoms do not correspond to anything in the world except by definition; therefore, they cannot be ancestral to anything nor can new species be placed within (other examples of the misuse of paraphyletic groups can be found in Medlin, 2009; Medlin and Sato, 2009; for commentary see Williams and Kociolek, 2010a).

5. Classification and the Palaeontological Dimension

Mostly based on new material, Nikolaev and Harwood proposed a classification of fossil diatoms primarily addressing relationships within the 'centric' diatoms, as traditionally conceived (Nikolaev and Harwood 2002a, b; Nikolaev et al., 2001). They placed all 'centric' groups in a single class, Centrophyceae, itself divided into six sub-classes: Archaegladiopsophycidae, Paraliophycidae, Heliopeltophycidae, Coscinodiscophycidae, Biddulphiophycidae and Rhizosoleniophycidae (Nikolaev and Harwood, 2002a, b; Nikolaev et al., 2001). Some of these sub-classes relate to some informal groups proposed in a classification by Mann, which was largely based on molecular evidence (Mann in Adl et al., 2005). Comparison between the fossil sub-classes and Mann's informal groups indicates areas of agreement (e.g. the 'Paralids') and disagreement (e.g. the 'Arachniodiscids') but Centrophyceae, like Coscinodiscophytina, appears paraphyletic, and Nikolaev and Harwood's Biddulphycidae (some non-centric 'centric' diatoms), included in Centrophyceae, is roughly equivalent to Mediophyceae, excluded from Coscinodiscophytina (Table 9, after Williams, 2007b, Tables 1 and 2).

Table 9. Comparison of two classifications of 'centric' diatoms.

Class: Centrophyceae	Subdivision: Coscinodiscophytina
Sub-classes:	Informal groups:
Archaegladiopsophycidae	
Paraliophycidae	'Paralids'
Heliopeltophycidae	
	'Arachniodiscids'
Coscinodiscophycidae	'Coscinodiscids', 'Melosirids'
Rhizosoleniophycidae	'Rhizosolenids', 'Corethrids'
Biddulphiophycidae	Class: Mediophyceae

Right-hand column is the classification of 'centric' diatoms after Nikolaev and Harwood (2002a, b; Nikolaev et al., 2001); left-hand column is the classification of 'centric' diatoms after Mann (in Adl et al., 2005). Table modified from Williams (2007b, Tables 1 and 2)

Table 10. Numbers of taxa in fossil classification, extinct and extant members.

	Total	**Extinct**	**Extant**
Subclasses	6	0	6
Orders	28 (12)	6 (3)	22 (9)
Families	70 (31)	25 (17)	45 (14)
Genera	247 (44)	126 (31)	121 (13)

Numbers in brackets are monotypic taxa

The degree of equivalence might be understood and interpreted as a crude measure of congruence between the two different classifications. To investigate, the congruence of classification properly, appropriate evidence is needed. Of significance, would be to know the level of extinction in Nikolaev and Harwood's Centrophyceae, because however much effort is expended, molecular data cannot be easily gained in any meaningful quantity from most extinct organisms (there are some exceptions and these are usually restricted to recent divergences, e.g. Kaplan, 2010). None of Nikolaev and Harwood's six sub-classes is entirely extinct, but at the order level, 6 out of 28 (22%), for families, 25 out of 45 (55%) and for genera, 126 out of 247 are extinct (51%) (Table 10). While Nikolaev and Harwood's classification contains a number of artefacts (there are many monotypic taxa, e.g. see Table 10), these percentages suggest that any revised classification concerning the relationships of *all* diatoms requires study and interpretation of silica morphology as it will be the primary (if not only) source of evidence of the relationships of extinct organisms to their extant relatives. The important role of fossil organisms in understanding phylogenetic relationship has been noted for a variety of other organisms (review in Donoghue et al., 1989). Their general importance is as specimens first, fossils second.

6. Methods and Classification: Character Conflict and Cladistics

6.1. METHODOLOGY

As noted above, Schütt began his discussion of diatom classification by comparing Pfitzer's classification, which was based primarily on plastid structure, with H.L. Smith's, which was based primarily on frustule characters (Pfitzer, 1871; Smith, 1872). Schütt discussed the overlap between some groups contained in the two classifications and proposed his own solution (the three classifications are summarised in Table 11). Although Schütt did not and would not have thought of it in such terms, he was asserting that Coccochromaticae was, in his view, not a natural group – in modern terms not monophyletic. At the very least, he was disagreeing with Pfitzer's assessment of what characters indicated what groups, what characters had significance. That is, a group of species with a '… granular endochrome …' conflict with a group of species with bilaterally symmetrical valves. By conflict, we mean that some species have both characters and two non-overlapping groups cannot be created. Character conflict is perhaps the most common source of problems in systematics. Previously (Williams and Kociolek, 2010b), we suggested three ways in which character conflict might be solved:

1. Choose a classification arbitrarily by favouring one particular dataset or character over another.
2. Assume all data so far examined are poor or of insufficient value and search for another source.
3. Treat character conflict as a problem in systematics that requires solution.

Schütt's classification divided diatoms into two primary groups, Centricae and Pennatae. His decision was not solely based on the evidence at hand but on a particular kind of argument. Such that, Schütt could circumvent the problem of conflicting data by arguing that valve symmetry was of greater importance in determining taxonomically valid groups of diatoms. Others used the same kind of argument but added a justification such as Hendey's view that a natural classification would relate 'structure to function', or Elmore's view that a natural classification would be based on 'characters having physiological significance'. These kinds of arguments do not solve the problem of classification. They simply avoid it. Thus, Schütt's classification can be

Table 11. Comparison of Pfizer's, H.L. Smith's and Schütt's classifications.

Pfitzer (1871)		**H.L. Smith (1872)**	**Schütt (1896)**
I. Coccochromaticae	a. Schalen centrisch gebaut	Cryptoraphideae	Centricae
	b. Schalen nach Umriss und Structur bilateral gebaut	Raphideae + Pseudoraphideae	Pennatae
II. Placochromaticae			

thought of as somewhat arbitrary as it depends upon making arguments to support his selection of characters from the available evidence rather than addressing the issue in any analytical sense. This kind of reasoning is evident in many of the classifications discussed above, as can be seen by the continual appearance, disappearance, and reappearance of a group of 'centric' diatoms. It is worth repeating that Schütt divided Centricae into two 'symmetry' groups: Eucyclicae, with well-defined, radially symmetrical valves, and Hemicyclicae which included some groups with distinct bilateral symmetry to their valves but lacking the central sternum ('axial area') of the 'araphid' (pseudo-raphid) diatoms.

Another solution often adopted is to believe (or argue) that all data so far gathered are poor or of insufficient value and because some data conflict (are not perfect), they can be rejected and a search for better, more perfect data undertaken. This view is often encountered when dealing with the usefulness or otherwise of morphological data which, because some characters provide conflicting evidence, *all* of that character source must be of insufficient value, or at least suspect. This viewpoint was recently expressed like so:

> … although SEM provided many new insights and suggested how some existing genera might be remodelled (e.g. Medlin & Round 1986 for gomphonemoid diatoms), in many cases evolutionary relationships were unclear because of character conflicts. (Mann et al., 2008, p. 17)

Thus, the search for new sources of data:

> … diatomists started to look for alternative sources of information, including cytological and reproductive characteristics. (Mann et al., 2008, p. 17)

After that (seemingly inconclusive) search, 'powerful molecular genetic tools have become available for determining evolutionary relationships' (Mann et al., 2008, p. 17). Thus, magic and perfection arrived (but see section on molecules above). Mann et al. believe that, along with character conflict, taxonomic philosophy was also at fault:

> … because the prevailing taxonomic philosophy was then phenetic, with its requirement that taxonomic judgments should reflect 'overall similarity' based on all available evidence … the phenetic approach has been discredited for phylogeny reconstruction. (Mann et al., 2008, p. 17)

A search of the pertinent diatom literature reveals only one study that performed a phenetic analysis: Kociolek and Stoermer (1986). Thus, it is evidently wrong to state that 'the prevailing taxonomic philosophy was then phenetic'. Of course, the word phenetic was commonly used at that time – or rather misused – to mean the assessment of 'all evidence'. But all taxonomic philosophies use all available evidence (Kitching et al., 1998). Phenetics was never concerned with 'phylogeny reconstruction'. It was always offered as a method of classification that deliberately did *not* try and discover phylogenetic relationships (Sneath and Sokal, 1973). Phenetics (in its proper sense) was concerned with measuring 'overall similarity', as determined by various computer algorithms, for clustering data

rather than attempting to resolve character conflict – in short, phenetics disregarded character conflict altogether and simply clustered groups together. With respect to any 'prevailing taxonomic philosophy', within diatom systematics, it is perhaps more accurate to suggest that there was none, other than the subjective assessment of any particular characters' importance relative to some set of beliefs concerning how to determine that importance, like Schütt (or Hendey or Elmore) above (or, ironically, as expressed in Round et al., 1990). An alternative to phenetics was cladistics, a taxonomic philosophy primarily concerned with monophyly, phylogeny, character conflict and its resolution – with respect to all available data (Williams and Ebach, 2007). It is worth mentioning that during the period from 1985 to 1995, there are at least ten cladistic character studies on diatoms. Mann et al. (2008) do not mention cladistics in their history (but then neither did Round et al., 1990, as was pointed out by Kociolek, 1991 – but they did champion the 'discredited' phenetics, which, in 1990, was already fading fast, if not having vanished entirely from mainstream systematics; see Kitching et al., 1998); hence the history of systematics presented in Mann et al. (2008) is at best mistaken revisionism, at worse simply false in all its detail.

6.2. CHARACTERS AND THEIR INTERPRETATION

Previously, we offered a simple example of the resolution of character conflict using cladistic reasoning (Williams and Kociolek, 2010b). Here we expand on the problem as represented in Table 11, and deal with characters that support the various arrangements.

We previously noted that plastids might be treated in different ways. It is not a case of simple observation and recording details of the structures. Plastids might be thought of as a character with two 'states', two conditions: 'many small plastids' and 'few large ones'. These might suggest alternatives, the first evidence for one group (Coccochromaticae), the second evidence for another (Placochromaticae). This approach treats characters as if they were simply just observations – some plastids are small and occur in numbers in each cell, others are large and occur with only a few per cell. But both characters are plastids. From an evolutionary perspective, one kind of plastid may be considered a modification of another. For example, a 'large plastid' (placochrome) may be considered a modified form of a 'small plastid' (coccochrome), the coccochrome condition being primitive relative to the derived placochrome condition. Thus, while these data still yield evidence for two groups, they differ from the straightforward bipartite subdivision. As the coccochrome condition is considered primitive, it is really a character of *all* diatoms as it includes those diatoms with the modified placochrome condition. In turn, the placochrome condition picks out a subgroup within diatoms: raphid diatoms plus some 'araphid' diatoms. A consequence of understanding characters in this way means that some diatom groups previously recognised ('araphid' diatoms) require dismembering as only some

'araphid' diatoms have the placochrome condition. Thus 'araphid' diatoms cease to be a viable (real) group – they are not monophyletic, as predicted by Williams and Kociolek (1988).

Interestingly, Round et al. (1990) placed two families, Plagiogrammaceae and Cymatosiracaceae, with bilaterally symmetrical valves in the 'centric' diatoms (of which more later). Plagiogrammaceae was placed in the 'centric' order Triceratiales – among the 'centric' diatoms (Round et al., 1990). Alongside *Plagiogramma*, Round et al. also included in the family Plagiogrammaceae *Glyphodesmis* (Round et al., 1990, pp. 240–241) and *Dimerogrammopsis* (Round et al., 1990, pp. 242–243). Kooistra et al. (2004) recently proposed the valid name *Talaroneis* Kooistra et Stefano for *Dimerogrammopsis* and presented molecular evidence to show it to be a member of an 'araphid' lineage – meaning that it is considered more closely related to an 'araphid' taxon than to any 'centric' taxon (see Kooistra et al., 2004; Sorhannus, 2004). Regardless of their attempt to define 'araphid' diatoms, the remarks of Kooistra et al. (2004) concerning the misplacement of genera in Plagiogrammaceae (as well as the family itself) are well taken. Surprisingly, if anything, the data presented in Round et al. (1990) suggest taxa in Plagiogrammaceae have the placochrome condition, evidence that also suggests they are better considered part of the pennate diatom group. With respect to Plagiogrammaceae, this view was predicted from morphology by Kociolek (1991) and more recently received further support from molecular data (Sato et al., 2008a, b).

These kinds of hypotheses concerning characters are not propositions concerning truth (cf. Kooistra et al., 2004, p. 62). They are statements concerning what groups (taxa) are supported by the available evidence. We previously contrasted the evidence provided by plastid structure with evidence derived from the valve sternum (defined here as a centrally placed zone of demarcation often at the centre of the valve) (Williams and Kociolek, 2010b). Some diatoms have a sternum, others do not. Thus, it is tempting to understand this character in simple terms too: presence of a sternum, absence of a sternum. However, it is clear that all raphid diatoms also have valves with a sternum, even if it is integrated closely with the raphe system. In this case, the character sternum characterises all pennate diatoms, raphid and 'araphid' alike. In addition, another character might be 'sternum plus raphe', characterising a subgroup within pennate diatoms, the raphid diatoms. It is thus futile to try and define 'araphid' diatoms using the sternum as a character (Kooistra et al., 2004, p. 62; Medlin, 2009, p. 500), even if defined by lacking the raphe:

> Pennate diatoms, in turn, are subdivided into two distinct groups: raphid pennates, which possess one or two slits (comprising the raphe system) that are either integrated within the sternum or associated with it, and araphid pennates, which lack a raphe. (Chepurnov et al., 2008, p. 98)

In summary, two groups might be derived from the character sternum: all pennate diatoms (raphid + 'araphid' diatoms) and a subgroup, the raphid diatoms. Again, this example shows that 'araphid' diatoms have no data to support them as a taxonomic (phylogenetic) group.

The conventional view is that if a diatom valve has a sternum, it will, by definition, exhibit bilateral symmetry. This appears to be so. But the reverse is not: If a valve has bilateral symmetry, it need not have a sternum. In this light, it is interesting to reconsider symmetry (not withstanding the discussions in Kooistra et al., 2003a; Alverson et al., 2006; Medlin et al. 2008a, b). Using Schütt's classification as a guide, diatoms with bilateral symmetry (with or without a sternum) are all pennate diatoms *and* non-centric 'centric' diatoms, the latter equivalent (more or less) to Hemicyclicae and would include more recently studied groups such as Cymatosiraceae (Hasle et al., 1983) and Plagiogrammaceae (Kooistra et al., 2004) (and, as it happens, may account for the position of 'odd' 'araphid' diatoms, such as *Ardissonia* and *Toxarium* without the necessity of invoking multiple origins derived from mapped molecular trees, Kooistra et al., 2003a; Alverson et al., 2006; Medlin et al., 2008a, b; see also Mayama and Kuriyama, 2002 for discussion of developmental patterns in valve formation).

Are there any general conclusions that can be drawn from diatom plastid structure and valve symmetry? There seems to be three:

1. Neither character supports a 'centric' or 'araphid' diatom group.
2. Support is for *new groups* not yet named in any classification.
3. There is no conflict between the two characters (as in the contrasting schemes of Pfitzer, H.L. Smith and Schütt), and these morphological data broadly agree with current molecular data – that is, there is nothing 'wrong' with the morphological characters, just the determination and recognition of the groups they 'define' (summarised in Table 12).

The process might be taken a step further by adding more characters such as those derived from the different processes of reproduction, touched on above. How useful has sexual reproduction been in providing evidence for relationships among diatoms? Consider this passage from a recent review:

> The use of the sexual phase as a primary source of information to determine systematic relationships is less worthwhile than it was, because of the introduction of molecular systematic methods, which can provide more taxonomic characters more quickly than reproductive biology or morphology or cytology. (Chepurnov et al., 2004, p. 137)

This is a standard argument for rejecting one data source (reproduction) in favour of another (molecular): the original data (reproduction) had promise but did not

Table 12. Comparison of plastid and sternum characters as homologies.

Primitive		**Derived**	
'… granular endochrome …' → Coccochromaticae		'… lamellate endochrome …' Placochromaticae	
	Primitive		**Derived**
	Sternum	→	Raphe
	Pseudoraphideae		Raphideae

deliver; molecular data are likely to be more reliable because they provide 'more taxonomic characters'. They continue:

> In the past, however, knowledge of sexual behavior was sometimes crucial in solving important taxonomic problems. Perhaps the best example was the discovery that the Cymatosiraceae have flagellate gametes, which prompted a reevaluation of their classification and the recognition that they are centric diatoms, despite their elongate shape. (Chepurnov et al., 2004, p. 137)

In general, as we and others have shown, molecular data agree with morphological data that 'centric' diatoms are not a group that exists in any sense of the word. Yet 'flagellate gametes' (oogamy) provides support for stating that Cymatosiraceae 'are centric diatoms, despite their elongate shape'. Something is wrong. One might simplify the data presented in Chepurnov et al. (2004). Broadly speaking, there are three general characters derived from modes of sexual reproduction: oogamy, anisogamy, isogamy (we acknowledge that the situation is more complex than this simple division). And these characters are seen to conflict with data derived from the plastids, the sternum (and raphe) and molecules.

'Centric' diatoms	Pennate diatoms	
	'Araphid' diatoms	Raphid diatoms
Oogamy	Anisogamy	
		Isogamy

The characters might also be represented as a sequence of change, a transformation:

'Centric' diatoms	Pennate diatoms	
	'Araphid' diatoms	Raphid diatoms
Oogamy	→ Anisogamy	→ Isogamy

However, there are overlapping elements: Anisogamy occurs in both 'araphid' and raphid diatoms; oogamy occurs in 'centric' diatoms, some bilaterally symmetrical diatoms that have been placed within 'centric' diatoms (Cymatosiraceae, non-centric 'centric' diatoms, Hasle et al., 1983) and some 'araphid' diatoms (*Rhabdonema*, von Stosch, 1958; see Medlin and Sato, 2009, fig. 1). That is, modes of sexual reproduction do not correspond to any of the old accepted groups, 'centric' or 'araphid' diatoms. How can sense be made of these data? Once again, the problem is not with the characters but with the groups. Congruence between datasets can be achieved by placing Cymatosiraceae in the pennate diatoms, as they share bilateral symmetry of their valves (a derived character), and by dividing the 'araphid' and raphid diatoms that share anisogamy from the isogamous raphid diatoms (see Medlin and Sato, 2009, fig. 1). This strategy, once again, yields a non-monophyletic 'centric' and 'araphid' diatom group. Thus, Cymatosiraceae, nor any other taxon for that matter, cannot belong to groups such as 'centric' diatoms as they have no existence, and oogamy, supposedly a defining character for 'centric' diatoms, is in fact primitive (plesiomorphic) and useless as evidence for phylogenetic relationships (Kociolek et al., 1989, see

Edlund and Stoermer, 1997, for a cladistic summary of reproductive processes and diatom phylogeny, especially their fig. 14 and the character data appended to Medlin and Sato, 2009, fig. 1). In a study on the evolution of meiotic patterns in 'centric' diatoms, Mizuno (2006) concludes:

> Diatoms with type 1 oogenesis and hologenesis type spermatogenesis, which are inferred to be primitive among extant centric diatoms, are the bipolar taxa *Attheya decora* West, *Odontella aurita* (Lynbg.) C.A. Agardh, *Odontella mobiliensis* (Bailey) Grunow, *Odontella regia* (Schultz) Simonsen and *Odontella sinesis* (Grev.) Grunow, and the multipolar *Lithodesmium undulatum* Ehrenb …. (Mizuno, 2006, pp. 62–63; see also Hoban, 2008)

Thus, if anything, Cymatosiraceae is the 'best example' of the use of reproductive characters demonstrating, instead, how plesiomorphy (oogamy) can mislead by identifying paraphyletic groups ('centric' diatoms), a fact Chepernov et al. tangentially allude to: 'In addition, there is an urgent need to reassess the centric group in relation to sexual reproduction, now that it is clear that the centrics are paraphyletic' (Chepurnov et al., 2004, p. 137; see Edlund and Stoermer, 1997; Medlin and Sato, 2009). The same situation pertains to the use of auxospore characters. As summarised in Medlin and Sato (2009, fig. 1), it is clear that scales are shared by the radial and bilateral 'centric' diatoms but it is not clear that scales are homologous to the properizonial bands, as both are depicted as occurring in the bilateral 'centric' diatoms (Medlin and Sato, 2009, fig. 1). However, it seems a reasonable assumption that scales and some bands *are* homologous and that scales are primitive, hence fail to be a character of 'centric' diatoms. Properizonial bands are a character of bipolar 'centric' diatoms plus all pennate diatoms; longitudinal perizonial bands are a character of all pennate diatoms and transverse perizonial bands, a character of some pennate diatoms (Medlin and Sato, 2009, fig. 1).

Thus, a cladistic approach to systematic problems analyses the conflict by searching for the cause of incongruence (the disagreement between characters). That is, its primary assumption is that characters – plastids, bilateral symmetry, sexual reproduction, auxospore morphology and so on – have primitive and derived components; only the derived components are informative and the informative component may be for groups of species yet recognised or named. Thus, a study of characters and their analysis yields taxonomic groups regardless of the source of data.

Of course, investigations of this kind yield new problems, further issues to solve – this is the very nature of systematic investigations. Consider the raphe once again. Above, it was viewed as a modified sternum. But what of rimportulae, how do they relate to the raphe? Suppose, as some have, polar rimoportulae in raphid diatoms such as *Eunotia* is a primitive (undeveloped) raphe (Hustedt, 1926; Berg, 1948; Kolbe, 1956). A consequence is the character rimoportula, being an unmodified raphe, is not a character in a phylogenetic sense and does not discriminate groups. Thus, the presence of rimoportulae in 'centric' and 'araphid' diatoms indicates nothing of their relationships and the best we can offer is that 'presence of rimoportulae' is a character of *all* diatoms. This conforms to current understanding, otherwise, if rimoportulae where considered a derived feature, a

group composed of all 'centric' diatoms, all 'araphid' diatoms and all eunotioid diatoms, would have been recognised. As far as we are aware, no such group has ever been proposed. With respect to the rimoportulae/raphe character, what problem has been revealed? If the rimoportula is considered a primitive raphe and one is a modified form of the other, then how can Eunotoid diatoms possess both? Of course, one might offer the view that the raphe is really a suite of characters and that its overall structure is the result of a series of fusion events (Mann, 1984); or that there were many rimoportulae, and in eunotioid diatoms some remain unmodified; or the rimoportulae, on modification, was retained as only part of the raphe, the helictoglossa perhaps, and so on (complete systems of classification have been proposed based on the raphe system and its relations: Jurilj and Jerković 1973). But these are simply stories empty of any empirical content. But there may be a problem worthy of investigation: What is the character? – rimoportulae + raphe or rimoportulae + sternum + raphe, or something else? The question relates to the determination of characters and the groups they pick out, such that investigations lead to further knowledge about the characters themselves (above and beyond mere observations) and, more importantly from the perspective of classification, the groups they recognise.

7. Further Comment on Taxon Numbers

Julius entitled a section in his review of diatom evolution 'Diversification Since the Cretaceous and the Overwhelming Nature of the Data' (Julius, 2007, p. 27). Assessing the number of possible diatom species (perhaps 200,000) and those already described (24,000), Julius noted that only 12% are currently known and of those a small proportion from SEM examinations (Julius, 2007). It is worth examining a few more numbers.

As noted above, in the mid-1930s, Agardh (1830–1832) was dealing with no more than 100 or so species, Kützing (1834a, b) with around 120 species. From then on, the descriptive phase in diatom studies was primarily focused at the species level, and 110 years later, after Hustedt's many floras (from 1920 to 1950, and onwards), nearly all taxonomic effort was directed towards species (some details are summarised in Williams and Reid, 2007; Julius, 2007, especially his table 1). But after 1980, a great many new genera were being described. We examined the numbers of genera described for the period 1980–2008. This period was chosen as it begins just after Simonsen's revised classification (1979); the midway period (1990) is marked by Round et al. (1990) and ends at the dawn of the molecular age (Medlin and Kaczmarska, 2004). For 1980–1989, an average of ≈10 genera per year was being described; for 1990–1999, an average of ≈17 genera per year was being described; and for 2000–2008, an average of ≈8 genera per year was being described. Some years are higher than others because of the publication of major works. For example, in 1990, two major contributions were published: Round et al. described 19 new genera and Gersonde and Harwood (1990) described 12 new genera (figures summarised in Table 13).

Table 13. Number of genera described for the period 1980–2008 (post Simonsen, 1979).

Year	No. genera	Year	No. genera	Year	No. genera	Totals
1980	8	1990	44	2000	19	
1981	1	1991	4	2001	12	
1982	4	1992	6	2002	11	
1983	12	1993	12	2003	8	
1984	13	1994	12	2004	5	
1985	11	1995	6	2005	12	
1986	15	1996	27	2006	8	
1987	12	1997	33	2007	3	
1988	19	1998	18	2008	5	
1989	9	1999	17			
Total	104		179		83	366
Average	≈10		≈17		≈8	≈11

Some years are higher than would be expected because of the publication of a major work. For example, in 1990, Round et al. described 19 new genera and Gersonde and Harwood (1990) described 12 new genera. However, the figures offer some understanding of the rate of description: for 1980–1989, an average of ≈10 per year; for 1990–1999, an average of ≈17 per year and for 2000–2008, an average of ≈8 per year. Fourtanier & Kociolek, Catalogue of Diatom Names, California Academy of Sciences, Online Version, accessed October 2010

This can be compared to the number of genera described during the period 1805–1975, marking the beginning of diatom taxonomic studies through to the pre-Simonsen classification period. Although it would be possible to count every year, we opted to sample at 5 year intervals. These figures offer some idea of the rate of description, which is around an average of ≈4 per year (Table 14).

Again, the figures are possibly biased by the choice of year. For example, 1845 saw 20 new genera described, most from Ehrenberg (1845, 9 genera), and 1895 saw 10 new genera described, most from Cleve (1895, 6 genera) (see Table 14). However, excluding the high figures for both 1845 and 1895, there is still an average of ≈3 genera a year. If 1844 had been chosen as target year instead of 1845, the number of new genera described would be 57, most from Kützing (1844, 26 genera – although some are subgroups, probably intended as sub-genera) and Ehrenberg (1844, 8 genera), increasing (possibly artificially) the average to ≈6.

The purpose of exposing these numbers is simply to give another dimension to diatom diversity and how it is currently being recognised. If one compares diatoms with a reasonably well-known group such as mammals, the figures for species relative to genera are revealing. For extant mammals, around 5,000 species are placed in 1083 genera (O'Leary et al., 2004); if we assume for diatoms there are around 12,000 known species placed in 350 genera (see Table 15, which includes estimates for extinct as well as extant genera). With respect to names, there are around 62,000 for both diatoms and fishes (Eschmeyer, 1998; Fourtanier and Kociolek, 2010), of which for fishes there are 12,000 generic names, but for diatoms there are only a tenth of that number, 1,200. Even so, there is every indication that progress is being made at generic level for diatoms. Interestingly, Mammalian genera are placed in a greater number of higher taxa (O'Leary et al., 2004). Diatom classification lacks detailed

Table 14. Number of genera described per year in 5 year intervals for the period (1805–1975).

Year	No general
1805	1
1815	0
1825	1
1835	5
1845	20
1855	2
1865	9
1875	2
1885	4
1895	10
1905	6
1915	0
1925	6
1935	2
1945	3
1955	0
1965	0
1975	6

Figures are biased by the choice of year. For example, 1845 has 20 genera, most described by Ehrenberg (1845, 9 genera), 1895 has 10, most described by Cleve (1895 has 6 genera). If 1844 had been chosen, the number of genera described would be 57, most from Kützing (1844, 27 genera) and Ehrenberg (1844, 8 genera). However, the figures in the table do offer some idea of the rate of description, around an average of ≈4 per year, including figures for 1845 and 1895, ≈3 excluding figures for 1845 and 1895. Fourtanier & Kociolek, Catalogue of Diatom Names, California Academy of Sciences, Online Version, accessed October 2010

Table 15. Comparison of numbers of species and genera of diatom for mammals.

	Genera	Number of species
Diatoms (after Round et al., 1990)		
Extant	350	12,000
Extinct	150?	5,000?
Mammals (after O'Leary et al., 2004)		
Extant	1,083	5,000
Extinct	4,076	20,000

hierarchical structure (although Round et al. provided many new taxonomic levels, as noted above, many are entirely redundant and meaningless). It now seems appropriate to consider naming taxonomic levels higher than genera when classifying any group of species so that diatom diversity can be better and more economically captured (Williams, 2009). However, to name taxa at any hierarchical level, one might invest in methodology rather than guesswork (Williams, 2009).

8. Conclusions

Diatom classification has progressed since the early days of Agardh's and Kützing's first attempts to bring order to this group of organisms, some 180 years ago. Then, around 50 species were arranged in a handful (9–12) of genera; now, the number of both categories – species and genera – is much larger, with c. 500 genera and at least 15,000 species (see Table 15). The increase in numbers of species recognised does not necessarily mean that each taxon is understood to any great degree, many of the species being described from few specimens and having only ever been encountered a single time. Species to one side, it is doubtful that many diatom genera are demonstrably monophyletic. By this we mean, they have their own (distinct) synapomorphies, defining characters (morphological or molecular). To determine synapomorphies, one needs to enhance and develop the various concepts of characters, their definition, and relations (we treated some of these issues above under 'Kinds' of Data). Two things need to be noted. First, to develop and refine notions of synapomorphy, more detailed studies on morphology are required. Second, synapomorphies are not determined from a pre-existing phylogenetic tree, regardless of the source or quality of the data used to derive that tree. For example, it has been noted that for *Sellaphora* 'Close examination will probably demonstrate that at least some of the new groupings revealed by molecular analysis possess synapomorphies in aspects of frustule morphology and structure, but at the moment these are not obvious' (Mann et al., 2008, p. 22). Leaving aside the notion of probability (anything is probable; see above), characters that correspond to molecular trees may or may not be true synapomorphies (by true, we mean determined from analysis rather than simple comparison; see Methods and Classification: Character Conflict and Cladistics). The only way to determine synapomorphy is through observation and analysis, not subservience to other more 'precious' data sources (for a recent excellent critique, see Mooi and Gill, 2010).

This should not be taken as advocacy of morphology over molecules. We envision new data arising from various sources. We have high expectations of new discoveries in new fossil taxa, for example, as well as further discoveries using molecular data. To allow these data to be useful beyond their immediate publication, they need to be more accessible, with sequences and alignments freely available for those interested in exploring them further.

Synapomorphies determine natural groups; natural groups are monophyletic – diatom classification should adopt the search for monophyly (and synapomorphy) as the norm. It is almost universally accepted that non-monophyletic groups are useless. Monophyletic groups are both explanatory and predictive. Morphology and molecules both confirm that 'centric' and 'araphid' diatoms are not natural groups (although recently, Ravin et al., 2010, did find a monophyletic 'araphid' clade using data from *cox*1 gene).

Finally, classifications are the basis for determining phylogenetic relationships, deriving taxon lineages and exploring various aspects of evolutionary biol-

ogy. Prior to the success of cladistics in systematic biology, phylogenies were worked out (or derived directly) from fossil taxa and their stratigraphic position (Williams and Ebach, 2007). The paucity of credible fossil lineages encouraged a scenario-based approach, whereby stories were invented to account for taxon relationships (or to account for missing fossils). Remarkably, molecular data relevant to diatom systematics is barely 20 years old, yet numerous papers have yielded various speculative phylogenetic scenarios:

> Thus, existing hypotheses of diatom origins tend to agree that the prediatom or 'Ur-diatom' developed, not in pelagic habitats, but in shallow marine environments, perhaps with the intercalation of a freshwater or terrestrial phase. (Sims et al., 2006, p. 363)

> Most diatomists believe the freshwater diatom flora evolved from marine taxa invading freshwaters, largely because the records of fossil marine diatoms extend some 65 Ma before those of the freshwater. (Sims et al., 2006, p. 388)

> B. Why Did Chromists Win Over Prasinophytes or Red Microalgae?
>
> C. Why Did Heterokontophytes Win Over Haptophytes and Dinoflagellates?
>
> D. Why Did Diatoms Win Over Other Heterokontophytes? (Kooistra et al., 2007, p. 277)

> Molecular phylogenies show that the araphid pennate diatoms evolved from a lineage of centric diatoms …, and the earliest known araphid fossils are from the Late Cretaceous …, whereas centrics are known from the Jurassic … (Chepurnov et al., 2008, p. 103, ellipses represent omitted references)

> The last common ancestor of *Thalassionema* must have reduced the thickness of its valves, and as a consequence of that had to reduce or eliminate the chambers. Chamber reduction probably resulted in its turn in a reduction of the elaborate chamber occlusions as seen in *S. toxoneides* into a single row of occluded pores along the perimeter of the valve face. (Kooistra et al., 2009, p. 13)

Such views (beliefs, 'perhapses', probabilities, possibilities, 'must-haves') are merely window dressing and have no discernable scientific value. Diatom classification is made more scientific by the search for synapomorphies (whatever the source of data) and the concomitant discovery of monophyletic groups. It would be a pity to drape progress in determining diatom interrelationships and their classification with pointless scenario-building and speculative evolutionary narratives.

In summary, we understand diatom classification to have progressed, albeit somewhat erratically. Consideration of a few principles, if accepted as fundamental to the science of systematics (classification), might make future progress less episodic:

1. Characters need to be explicitly determined
2. Synapomorphy recognition and analysis
3. Only demonstrable monophyletic groups recognised
4. Analyses of *all* data sources made explicit and repeatable

If these few principles are adhered to, then diatom classification will develop along more 'natural' lines, rather than having to be reinvented every 10 years, as is the case at present.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 93–118
DOI 10.1007/978-94-007-1327-7_4, © Springer Science+Business Media B.V. 2011

A REVIEW OF THE EVOLUTION OF THE DIATOMS FROM THE ORIGIN OF THE LINEAGE TO THEIR POPULATIONS

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1. Introduction

The diatoms are one of the most easily recognizable groups of major eukaryotic algae, because of their uniquely silicified cell walls (frustules), which consist of two overlapping thecae, each in turn consisting of a valve and a number of hoop-like or segmental girdle bands (Round et al., 1990). Well-preserved and diversified frustules are found in one of the earliest known deposits of fossil diatoms, from the early Albian (Lower Cretaceous), of what is now the Weddell Sea, Antarctica, but these diatoms bear little resemblance to modern diatoms in their morphology (Gersonde and Harwood, 1990), although it is clear that the diatoms were very highly developed and diversified even at that time.

2. Origin of the Heterokont Lineage

Molecular sequence data show that diatoms are heterokont algae. Pigmented heterokonts are chlorophyll *a*+*c* containing algae whose motile cells, with the exception of the diatoms, have two heterodynamic flagella, one covered with tripartite hairs and the other smooth (Van den Hoek et al., 1995). In diatoms, the flagellar apparatus is reduced or absent; indeed, only the spermatozoids of the oogamous "centric" diatoms are flagellated, and these are uniflagellate (von Stosch, 1950), lacking all trace of the smooth posterior flagellum.

Because photosynthesis has played such a fundamental role in shaping the biosphere, the origins of the plastids have remained one of the most intriguing and well-researched topics in biology (see reviews in Bhattacharya and Medlin, 1995; Yoon et al., 2002; Keeling, 2004). The origin of the initial photosynthetic eukaryote is believed to have involved the phagotrophic uptake of a cyanobacterium by a heterotrophic host cell. This event resulted in the origin of the green algae, the red algae, and the glaucophyte algae. Following this event, a secondary endosymbiotic event occurred, in which one of the primary endosymbiotic algae was engulfed by a second heterotrophic host. If the engulfed alga was a green alga,

then the resultant new eukaryote cell became a euglenoid or chlorarachniophyte alga. If the engulfed cell was a red alga, then the resultant new eukaryotic cell became a cryptophyte, haptophyte, heterokont, or dinoflagellate alga. Even now that both primary and the red lineage secondary endosymbioses seem to have happened only once (Yoon et al., 2004), it is clear that an early timing of this event (1.3 Ga) does not match the fossil record of the phytoplankton that are the modern components of the red algal secondary endosymbiotic event (Lipps, 1970). Clearly, the host lineages did not take immediate advantage of their newly acquired organelle and photosynthetic function. All of the early divergences in the heterokont tree are heterotrophic and the cells appear to have lost the plastid from their secondary endosymbiosis. There is a final divergence in this lineage of all of the autotrophic golden brown and brown algae, which include the diatoms. To complicate further the endosymbiosis story, there is also growing molecular evidence that some of the lineages with the red algal plastid, especially the diatoms, may have originally had a green plastid, which was later exchanged for a red algal one (Petersen et al., 2007; Frommolt et al., 2008; Mustafa et al., 2009). It is presumed that both types of plastids were present in the cell at the early origin of secondary endosymbiosis. If this hypothesis is found to be true, then some ecological event is likely to have driven the elimination of the green plastid in favor of the red one. None of the clocks with close sister groups to root the diatom origin have placed their origin earlier than 250 Ma, which likely corresponds to the Permian–Triassic (PT) extinction. Thus, the heterokont algae to which the diatoms belong likely radiated at the PT boundary as did the dinoflagellates and the haptophytes (Medlin, 2008, 2011), although at different taxonomic levels. Ocean trace metal chemistry changed at the PT boundary at the extinction event caused by the volcanic eruption in China and this likely gave the host plants with a red algal plastid the adaptive advantage that they needed to radiate (Falkowski et al., 2004a, b). With the many open niches that appeared at the end of the PT extinction event, they would have been able to evolve rapidly at that time (Medlin et al., 1997a). Red and green algal plastids differ greatly in their need for certain trace metals. The abundance of Fe after the PT boundary favors the growth of the red algal plastid, which has the Fe-containing cytochrome C6 in its photosynthetic electron carrier complex instead of the Cu-containing plastocyanin found in the photosystems of other algae (Falkowski et al., 2004a, b). Thus, it is most likely that the PT mass extinction event is the event that triggered the elimination of the green algal plastid from the cell in favor of the red plastid, which resulted in the radiation of the heterokont algae and other members of the modern phytoplankton. The diatoms are one of the major contributors to this radiation and have continued to rise in importance ever since.

3. Origin of the Diatoms

Today, the diatoms are found in almost all aquatic and most wet terrestrial habitats. Existing hypotheses of diatom origins tend to agree that the pre-diatom or "Ur-diatom" developed from a scaly ancestor, not in pelagic habitats, but in shallow marine environments and were tychoplanktonic (see review in Sims et al., 2006). Sexual reproduction studies of the diatoms have confirmed that early stages of the

auxospore, the specialized zygote of the diatoms, have a covering of silica scales in many genera. Different groups of diatoms have additional bands added to the developing auxospores but in most of the initial stages of all auxospores of all diatoms studied to date is a rounded cell covered with silica scales. Several heterokont algae, such as the Dictyochophyceae, Synurophyceae, Chrysophyceae, Parmophyceae, and Xanthophyceae (van den Hoek et al., 1995; Graham and Wilcox, 2000) produce silica structures, either as resting stages or as part of their vegetative cell. Because these groups are spread across the heterokont phylogeny (Medlin et al., 1997b), the ability to metabolize silica was probably inherited from the heterotrophic heterokont ancestor. Scales are present on the reproductive stage of the Labyrinthuloides, which are earlier divergences in the heterokont lineage (Medlin et al., 1997b). Phylogenetic analyses have documented that the closest sister group to the diatoms are the Bolidophyceae, uniflagellated picoplankters (Guillou et al., 1999). Bolidophytes lack any silica structures or scales and most likely lost the ability to make silica elements and scales secondarily. However, the most recent discovery from molecular data that the Parmales, which do have silica cell walls, are embedded in Bolidophyceae (Ichinomiya et al., 2011) would suggest a different interpretation. It has been proposed that the naked haploid flagellated bolidomonads are a different life cycle stage from the diploid silica walled palmales cells.

Most recently, Harwood et al. (2004) have proposed that diatoms arose in terrestrial habitats because a new early diatom deposit (175 Ma) had been found in Korea that seems to be terrestrial in origin. This would appear to conflict with molecular data, because the Bolidophyceae, their true sister group, are an exclusively marine group of picoplankton. In keeping with this proposed terrestrial origin, Medlin (2004) has proposed a scenario in which "Ur-diatoms," abundant as non-silicifying unicells in coastal waters, could have become stranded in isolated tidal pools as eustatic seas retreated after flooding the continents. When these large saline pools began to dry up over considerable time periods, the unicellular, flagellated Ur-diatom, if they survived the desiccation, had to adapt to a semiterrestrial habitat. The ability to metabolize silica and the production of thick silica walls could have evolved at this time as protection against desiccation (and higher salinity) and to put the cells into a temporary resting state (Medlin, 2002) until the areas were reflooded. Thus, a simple naked biflagellate cell initially evolved or retained silicon metabolism, which prevented the cell from aging and thus aided its survival by placing it in a prolonged resting state while it was stranded in the tidal pools. Medlin (2002) has reviewed the literature that shows that mammalian cells grown on a silica substratum are placed in a temporary resting state and she proposed that this same benefit of being placed in a temporary resting state was the force driving the diatoms to metabolize silica. The evolution of the diatom vegetative cell from a resting cell stage was originally proposed by Pascher (1921) and later expanded by Mann and Marchant (1989) who proposed that the Parmales could be a close relative of the diatoms. If this scenario is true concerning the placement of the cells in a temporary resting state, then the evolutionary scenario by Pascher and Mann and Marchant bears some merit, especially in light of the new discovery of the phylogenetic placement of the Parmales (see above).

With further sea-level changes, the early diatoms would have recolonized coastal waters, but their newly developed heavy silica walls would have made them too heavy for a planktonic existence and restricted them to a benthic, nearshore existence. To judge from their morphology, most of these early diatoms would have been chain formers, and Crawford and Sims (2007) proposed that an additional function of a chain was to keep cells in close contact to prevent dispersal in what might have been a turbulent environment. If the sexual life cycle of the diatoms was still uncertain and in early stages of evolution, then the close proximity of mating types would have been beneficial. Kooistra et al. (2008) have argued that isogamy in the plankton is not as successful as oogamy, and that this may explain why there are so few planktonic diatoms with pennate morphology.

In this respect, *Paralia sol* as the first diatom lineage in the radial centric clade in the molecular trees is a reminder of these early heavily silicified, chain-forming diatoms that are benthic, only rarely being suspended up into the plankton with increased turbulence. *Paralia sol* has been transferred to *Ellerbeckia* (Crawford and Sims, 2006), a terrestrial genus, so now the first divergence in the radial centrics is of a genus with both marine and terrestrial representatives. In the proposed ancestral terrestrial habitat for the "Ur-diatoms" (Harwood et al., 2004), *Ellerbeckia* could be, as the first divergence in the molecular tree, the most likely candidate for survivors of this ancient terrestrial assemblage.

4. Deep Divergences in the Phylogenetic Tree

Most diatomists have long assumed that the diatoms contain two groups: the centrics and the pennates, which can be distinguished by their pattern centers or symmetry, mode of sexual reproduction, and plastid number and structure (Round et al., 1990). The oogamous centric diatoms, with radially symmetrical ornamentation on their valves and with numerous discoid plastids, are distinct from the isogamous pennate diatoms with bilaterally symmetrical pattern centers and generally fewer, plate-like plastids. These groups are known to most aquatic and cell biologists under these terms, and each term conveys a distinct image of a particular type of diatom valve. It is important to remember that these terms are descriptive and have no taxonomic value.

Historically, centric and pennate diatoms have been separated as two classes or orders. Williams (2007) has traced the historical classification of the diatoms. Round et al. (1990), however, recognized three classes: Coscinodiscophyceae (centric diatoms), Fragilariophyceae (araphid pennate diatoms), and Bacillariophyceae (raphid pennate diatoms) – giving equal ranking to the raphid pennate diatoms with a double slit opening (raphe) in the cell wall for movement and the araphid pennate diatoms without this slit. Recently, Medlin and colleagues (literature summarized by Medlin and Kaczmarska, 2004) have divided the diatoms into two groups on the basis of molecular sequence data. What was initially called "Clade 1" in Medlin's early molecular work (Medlin et al. 1993, 1997a, 2000a)

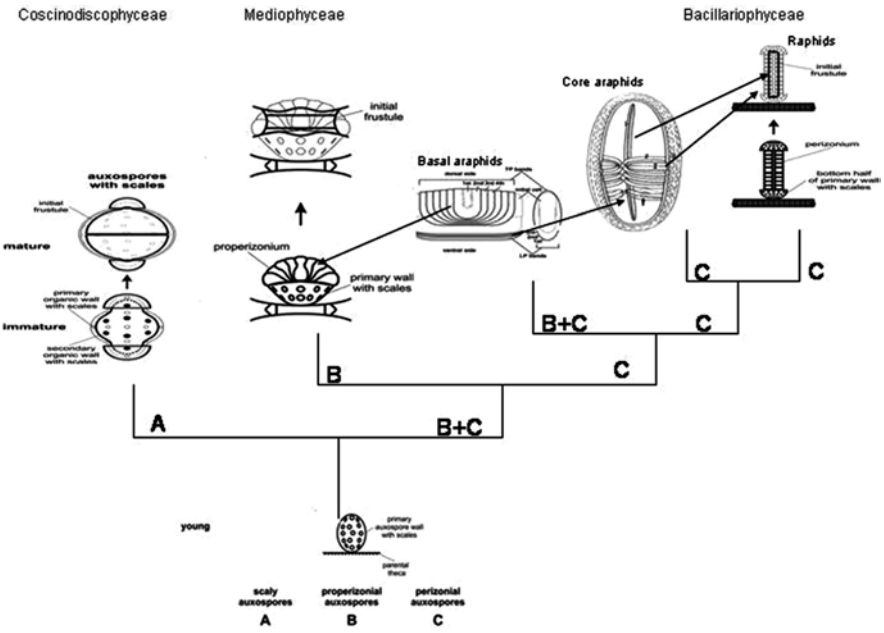

Figure 1. Correlation of the type of auxospore with the new classification of the diatoms (From Medlin and Kaczmarska, 2004; redrawn from Medlin and Kaczmarska, 2004; Kaczmarska et al., 2001; Medlin and Sato, 2009).

contains those centric diatoms with essentially radial symmetry of valve shape and structure. "Clade 2" consists of two groups, the first of which contains the bi- or multipolar centrics and the radial Thalassiosirales ("Clade 2a") and the second, the pennates ("Clade 2b"; Fig. 1). Morphological and cytological support for these clades was reviewed in Medlin et al. (2000a) and taxonomically formalized in Medlin and Kaczmarska (2004). Clades 1 and 2 are now recognized at the subdivision level as the Coscinodiscophytina and Bacillariophytina, respectively, and Clades 1, 2a, and 2b are now recognized at the class level, as the Coscinodiscophyceae, Mediophyceae, and Bacillariophyceae (Medlin and Kaczmarska, 2004). These classes are not recovered as monophyletic if alignments are not performed using the secondary structure of the ribosomal RNA genes as a guide or if single outgroups are used (Medlin et al., 1993, 2008b; Sato 2008; Medlin, 2010a).

There are good correlations between the major molecular clades and certain cytological features. For example, the Bacillariophytina have a perinuclear arrangement of the Golgi apparatus, whereas in the Coscinodiscophytina, the Golgi stacks are usually in Golgi–endoplasmic reticulum–mitochondrion (G–ER–M) units (available data summarized in Medlin et al., 2000a and Medlin and Kaczmarska, 2004). However, there are some exceptions in each group (see Schmid, 1988). Labiate processes in the Coscinodiscophytina, where present,

are located in a ring around the valve perimeter or scattered over the valve face. Several genera possess spines at the edge of the valve face, which interlock firmly with similar structures on the valve face of the adjacent sister cell and link cells firmly into chains. External cribra are found in these diatoms with loculate areolae, with one possible exception of *Endictya*, which is certainly misclassified because it has eccentric areolation. There is a central structure in the mediophycean valves that may be a labiate process, a strutted process, or a sternum. Internal cribra dominate in the mediophycean diatoms, especially if the valves are loculate as in the Thalassiosirales and *Triceratium*. One possible exception is *Eupodiscus*. In the simple poroid mediophycean valves, the cribrum appears to be at the internal valve surface, for example, *Lampriscus*. Chain formation in the bipolar mediophycean diatoms tends to be less robust than in the Coscinodiscophytina.

The best independent, nonmolecular support for three classes comes from auxospore structure (Fig. 1), the specialized zygote of the diatoms that swells to restore the cells to their original cell size, which has diminished with progressive vegetative division (Kaczmarska et al., 2001; Medlin and Kaczmarska, 2004). Isodiametric auxospores that can swell in all directions and have only scales are characteristic of the class Coscinodiscophyceae, anisodiametric auxospores with scales and hoops or bands (a properizonium) to restrict the swelling to bipolar or multipolar directions are found in the class Mediophyceae and anisodiametric auxospores that form a complex tubular perizonium, usually consisting of transverse hoops and longitudinal bands, are found in the class Bacillariophyceae. Among the Mediophyceae, the Thalassiosirales have retained the scaly isodiametric auxospores of the Coscinodiscophyceae, but they are firmly placed in the Mediophyceae because of the process in the valve center, the perinuclear arrangement of the Golgi bodies, and the internal cribrum in the loculate areolae. Among the pennates, the araphids have two different types of auxospores, which correlates with the molecular tree, and the raphids have only one type of auxospore.

Although Simonsen (1979) has been cited as the first person to show that there is a grade of clades from centrics to pennates (Theriot et al., 2009), this is in fact a misinterpretation by Theriot et al. (2009) of the phylogenetic tree Simonsen drew in his paper. In fact, Simonsen shows a deep basal dichotomy in the diatoms that does not represent segregation into centrics vs. pennates evident in the classification system provided in Simonsen (1979, pp. 48–53), neither does it show the grade of clades. Simonsen did advocate a formal separation of these groups at the order level (Centrales and Pennales), beneath the Class Bacillariophyceae (Simonsen, 1979, p. 11). However, in his phylogenetic tree, he illustrates two separate lineages within the centrics, the radial vs. non-radial diatoms. Recognition of two separate groups of radial centrics versus non-radial centrics plus pennates shown on that figure is very similar to the basal dichotomy we recovered between the subdivisions Coscinodiscophytina and Bacillariophytina. Simonsen's suborder Coscinodisciineae included the Thalassiosiraceae, which we know now from molecular data belongs to the bipolar centric group and not the radial centric group. Thus, except for the Thalassiosiraceae, the first divergence in the tree by

Simonsen is essentially that of the proposed subdivision Coscinodiscophytina from Bacillariophytina (Medlin and Kaczmarska, 2004). Furthermore, Simonsen (1979) also defines Coscinodisciineae by one of the characters that we have used, viz., the marginal ring of processes (Simonsen,, 1979, p. 45). In the second branch of the first divergence, we find a polytomy between the suborders Rhizosoleniineae and Biddulpiniineae. These groups are defined in Simonsen's key as those diatoms that do not have marginal rings of processes. He would not have known as we do now from morphogenetic data that the labiate process of *Rhizosolenia* begins on the margin and moves to the center, thus it was only logical for Simonsen not to include the Rhizosoleniaceae in Coscinodisciineae. As well, Simonsen's (1979) Biddulpiniineae are not a grade of clades but are relegated to two clades and one of them is the Eupodiscaceae from which the pennates arise.

The rate of evolution in coscinodiscophycean diatoms has been calculated using two different means of calibrating molecular trees: (1) by fossil dates for the entire clade (Kooistra and Medlin, 1996) and (2) by biomarker compounds for the clade containing *Rhizosolenia* (Sinninghe-Damsté et al., 2004). Both methods suggest that these diatoms are evolving very quickly (1% per 21.5 and 14 Ma for the rRNA gene, respectively) and this could explain why the morphology of the diatoms changes so rapidly across the Cretaceous, between Lower and Upper Cretaceous floras.

Molecular clocks are proving to be very useful tools for unraveling the evolution of protistan taxa. They have been used to reconstruct biogeographic histories, divergence times of many protists ranging from their origins to the divergence of cryptic species. Microalgae, such as diatoms, dinoflagellates, and coccolithophores, have mineralized walls that preserve well. These microalgal groups have better-preserved fossil records than their metaphyton and metazoan counterparts and molecular clocks made using calibrations from microalgae are consequently better calibrated (Berney and Pawlowski, 2006). Many microalgal groups suffered greatly at mass extinctions only to reradiate after the event, whereas others pass through the events relatively unscathed. Mass extinctions are important to macroevolution not only because they cause a sharp increase in extinction intensity over ambient levels, but also because they bring a change in extinction selectivity and these quantitative and qualitative shifts often set the stage for evolutionary recoveries and radiations (Jablonski, 2005).

Several workers have constructed molecular clocks for the diatoms, and most are concerned with dating the origin of the group and the diversification of its major clades, which would be recognized at the class level when these are recovered as monophyletic groups. Kooistra and Medlin (1996) made the first molecular clock for diatoms using a linearized tree where the rate of evolution was averaged across the tree using other heterokonts as the outgroup. Using the Hillis and Morris model (Hillis et al., 1996) for their clock, they calculated an average age and an earliest possible age given a 95% confidence interval around any undated node. In this clock, the origin of the diatoms was estimated to be 164–266 Ma (average to earliest). The major clades that constitute the two subdivisions of the

diatoms were estimated to have diverged between 120 and 200 Ma (average to earliest). In Clade 2, the bipolar centrics and the pennates diverged between 86 and 159 Ma. Phillippe et al. (1994) used a relative rate test to estimate the rate of evolution between the diatoms and other eukaryotic groups. They used the ciliates as the sister group to the diatom, and the branch length leading to the origin of the diatoms used for the relative rate test in that paper corresponded, in fact, to the origin of the heterokonts, which is the division to which the diatoms belong. Because too distant an outgroup was used to root the ingroup, they erroneously concluded that there was a 300-Ma gap in the diatom fossil record. In his first clock paper, Sörhannus (1997) used the chrysophytes as the nearest sister group to the diatoms, which are likely still too far away from the ingroup, but this gave a more reliable date of 330–400 Ma for the origin of the diatoms based on two genes. His most recent clock (Sörhannus, 1997) is based on a single gene, the SSU rRNA gene with the true sister group of the diatoms, the Bolidophyceae as the outgroup and uses a relaxed molecular clock (PATHd8), which was calibrated sequentially from single dating points. His dates for the origin of the diatoms range from 250 to 183 Ma, which is similar to that of Kooistra and Medlin (1996).

Medlin (2010b) and Sato (2008) using four genes and the program, Multidivtime, with designated maximum and minimum divergence times of 250 and 190 Ma, respectively, found much older divergence times for all of the classes, especially the pennates (Fig. 2). Their clock using an ML tree as input suggests that the radial centrics, Class Coscinodiscophyceae, emerged from 180 to 240 Ma and the bipolar centrics, Class Mediophyceae diverged from the Class Bacillariophyceae at 183–238 Ma (minimum to maximum, respectively). These results also indicate that the early divergence of the pennates into three major clades, basal araphid, core araphid, and raphid diatoms, took place over a very short period. All major clades (~ orders or families) of araphid diatoms appeared by the end of the Cretaceous in all analyses. Thus, the molecular diversification of the diatoms appears to be much earlier than the first appearance of these taxa in the fossil record at 180 Ma. However, modern diversifications of the genera in these lineages usually coincide with the first appearances of the extant genera. The reconciliation of molecular diversification with first appearances of selected genera of diatoms is discussed in Sörhannus (2007), Kooistra and Medlin (1996), Sims et al. (2006), Medlin (2010b).

The recovery of the three new classes as monophyletic groups has been controversial and will not be recovered as such unless multiple distant outgroups are used (Medlin and Kaczmarska, 2004; Sato 2008), as well as a secondary structure alignment for the ribosomal RNA genes (Medlin et al., 1993, 2008b; Medlin, 2009). Nevertheless, one or more of the three classes have been recovered in some trees using some types of analyses and with different genes by many different workers [small subunit ribosomal RNA (SSU rRNA) in all papers by Medlin, in some of the trees in Cavalier-Smith and Chao (2006), Choi et al. (2008), Rampen et al. (2009), Sinninghe-Damsté et al. (2004), Sorhannus (2004); large subunit ribosomal RNA (LSU rRNA) in

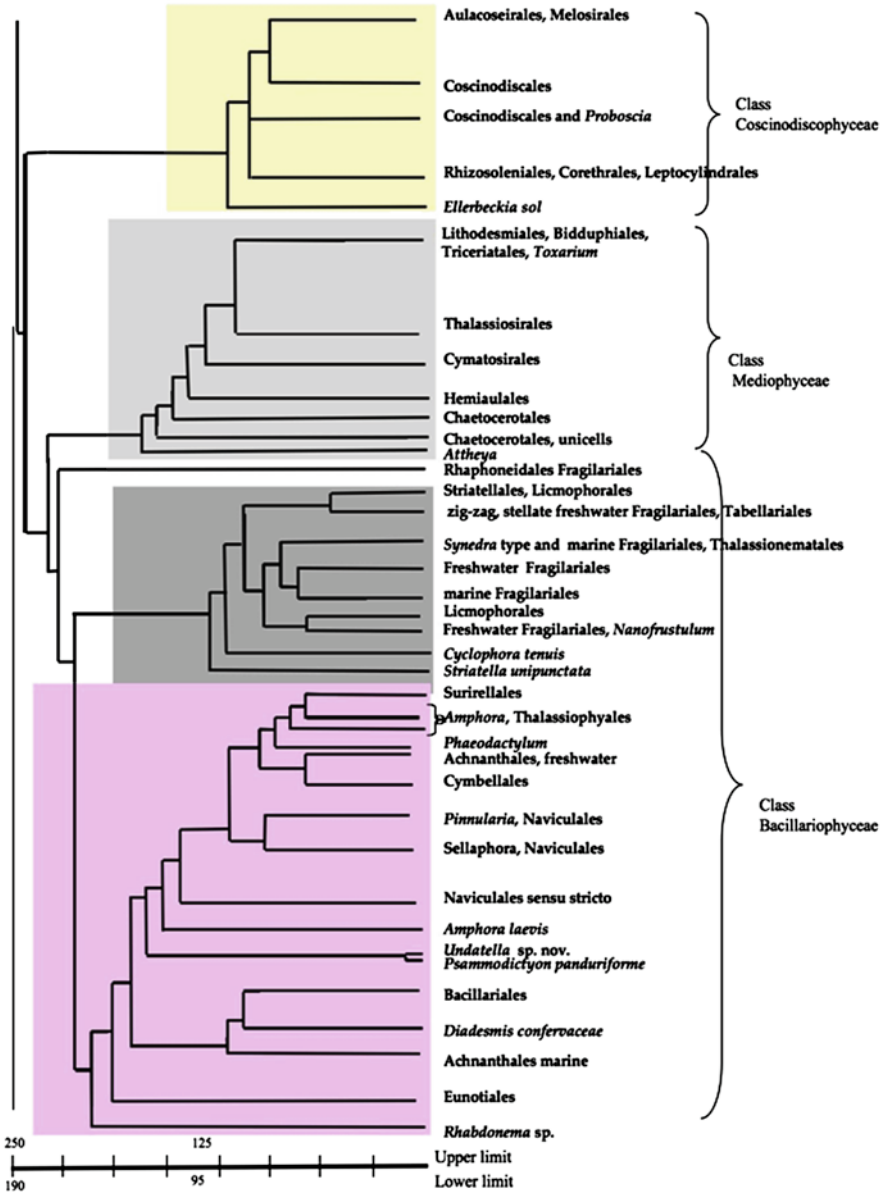

Figure 2. Time line for the divergences of classes and orders in the diatoms, with the root of the diatom lineage constrained at 250 Ma for a maximum time of origin or 190 Ma for a minimum time of origin.

Sörhannus et al. (1995); 16S, TufA, *Rbc*L in Medlin et al. (1997b); Cox 1 in Ehara et al. (2000); and *Rpo*A in Fox and Sorhannus (2003)]. This is in contrast to the claims made by Williams and Kociolek (2007) that these classes have not been found as monophyletic groups other than in the works of Medlin and coworkers. A full reference to all molecular studies at all taxonomic levels can be found in Mann and Evans (2008) with a discussion of what remains as open questions about diatom evolution.

When a single outgroup is used in the phylogenetic analysis, the two centric classes are not monophyletic (Medlin and Kaczmarska, 2004, fig. 2), but this type of analysis can be useful to see the sequential divergences of the subclades/orders within each class. For example in fig. 2 of Medlin and Kaczmarska (2004), the first coscinodiscophycean taxon to diverge is that of the terrestrial genus *Ellerbeckia* and the last mediophycean taxon to diverge before the pennates is that of the Cymatosirales.

Williams and Kociolek (2007) have also claimed that the terms centric and pennate cannot be used. On page 313, Williams and Kociolek write "A natural classification system based on these new phylogenies [those of Medlin and Kaczmarska, 2004] will radically change the diagnoses of group names used historically, requiring us to discontinue some long-used and familiar names; this should alter the way we teach diatom classification and systematics to future generations, allowing the study of a wide array of scientific questions and problems." Centric and pennates can still be used in a morphological/descriptive/adjectival sense because of their morphogenetic patterns and their mode of reproduction, but it is true that they cannot be used as **taxa** because centrics are not monophyletic. The terms centric and pennate diatoms, including araphid and raphid ones, can be continued to be used in a descriptive sense and there is no need to modify the way we teach diatom classification other than to include the most recent data on auxospore formation, which continue to define the classes and sublineages of the diatoms (Figs. 1 and 2). These terms convey a distinct diatom morphology (centric = areolation radiates from the central annulus and pennate = areolation is bilaterally arranged to the sternum with or without a raphe in the sternum) and type of sexual reproduction (centric = oogamous reproduction and pennate = isogamous reproduction). Thus, in teaching diatom classification, we only have to add that there are two types of centrics, one group with only scales on their zygotes or auxospores, which enable the auxospore to expand in all directions, i.e., the radial centrics or Coscinodiscophyceae and another group of centrics that have both scales and hoops so that their auxospore is restricted in its expansion in a bipolar or multipolar direction, i.e., the bipolar centrics or the Mediophyceae. The only exception to this are the Thalassiosirales, which have only scales on their auxospores, but are placed in phylogenetic analyses in the Mediophyceae because they share other diagnostic features of the group, most importantly, a structure in the annulus and internal cribra in loculate areolae. Pennates can be defined as is done historically.

5. Mid-Level Clade Divergences

It seems that features of the living cell, for example, the zygote morphology and its development define and better support the deeper branches of the tree, whereas the details of the silica cell wall, upon which the classification and the systematics of the diatoms are based, can only be used to define the middle branches to the tips of the tree.

In the coscinodiscophycean diatoms, all of the clades recovered in this class correspond to orders as presently defined in the classification system (Fig. 2, Medlin and Kaczmarska, 2004). Following the divergence of the terrestrial genus, *Ellerbeckia*, there is a basal separation between those diatoms with an extended pervalvar axis and those not. Those with an extended pervalvar axis include *Corethron* and *Rhizosolenia sensu stricto*. Coscinodiscales is sister to Aulacoseriales/Melosirales. Of these orders only the Aulacoseirales has been investigated in detail with both a molecular and morphological analysis (Edgar and Theiot, 2004). *Aulacoseira* is composed of five major clades: (1) an *A. crenulata* and *A. italica* clade, which is the most basal, (2) an *A. granulata* complex clade, (3) an *A. ambigua* clade, (4) an *A. subarctica* and *A. distans* clade, and (5) an *A. islandica* clade that also contained endemic species from Lake Baikal, Siberia, and many extinct *Aulacoseira* taxa.

Basically, the same can be said for the mediophycean diatoms, and perhaps the only clade where the taxa are not completely sorted into accepted orders is the clade with Biddulphiales and the Triceratiales. The radially centric order, Thalassiosirales with only a scaly auxospore, lies within the Mediophyceae, and their true sister group with high bootstrap support are the Lithodesmiales. Their inclusion within this class appears contradictory to the structure of the auxospore being the defining character of the classes because they retain the auxospore structure of the Coscinodiscophyceae, but the Thalassiosirales have the perinuclear arrangement of their Golgi bodies, a central process and an internal cribrum that unites them with the Bacillariophytina and other mediophycean diatoms. Kaczmarska et al. (2005) have presented several hypotheses for the origin of the strutted processes of the Thalassiosirales, which is the unique structure defining this order. In Medlin and Kaczmarska (2004), a scenario in which a central process, such as that found in *Praethalassiosiropsis*, could evolve into a modern labiate process was proposed. The presence of multiple types of central processes in the lithodesmioid diatom, *Mediopyxis* (Kühn et al., 2006), lends some support to a hypothesis that the central labiate process of the Lithodesmiales could have evolved into the central strutted process (Medlin and Kaczmarska, 2004). Within the Thalassiosirales, the families Thalassiosiraceae, Skeletonemaceae, and Stephanodiscaceae and the genus *Thalassiosira* Cleve are paraphyletic (Medlin and Kaczmarska, 2004). The recent separation of *Discostella* from *Cyclotella* (Houk and Klee, 2004) has been validated by molecular studies from both SSU and LSU data sets (Jung et al., 2009).

Two groups of diatoms that have been traditionally placed in either centrics or pennates have now been correctly placed with molecular analyses. *Dimeregramma*, *Talaroneis*, and *Subsilicea* have parallel ribs on their valve faces, apical pore fields, and what appears to be a sternum, as in araphid pennate diatoms, but they totally lack labiate processes and have been returned from being placed in the centrics (Round et al., 1990) to the pennates based on molecular analyses (Kooistra et al., 2004). *Toxarium* (Kooistra et al., 2003) has been considered a pennate diatom because of its elongate shape and benthic habitat, but the best-known species *T. undulatum* lacks a midrib, parallel ribs, or any trace of labiate processes. The pattern center here is neither a simple annulus nor a sternum. Instead, *Toxarium* seems to have a pattern center lying at or near the valve face–mantle junction and running around the whole perimeter of the valve face. This subtends rows of pores outward, on the mantle and encloses a more or less irregular scatter of pores on the valve face. A similar arrangement is found in *Climacosphenia*, *Synedrosphenia*, and *Ardissonea*, which are closely related to *Toxarium* (they share similar girdle and chloroplast morphology), except that on the valve face, the pores are organized into rows perpendicular to the peripheral pattern center, which has been called a bifacial annulus (Mann, 1984) because it subtends ribs on both sides of a line rather than radially, as in a true annulus. These genera also lack labiate processes and well-defined apical pore fields (Kooistra et al., 2003). Molecular analyses (Kooistra et al., 2003; Medlin et al., 2008b) place all of these genera in a well-supported clade in the bipolar centrics, and the elongated pennate shape has thus clearly evolved twice (Medlin et al., 2008b) as opposed to once as proposed by Alverson et al. (2006). With their placement in the bipolar centrics, the araphid diatoms become better defined: bipolar valves with a sternum lacking a raphe slit and with labiate processes and apical pore fields, except where secondarily lost, for example, in *Staurosira*. *Psammodiscus* is a final example of a diatom that does not easily fit into a centric or pennate group. It has circular valves and radial symmetry, but neither has an annulus nor a sternum. It has been suggested that *Psammodiscus* is related to *Rhaphoneis* and its allies, but that the sternum has been reduced to a point (Mann, 1984; Round et al., 1990). Its phylogenetic position has not been tested with molecular data.

The pennates are always recovered as a monophyletic group. There are two groups of araphid diatoms and one raphid clade in all molecular analyses. The two araphid diatom clades have been termed basal and core araphids (Sato, 2009). Sato (2008) and Medlin and Sato (2009) have shown that the basal araphids have both a properizonium and a perizonium in the same auxospore (a combination of bipolar centrics and raphid pennates), whereas the core araphids have only a perizonium, in common with the raphid pennates (Fig. 1). The modern classification of the araphid diatoms will need to be extensively revised because this group is paraphyletic. Within the Fragilariales, most of the new genera separated from *Fragilaria* are shown to be paraphyletic, but those separated from *Synedra* appear to be genetically distinct (Medlin et al., 2008a). More *Synedra*-like taxa should be examined to confirm this.

Raphe slits that occur in *Eunotia*, *Actinella* and *Semiorbis*, and *Eunophora* are much shorter than in most raphid diatoms and are not fully integrated into the sternum. Furthermore, labiate processes are present in most of the *Eunotia* group, whereas they are apparently never found in raphid diatoms with a fully developed raphe system. Greater integration between the pattern center and raphe is present in *Peronia*, which is closely related to *Eunotia*. Comparative morphology has suggested that the raphe originated from the labiate process (Hasle, 1974) and that it only later became associated with and incorporated into the pattern center or annulus/sternum. Thus, the *Eunotia* group has traditionally been considered to represent an intermediate stage between araphids and raphid diatoms. Most molecular analyses place them as the first divergence in the raphid diatoms. The type of analysis affects the position of the Eunotiales, regardless of the alignment (Rimet et al., 2011)

Among the raphid diatoms, most orders and the families therein are recovered as monophyletic groups, but there are some genera/orders that are not monophyletic and revisions will be needed here as well. Many well-documented genera, viz. *Diatoma, Fragilariopsis, Seminavis*, and *Pleurosigma*, arise from within other genera, making the parent genus paraphyletic. This is problematic for pure cladists to accept, and it appears that within the diatoms, paraphyletic genera will have to be accepted unless extensive generic redefinitions are done.

Medlin and Kaczmarska (2004) produced the first phylogenetic tree with many raphid diatoms. Bruder and Medlin (2007, 2008a, b) and Bruder et al. (2008) have produced an assessment of raphid pennate diatoms using a three-gene phylogeny concentrating primarily on naviculoid diatoms and increased substantially the number of taxa investigated over that in Medlin and Kaczmarska (2004). Bruder and Medlin's first study focused on species of *Placoneis*, a genus that was separated from *Navicula,* which, based on its chloroplast morphology, was placed within the Cymbellales. The phylogenetic analyses clearly confirmed this, but the relationships between the different species varied with different genes. *Navicula hambergii* was shown to belong to *Placoneis*. In their second study, *Hippodonta* was consistently placed as sister group to *Navicula s. s.*, supporting it as a genus separate from *Navicula*. There were three distinct, well-supported clades within *Navicula s. s.*, but an investigation of their valve morphology revealed no morphological features that would support these clades and clearly more work is needed with both improved taxon sampling and morphology, perhaps from living cells to ascertain subclade relationships within the Naviculales. In their third study, *Gomphonema*, *Cymbella*, *and Encyonema* were recovered each as monophyletic groups.

Cymbelloid diatoms were divided into two clades, which corresponded to the genera of *Cymbella* and *Cymbopleura* supporting the morphological interpretation of the genera by Krammer (2000). *Gomphonema* was also divided into two groups based on external areolae coverings, and it was suggested that more taxa should be investigated here to determine if *Gomphonema* naturally sorted into two groups.

Relationships of cymbelloid and gomphonemoid diatoms were discussed by Pfitzer (1871) Lauterborn (1896), Cleve (1894–1895), and Cox (2002). Pfitzer

proposed 22 families. Family II, the Cymbelleae Kütz, included genera grouped together because of their lateral concavo-convex asymmetry viz., *Brebissonia* Grun., *Anomoeneis* Pfitzer, *Cymbella* Ag., *Cocconema* Ehrbg., and *Encyonema* Kütz. Family VI, the Gomphonemeae Kütz. contained Gomphonema Ag. and *Sphenella* Kütz. from which all species have been removed to other genera, chiefly to *Gomphonema.* Both of these families are asymmetric in valve and girdle views, but the third of Pfitzer's genera, *Rhoicosphenia* Grun., was of similar valve asymmetry but symmetrical in girdle view. Cox (2002) reviewed how morphogenesis can be used in taxonomic interpretation of relationships between genera and used Cymbellales to illustrate her conclusions.

Amphora was not monophyletic in the molecular analysis of Bruder and Medlin (2008b) and the two groups recovered corresponded to the subgenera *Amphora* and *Halamphora*, which are two of the nine recognized subgenera established by Cleve (1894–1895). *Mayamaea* was consistently recovered as the sister taxon to a *Pinnularia*/*Caloneis* clade. *Craticula* and *Stauroneis* were also consistently recovered as sister taxa, but the addition of *Navicula intergra* to the base of the *Stauroneis* clade suggested that a new genus was warranted to accommodate this taxon. *Prestauroneis* was described to accommodate this taxon (Bruder et al., 2008).

Neither *Pinnularia* nor *Caloneis* were monophyletic, thus supporting earlier contentions that the genera could not be separated (Round et al., 1990). However, their molecular analysis did reveal two well-supported clades, which corresponded to the division of the two genera following Krammer and Lange-Bertalot (1985), using the degree of opening of alveoli as a criterion for separating species in the two genera. The divisions of the genus by Cox (1988) on the basis of plastid morphology were not supported by the molecular analyses.

The monoraphid diatoms were first grouped together by Kützing (1844) within the Monostomaticae and later by Cleve (1894–1895) within the Achnantheae, but freshwater and marine monoraphids do not form a monophyletic group in the rRNA phylogenies, although individual genera were monophyletic. Marine *Achnanthes* species are consistently placed sister to the Bacillariaceae with high bootstrap support. Finding morphological support for this sister relationship is difficult but both groups appear to have their cribrum in their poroid areolae close to the external surface of the valve. *Achnanthidium* and its freshwater relatives are sisters to both marine and freshwater *Cocconeis* spp., which are placed inside the Naviculales in agreement with the inclusive classification of the order by Cleve (1894–1895). Cladistic analyses place these genera in entirely different clades and with entirely different sister groups (Kociolek and Stoermer, 1993; Cox and Reid, 2004), which illustrates how misleading cladistic analyses can be with no molecular analyses to guide outgroup selection or character polarization.

Canal raphid diatoms are also not monophyletic. Bacillariales are one of the early divergences in the pennates, whereas the Surirellales are a later divergence (Medlin and Kaczmarska, 2004). The latter group evolves through a sequence from *Amphora* to *Entomoneis* to *Surirella* as the canal raphe evolves from a

naviculoid type to a canal type that is arched over the valve and then to one that completely surrounds the valve. Ruck and Kociolek (2004) performed a cladistic analysis using nitzschioid diatoms as the closest outgroup, assuming that this group was the ancestor of the circumferential raphe in contrast to that shown by molecular data. It is unclear if using an amphioid diatom as the outgroup would recover the same paraphyletic (not polyphyletic) nature of the surirellioid genera as found in Ruck and Kociolek (2004). The most interesting finding from their study was a clear separation of marine and freshwater taxa. A more in-depth analysis of this family based on morphology and multigenes is being conducted by Ruck and Theriort (2011).

Symmetry of the frustule and orientation of the movements of the protoplast and particularly the plastids during the cell cycle is also important and has been since Pfitzer (1871) and Lauterborn (1896) studied live cells of many taxa. Pfitzer split the diatom genera into two groups according to the number of plastids, a group with one or two plastids and another with many. His second split of the first group was according to the position of the plastid in the cell, the position of the raphe, and the number of auxospores. His group with many plastids included zygomorphic and radially symmetric or centric forms. Lauterborn (1896) was one of the earliest microscopists to view mitosis and chromosome movement in living cells. Mereschkowsky (1902–3) followed the example of these two authors and examined the "endochrome" of 125 species or varieties of marine diatoms. His classification divided the diatoms into mobile and sexual species (raphid pennates) and nonmobile and asexual species (araphids and centrics). He divided the centrics into two groups (discoid and biddulphioid forms, see the two new groups of centric diatoms *sensu* Medlin and Kaczmarska (2004) to which this grouping is virtually identical except for the Thalassiosirales, which are in the Mediophyceae). Even today, few species or even genera have been followed through the cell cycle in any detail and it is unknown what, if any, evolutionary significance this may have (Mann, 1984, 1985).

6. Cryptic Species

As molecular data have explored the boundaries between species, it has been commonly found that many of our cosmopolitan species are actually species complexes, substantiating the contention that the diatoms are under classified (Mann, 1999). Cryptic species can be most easily defined as a complex of morphologically identical or nearly identical strains that be separated into distinct groups genetically and thus, can be termed sibling species. The minute details separating *Sellaphora pupula* in several varieties were first correlated with breeding data to divide the varieties according to a biological species concept (Mann, 1989), and this was further supported by differences at the molecular level (Evans et al., 2007) so that a phylogenetic species concept could be met. Such data lends confidence to recognizing the value of small morphological differences. The diatom *Skeletonema costatum*,

now contains eight semi-cryptic species (Zingone et al., 2005; Sarno et al., 2005), each with a distinct biogeography (Kooistra et al., 2006). Hints that *Skeletonema costatum* contained more than one species was revealed with the isozyme analysis done by Gallagher in 1982. She found that her spring and autumn populations were more genetically distinct than sibling species of land plants, but if she had investigated her diatoms morphologically, she would have found that they were also morphologically distinct. From what we now know of the distribution of *Skeletonema* species complex (Kooistra et al., 2006), the diatoms most likely to be present in Narragansett Bay are *S. grethae*, *S. marinoi*, and *S. japonicum*, which are morphologically distinct.

Cyclotella menegheniana, considered by Finlay (2002) as a diatom that is everywhere, was shown by geometric multivariate statistics to be composed of three distinct morphospecies, which could be distinguished morphologically (Beszteri et al., 2005a). In addition, true *Cyclotella menegheniana* morphospecies are composed of more than eight cryptic, sibling clades/species using sequence data from three genes (Beszteri et al., 2005b) that cannot be separated morphologically. One of these eight populations (Cme1) was examined more closely with fingerprinting data and found to contain two populations, maintained 40 km apart within a tidal stretch of the Weser River and were sufficiently genetically distinct to be considered sibling species (Beszteri et al., 2007), although with sequence data, all strains within this population were found to be identical. Hints of this species complex were first seen in the restriction fragment length polymorphisms (RFLP) patterns of the plastid by Bourne (1992). Plastid DNA RFLPs of this morphospecies showed a comparatively large variation and paraphyly with respect to *C. cryptica*.

Experiments were performed on two morphologically distinct entities: *P. delicatissima* and *P. pseudodelicatissima*. Amato et al. (2007) investigated the genetic identity of two species of *Pseudo-nitzschia*. Each of the species comprised multiple genetically distinct and reproductively isolated taxa, all occurring in sympatry: *P. delicatissima* was composed of three phylogenetic and reproductively distinct groups, whereas *P. pseudodelicatissima* consisted of up to five. After these taxa had been defined both genetically and biologically via sexual compatibility, subtle ultrastructural differences were detected that could support their recognition as distinct morphospecies. Their findings not only show that cryptic genetic variants abound in sympatry, but also that they are reproductively isolated and, therefore, biologically distinct units.

Vanormelingen et al. (2007, 2008) have investigated multiple clones of *Eunotia bilunaris*, from several locations in Belgium, New Zealand, and Tasmania using internal transcribed spacer region (ITS) data and breeding studies and found it to be a cryptic species. The Belgium clones were composed of three groups (11–12% genetic differences) that corresponded well with their morphology (slender, robust, labile) and could interbreed, but the F1 hybrids were generally sterile and of an intermediate morphology. In contrast, the Australasian clones were morphologically and more genetically similar (~4% difference) and could produce viable F1 hybrids despite the greater geographical distances between locations.

A more in-depth review of cryptic species can be found in the chapter by Mann in this volume, and the contribution of molecular data to the species concept in diatoms can be found in Alverson (2008).

7. Genetic Diversity Below the Species Level

Medlin et al. (2000b) reviewed the diversity in marine phytoplankton as revealed by various fingerprinting methods and isozyme studies. In nearly every case, algal strains were genetically distinct, and far more diversity and population structure was documented than previously imaged from cells that had originally been considered homogenously distributed along their entire range. Thus, phytoplankton populations can be fragmented in large bodies of water and if they can have fragmented populations, then allopatric speciation can occur. It is also easy to understand how benthic populations can have fragmented populations. Since this review, microsatellites have been developed for several diatoms (Evans et al., 2004, 2005; Rynearson and Armbrust, 2000, 2004, 2005; Rynearson et al., 2006). Rynearson and Armbrust (op. cit.) studied the genetic diversity of the diatom *Ditylum brightwellii* in the Puget Sound estuary. Four genetically distinct and highly diverse populations were identified. Population one developed blooms in early spring and occupied the entire estuarine system and also appeared in the fall bloom. In late spring, a second population replaced the blooms in a different basin inside the estuary. Two other populations were identified inside the estuary in the fall, one in the upper basin of the estuary and the other in the lower basin. Population one has been identified repeatedly in both spring and fall blooms for seven years. Populations one and two had identical 18S rDNA regions and their ITS regions differed by ~1%. Distinct physiological characteristics, i.e., salinity tolerances, were associated with each genetically distinct population. Genetically distinct populations in the upper basin of the estuary were never found in the lower basin of the estuary despite a constant flushing rate from the upper basin to the lower basin.

In the freshwater diatom, *Sellaphora capitata*, MS revealed that only a small number of alleles from water bodies in Scotland, England, Belgium, and Australia could be found in all isolates (Evans et al., 2009), indicating a limited dispersal between populations, although all isolates could still interbreed.

The planktonic cosmopolitan diatom *Pseudo-nitzschia multiseries* also contains genetically distinct and highly diverse and distinct gene pools between North American and European populations (Evans et al., 2004), whereas a morphologically similar cosmopolitan species, *Pseudo-nitzschia pungens*, is also highly diverse but with little population structure both spatially over similar geographic areas and temporally over 2 years (Evans et al., 2005). Breeding studies from global isolates of this species show that all tested global isolates can interbreed (Chepurenov et al., 2005), and thus this species is the only example of a protist so far tested with molecular and breeding techniques that shows a global gene pool. A more recent study of more global isolates of *Pseudo-nitzschia pungens* has shown it to be composed of 3 clades as determined from ITS sequencing (Sabbe

et al., 2009). Two of the clades can interbreed and represent a global gene pool, but the third one appears to be reproductively isolated. Distinct populations corresponding to major oceanic/geographical water masses/area can be recovered (Casteleyn et al., 2010).

Using classical theoretical genetics methods, Wood et al., (1987) documented that speciation could take place over a relatively short timescale under sympatric distribution.

8. Comparisons Across Genomes

Two diatom genomes have now been completely sequenced (*Thalassiosira pseudonana* (Armbrust et al., 2004) and *Phaeodactylum tricornutum* (Bowler et al. 2008). Both have revealed unusual surprises about the diatoms. The discovery of an animal-like urea cycle was completely unexpected. The need for such a cycle was not well understood, and it was generally believed that the urea could just be a nitrogen storage product. Recently, the discovery of planktonic organisms' ability to substitute nonphosphate lipids for phosphate lipids in their membranes under phosphate limitations (Van Mooy et al., 2009) has provided clues to the unknown function of the urea cycle in diatoms with its side production of ornithine in the urea cycle, which is one of the compounds used in the nonphosphate lipids. The pennate genome revealed that there had been many lateral gene transfers from bacteria into the diatoms. The genome of the Antarctic sea ice diatom, *Fragilariopsis nana* (= *cylindrus*), is near completion.

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Biodata of **Edward C. Theriot**, Author, with Coauthors **Elizabeth Ruck**, **Matt Ashworth**, **Teofil Nakov**, and **Robert K. Jansen** of "***Status of the Pursuit of the Diatom Phylogeny: Are Traditional Views and New Molecular Paradigms Really that Different***?"

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 119–142
DOI 10.1007/978-94-007-1327-7_5, © Springer Science+Business Media B.V. 2011

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STATUS OF THE PURSUIT OF THE DIATOM PHYLOGENY: ARE TRADITIONAL VIEWS AND NEW MOLECULAR PARADIGMS REALLY THAT DIFFERENT?

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1. Introduction

1.1. DIATOM STRUCTURAL GROUPS

Diatoms have long been divided into two major structural groups, centrics and pennates. Major morphological elements of the cell wall of centrics are typically arranged around a central point or have no apparent organization, whereas pennates are typically elongate with their structures organized more or less perpendicular to a longitudinal rib or bar called a sternum. Pennates themselves are often divided into two groups, the raphid pennates (with a pair of slits, typically running through the sternum) and araphid pennates (those without such slits). The terms "radial" and "(bi-)multipolar" (or some variant thereof) have recently come into common usage to describe perceived differences in centric diatom bauplans. Most diatoms identified as radial centric diatoms have a circular or nearly circular outline, and lack structures such as paired pore fields which might impose some degree of bilateral symmetry on the markings of the valve. (Bi-)multipolar centric diatoms typically are noncircular (having elongated or other shaped valves) with two or more structures (e.g., pore fields, setae) arranged toward the edge of the valve, which also impart various symmetries to the cell. We will simply call these polar diatoms.

These structurally defined groups are sometimes given formal names which differ according to author and which may or may not reflect different opinions as to rank (e.g., class, phylum, or other rank). The centrics, for example, are called Centrales and given ordinal rank by some (e.g., Simonsen, 1979) or

Coscinodiscophyceae and given class rank by others (e.g., Round et al., 1990). There are a few genera whose assignment to one structural group or another has been uncertain or debated, but most taxa seem to be placed in the same broad structural groups by nearly all authors.

Diatomists have not only constructed classifications based on these structural groups, but have attempted to arrange the groups into various phylogenetic schema. The traditional approach to reconstructing diatom phylogeny has been to employ a noncanonical mix of informal phenetics, informal homology assessment, stratigraphic analysis, and evolutionary scenarios on morphological and ecological information. That is, there has not yet been a formal attempt to conduct cladistic analysis of morphology across diatoms as a whole.

The traditional approach has produced a wide variety of views on diatom relationships. For example, Steinecke (1931) drew a phylogenetic tree of the diatoms which had the centrics and pennates each as monophyletic sister taxa with raphid pennates as a monophyletic group nested within the paraphyletic araphids. In complete contrast, Simonsen (1979) drew a phylogenetic tree which had centric diatoms as paraphyletic, araphids as monophyletic, and raphid diatoms as paraphyletic. Round and Crawford (1981, 1984) argued that three major lineages (composed of centrics, araphid pennates, and raphid pennates) were derived independently from a pool of "Ur-diatom" forms, and so were each monophyletic. In short, these traditional, noncanonically derived phylogenies have concluded that centrics were monophyletic (or not), that araphids were monophyletic (or not), and that raphid pennates were monophyletic (or not).

1.2. ATTEMPTS AT A MOLECULAR PHYLOGENY

Formal analyses of the larger diatom phylogeny did not begin until the advent of gene sequence data (Medlin et al., 1993). Alverson and Theriot (2005), Mann and Evans (2007), and Theriot et al. (2009) summarized most of the available formal molecular phylogenetic analyses done on higher level relationships of diatoms.

Results from analyses of molecular data (mainly nuclear small subunit rDNA: SSU) resemble the traditional trees in at least one way. Different studies have yielded starkly different topologies (Medlin and Kaczmarska, 2004; Medlin et al., 1993, 1996a, b, 2000, 2008; Sims et al., 2006; Sorhannus, 2004, 2007). Many results suggest that radial centrics grade into polar centrics which grade into araphids which grade into raphid pennates and this seems to be a widely accepted view (Adl et al., 2005, 2007; Williams and Kociolek, 2007). However, Medlin and Kaczmarska (2004) proposed that diatoms were composed of only three major clades: the Coscinodiscophytina, including the radial centrics; the Mediophyceae, containing the polar centrics plus the Thalassiosirales; and the Bacillariophyceae, containing the pennates (CMB hypothesis). Strong statistical support for this result using SSU data has been claimed (Sims et al., 2006), but that support seems to be an artifact (Theriot et al., 2009).

The CMB hypothesis is not universally accepted. Williams and Kociolek (2007) argued that the variation in results from study to study suggested that the Coscinodiscophytina and Mediophyceae may not be monophyletic. Going a bit further, Adl et al. (2005) mark the taxa Coscinodiscophytina and Mediophyceae as (P) which the text indicates means "probably paraphyletic."

In fact, SSU data hardly distinguish between the two hypotheses at all (Theriot et al., 2009). Analysis of a dataset including over 600 diatoms recovered Coscinodiscophytina (radial centrics) and Mediophyceae (polar centrics plus Thalassiosirales) as grades rather than clades, but the most parsimonious trees were barely shorter than trees recovering the CMB hypothesis. For example, using only *Bolidomonas* as the out-group, trees recovering monophyly for each of these two centric groups were only 14 steps longer (out of more than 14,000 steps) than the shortest trees. Clearly, small changes in taxon sampling or alignment criteria could possibly result in any combination of one group or the other, or both, being monophyletic or paraphyletic. The strong statistical support for the CMB hypothesis reported by Sims et al. (2006) and Medlin et al. (2008) appears to be an artifact of an insufficient number of MCMC generations in Bayesian analysis (Theriot et al., 2009). We conclude that SSU data are, in fact, largely indecisive with regards to the CMB hypothesis.

A difficulty with the Theriot et al. (2009) analysis and indeed with most recent SSU analyses to date is that sampling has been heavily skewed toward pennates and two orders of centric diatoms (the Thalassiosirales and Rhizosoleniales). Even within these groups, sampling is heavily skewed toward a handful of genera. In particular, radial and polar centrics have been highly under-sampled relative to known diversity. Another issue is that the amount of data is probably overwhelmed by the size of the problem. One could hardly expect that about 1,000 informative sites would be able to fully and robustly resolve more than 600 taxa used by Theriot et al. (2009), which may have evolved for nearly 200 million years. Obviously, additional data are required to resolve the diatom phylogeny.

1.3. VARIATION IN MOLECULAR STUDIES PARALLELS THAT OF TRADITIONAL APPROACHES

In fact, variation in molecular trees has been extensive enough so that one molecular study or another has even limited support for each of the different traditional hypotheses discussed above. The key elements of Steinecke's hypothesis were recovered by Van de Peer et al. (1996): centrics and pennates were each monophyletic with high bootstrap support. Ehara et al. (2000) recovered a monophyletic araphid group with high bootstrap support using the *cox*I gene; Round and Crawford (1981, 1984) and Simonsen (1979) each proposed that araphids were monophyletic. Both of these molecular studies included only a handful of diatom exemplars (11 and 9, respectively) which likely biased results. However, even studies with broader taxon sampling have recovered a somewhat radical element of

Simonsen's (1979) hypothesis, nonmonophyly of the raphid pennates. Sato et al. (2008), for example, recovered an SSU tree in which *Eunotia* was grouped with two of the araphid genera (*Striatella* and *Pseudostriatella*), making the raphid pennates paraphyletic. They called that result "implausible" on the basis of morphological data.

Whereas the finding of paraphyly for raphid pennates was dismissed in this case, in part because it ran counter to morphological data (viewed informally), diatomists seem to accept, without question, other results that run counter to traditional ideas and/or morphological data. For example, the Thalassiosirales have the circular valve outline typical of radial centrics. They lack any sort of paired pore fields, setae, or other structures that suggest polarity (characteristics of polar diatoms). They have developmental characteristics (globular isometric auxospore shape/growth and lack a properizonium) of radial centrics, and lack the developmental characteristics of polar centrics (which have nonisometric auxospores and a properizonium). Mann and Evans (2007) were certain enough that the Thalassiosirales were polar centrics that they listed this result as one of the "successes" of recent efforts and concluded it is one of the things we now know with some degree of certainty.

It is unclear why diatomists allow morphological data to overrule molecular results in one case and not the other. In both cases, the morphological characters are complex. In both cases, the position of the diatom in question has been unstable in molecular analyses. The position of the Thalassiosirales within the polar centrics seemingly depends on taxon sampling, for example. Using the same analytical technique (Bayesian analysis) and a similar dataset (SSU, although alignments were probably different), three distinct results have been obtained. The Lithodesmiales have been recovered as the sister group to the Thalassiosirales in an analysis of 123 diatoms (Medlin and Kaczmarska, 2004); the Hemiaulales and the Lithodesmiales formed a clade with Thalassiosirales as its sister group in an analysis of 181 diatom sequences (Alverson et al., 2007); the Lithodesmiales grouped with the Biddulphiales, Triceratiales, and *Toxarium* to the exclusion of the Thalassiosirales with a dataset of unspecified size (Sims et al., 2006). Furthermore in that last analysis, Hemiaulales were separated from both the Thalassiosirales and the Lithodesmiales by two or more nodes. In all these studies, all relevant nodes but one had Bayesian support values of at least 0.88, with most having support values of 1.0 (the maximum value). In short, the position of the Thalassiosirales has been highly unstable within the polar diatoms. Given that instability, and given the complex morphological evidence that Thalassiosirales are radial centrics, not polar, the question is begged: "Just how plausible is it that Thalassiosirales are polar centrics?"

We do not mean to say that progress has not been made. Comparing studies with about a dozen diatoms (e.g., Van de Peer et al., 1996) to those with more than 600 (Theriot et al., 2009) is bound to result in different trees. Moreover, increasing sampling within the group of interest likely increases phylogenetic accuracy (Verbruggen and Theriot, 2008; Zwickl and Hillis, 2002). Different optimality criteria and different alignments may also lead to different results. Our goal is simply

to demonstrate that the results of molecular systematics have, like the traditional approach, resulted in a range of hypotheses through time, and that these hypotheses are, overall, strikingly similar to ideas argued from more eclectic and informal points of view. In short, it is not enough to simply say that morphology and molecules do not agree. They do. And they do not.

2. Objectives and Methods

Here we attempt to understand phylogenetic incongruence between molecular data and traditional hypotheses using a three-gene dataset (SSU, *rbc*L and *psb*C) developed by Theriot et al. (2010). That dataset was specifically designed to acknowledge issues with taxon sampling and amount of sequence data discussed above. It greatly increased taxon sampling in both the radial and polar centrics over the SSU dataset and more than doubled the number of potential characters. We constrained topologies to approximations of the hypotheses of Steinecke (1931), Simonsen (1979), and Round and Crawford (1981, 1984). Specifically, we tested the probability conferred upon the molecular data by trees in which centrics, radial centrics, polar centrics, araphid pennates, raphid pennates, alone or in some combination, were monophyletic. Also we tested various positions in the tree for the Thalassiosirales, as sister to Melosirales as implied by Simonsen (1979) or anywhere in the radial grade (but outside the remaining polar diatoms) as implied by a cladistic analysis of morphological characters (Theriot et al., 2009). We also tested recent molecular results indicating nonmonophyly of raphid diatoms (e.g., Sato et al., 2008).

Maximum likelihood analyses calculate the likelihood imparted to the data by a given tree. We can examine the likelihood imparted by various hypotheses to the three-gene dataset of Theriot et al. (2010), by constraining searches for trees to various topologies and comparing those to the best tree obtained in an unconstrained analysis.

Maximum likelihood trees were calculated using RAxML 7.04. The dataset had seven partitions (SSU, and first, second and third codons of each of the *rbc*L and *psb*C genes). In order to demonstrate the sensitivity of the tree to analysis parameters, we ran the data under the GTR+G+I model (GTRGAMMAI) as per that selected by ModelTest (Posada and Crandall, 1998; Posada and Buckley, 2004), and reran the analysis under GTR+G (GTRGAMMA) as per the suggestions of the RAxML manual (Stamatakis, 2006). The only difference between the two is that GTR+G+I specifies a percentage of sites as invariant (i.e., the gamma distribution of site variability is somewhat curtailed), whereas GTR+G allows for more subtle differences in variability across low variation sites. Other details of analysis, including running of ModelTest, are in Theriot et al. (2010).

We cannot reproduce exactly the hypotheses of Steinecke, Round and Crawford, and Simonsen for various reasons (inability to get sequence data from all living taxa, those studies all reference extinct forms [yet another issue – see Discussion], lack of specificity about taxa used in the traditional hypotheses), but we have

Table 1. Monophyly (m) or nonmonophyly (x) of major diatom structural groups as viewed by various auth ors.

Author	Centrics	Radial centrics	Polar centrics	Pennates	Araphid pennates	Raphid pennates
Steinecke, 1931	m	x	m	m	x	m
Round and Crawford, 1981, 1984	m	?	?	m	m	m
Simonsen, 1979	x	x	x	m	m	x
Sims et al., 2006	x	m*	m*	m*	x	m*
Ehara et al., 2001	x	m*	m*	m*	m*	m*
Van de Peer et al., 1996	m*	x	1	m*	x	m*
Sato et al., 2008	–	–	–	m*	x	x

An asterisk indicates that monophyly received statistical support (Bayesian posterior probability or bootstrap value above 70%). A dash (–) indicates that analysis did not include enough of these taxa except as out-groups

approximated them to various degrees in our constraint trees. We constrained ML analyses to the hypotheses presented in Table 1 in which groups are indicated in standard parenthetical form.

Analyses attempt to find the tree under a particular topological constraint that imparts the highest likelihood to the data. The likelihood score associated with this constraint is compared to that of the highest scoring tree calculated without any restraints. Differences between the two are tested using the SH test, which tests whether the likelihood conferred by the tree topology on the dataset is significantly worse or not. Many different tests are available. Generally, they give similar results. Overall, considering susceptibility to bias, type-1 and type-2 error rate, the two best tests appear to be the approximately unbiased (AU) test and the Shimodaira-Hasegawa (SH) test. We selected the SH test both for practical (it is available in RAxML) and theoretical reasons. The SH test is a good option when the number of candidate trees is not very large (Shimodaira, 2002); here we only make pairwise comparisons. Control of type-1 error is conservative, such that one will not miss the true tree in the confidence set, which is often larger than that for the AU test. The main failing of the SH test is that it is susceptible to increased error when the number of candidate trees is large, but this concern is ameliorated by the fact that we are analyzing a small population of trees in total, and each test was conducted as a pairwise test of the optimal tree against a select alternative hypothesis.

3. Results and Discussion

3.1. TEST RESULTS

The best tree obtained by Theriot et al. (2010), using the GTR+G+I model, had radial centrics grading into polar centrics (including the Thalassiosirales), polar centrics grading into araphids, and araphids grading into raphid pennates (not shown). *Striatella* was the sister group to raphid pennates. The best tree using the GTR+G model was broadly similar. It was virtually identical for centrics in that radial centrics graded into polar centrics and there were at least moderate bootstrap values for internodes among the radials, but no support for internodes separating branches on the polar grade (Fig. 1). The major difference between the GTR+G+I tree and the GTR+G tree is that the latter recovered *Striatella* as the sister group to *Eunotia*, making the raphid pennates nonmonophyletic (Fig. 2). This tree is the unconstrained optimal tree (UOT).

Among the various topologies tested under the GTR+G model, the best tree with raphid pennates constrained to monophyly was not significantly worse than the UOT (Table 2). The next best tree by likelihood score was that in which the araphid diatoms were constrained to monophyly. It was not significantly worse than the UOT. The next two best trees had nearly identical likelihood scores and neither was significantly worse than the UOT. In one test, the Thalassiosirales

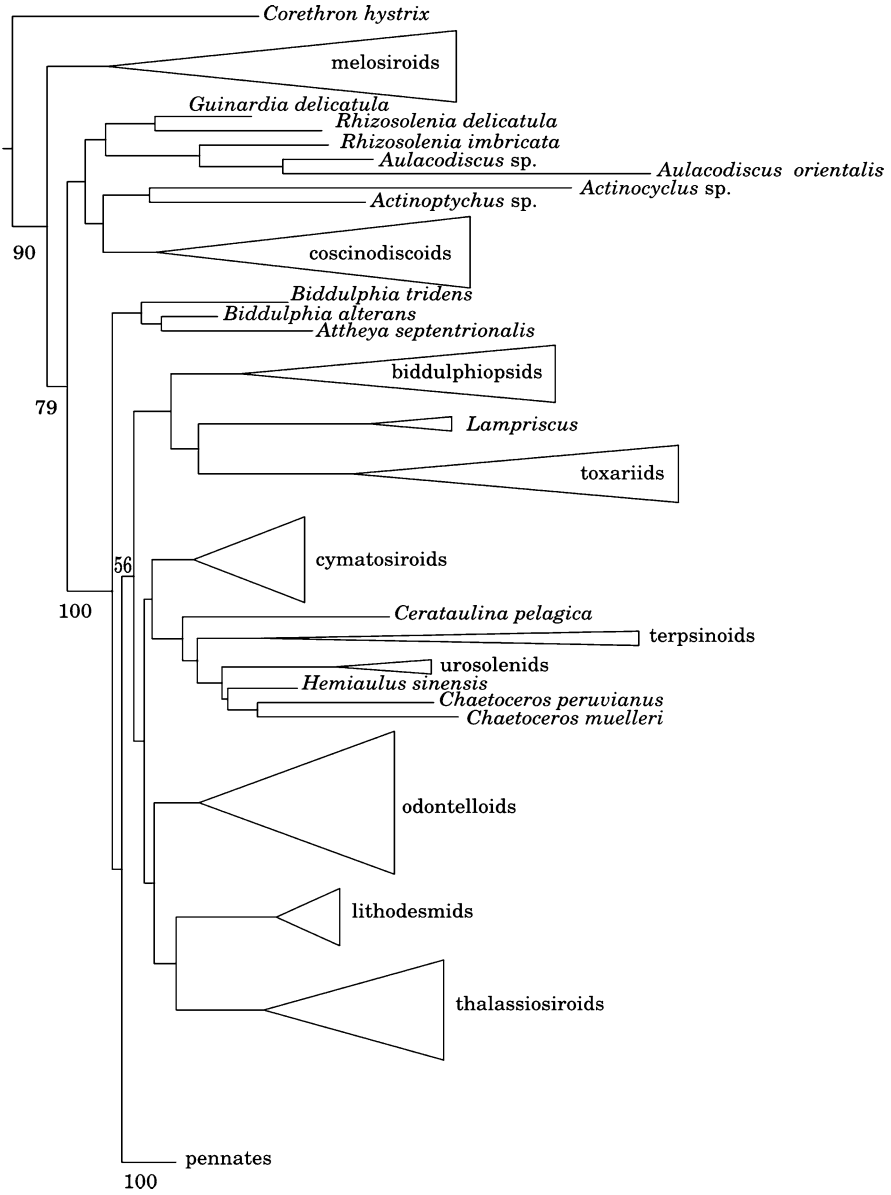

Figure 1. Maximum likelihood tree calculated under the GTR+G model from the three-gene dataset of Theriot et al. (2010). Centrics are shown here. Pennates are continued in Fig. 2. Bootstrap values are only presented for the backbone of relationships among major structural groups.

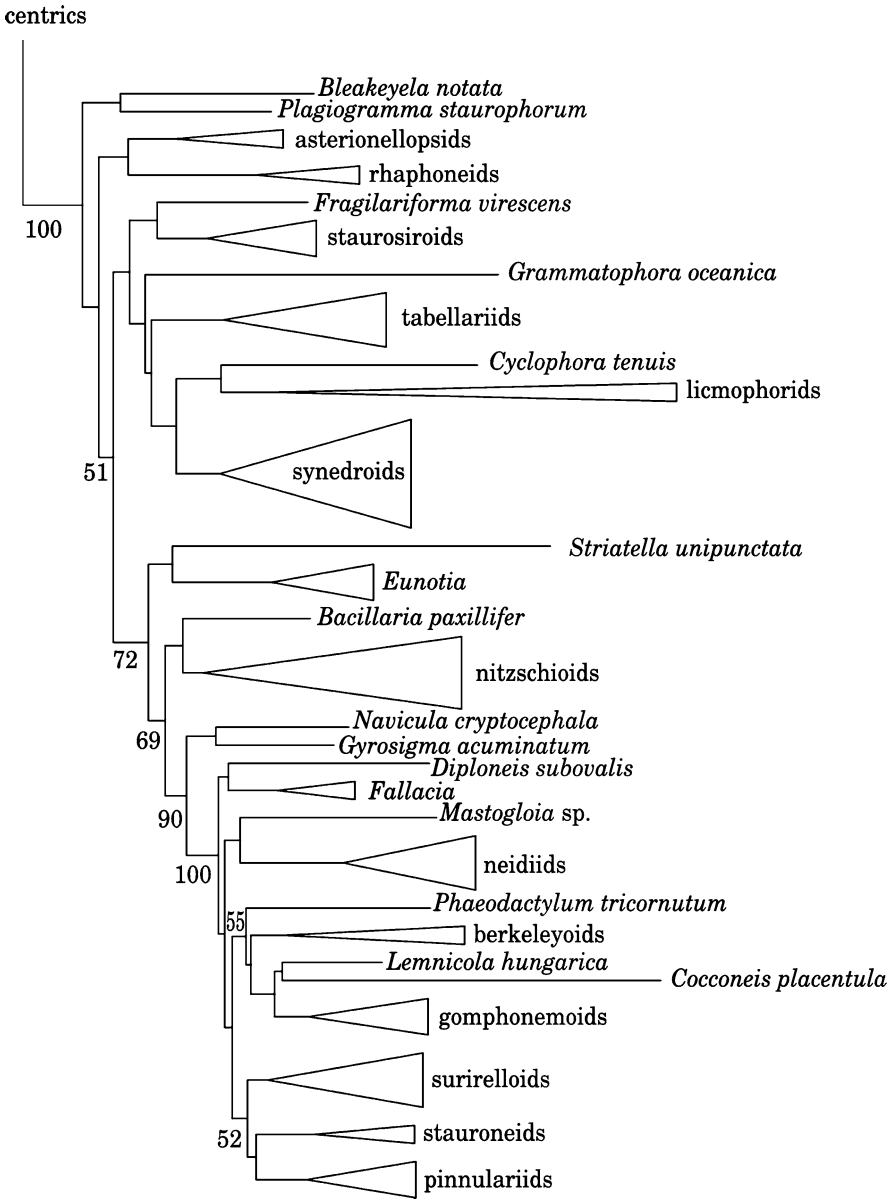

Figure 2. Maximum likelihood tree calculated under the GTR+G model from the three-gene dataset of Theriot et al. (2010). Pennates are continued from Fig. 1. Bootstrap values are only presented for the backbone of relationships among major structural groups.

Table 2. SH test results.

Constraints arranged by score from best constraint result to worst. ML score best unconstrained tree: −105461.16

Constraints	Likelihood	Difference	S.D.	Significant
(raphids)	−105465.02	3.85	24.13	N
(araphids)	−105484.46	23.30	19.33	N
(radials(thals(polars(pennates))))	−105496.63	35.46	19.22	N
((radials)((polars)(pennates)))	−105497.11	35.95	33.09	N
(radials(thals)((polars)(pennates)))	−105498.44	37.28	18.10	Y
(araphids *Eunotia*)	−105514.16	53.00	21.40	Y
((araphids)(*Eunotia*))	−105518.59	57.42	22.99	Y
((centrics)(pennates))	−105542.53	81.37	30.81	Y
((centrics)(araphids)(raphids))	−105558.05	96.89	34.34	Y
((thals)(melos))	−105575.11	113.95	27.89	Y
((thals)(melos)); ((*Eunotia*)(araphids))	−105633.14	171.98	35.66	Y

Standard parenthetical notation is used to indicate groupings. Paired parentheses indicate monophyly. A single open parenthesis indicates paraphyly. (((A)(B))C) indicates A, B, A + B, and A + B + C are all monophyletic, but C is paraphyletic. In contrast, (A(B(C))) indicates that A + B + C is monophyletic, B + C is monophyletic and C is monophyletic, but A and B are each paraphyletic
thals thalassiosiroid diatoms, *melos* melosiroid diatoms

were constrained, as part of the radial grade, outside of the polar diatoms. The other constraint kept radial centrics, polar centrics (plus Thalassiosirales), and pennates each as monophyletic (the CMB hypothesis). All other constrained topologies were significantly worse than the best tree. These included trees in which all centrics were constrained to monophyly, trees in which Thalassiosirales were constrained as sister to the Melosirales, and trees in which araphids plus *Eunotia* were constrained to monophyly.

Results were similar for the GTR+G+I model. Monophyly of raphid pennates was the optimal solution in this case, so we tested the reciprocal result (*Striatella* sister to *Eunotia*) which did not impart a significantly worse likelihood to the dataset. Thalassiosirales, as part of the radial grade, could not be rejected, nor could araphid monophyly or the CMB hypothesis. Again, all other tree topologies tested imparted significantly worse likelihoods to the molecular dataset.

3.2. TAXONOMIC CONSIDERATIONS

3.2.1. Centric Monophyly Appears Unlikely

Steinecke (1931) and the Ur-diatom hypothesis (Round and Crawford, 1981, 1984) each presented the centrics as monophyletic, and both hypotheses implied significantly worse likelihood scores for the molecular data (Table 2). Thus, both

of these hypotheses seem highly unlikely given the molecular data and method of tree reconstruction.

3.2.2. Araphid Monophyly Is Not Entirely Implausible

Of the topologies tested, the worst correspondence between a traditional hypothesis and molecular data (Table 2) was found for the constraint tree which we constructed to most closely represent Simonsen (1979). A monophyletic araphid group with *Eunotia* as the sister group (the Simonsen hypothesis), or a paraphyletic araphid group with *Eunotia* placed within the araphids (a "relaxed" Simonsen hypothesis) both produced significantly worse scores for the molecular data. However, the somewhat radical notion that araphids are monophyletic did not confer significantly worse scores upon the molecular data when no other constraint was placed upon their relationships to each or other diatoms. Thus, that component of both Simonsen (1979) and the Ur-diatom hypothesis that claims monophyly for araphids could not be rejected by our analysis.

3.2.3. Molecular Data Do Not Significantly Reject the Morphological Evidence That the Thalassiosirales Are Radial Centrics

Likewise, holding strictly to Simonsen's idea of thalassiosiroid relationships (here, the Melosirales plus Thalassiosirales as monophyletic) produced constraint trees which conferred significantly worse likelihood scores upon the molecular data. This, however, does not necessarily mean that molecular data reject any hypothesis in which Thalassiosirales are not embedded within polar centrics. Simply excluding the Thalassiosirales from polar diatoms and pennate diatoms, for example, was not significantly worse than the optimal tree. That is, molecular data do not reject the idea that Thalassiosirales are part of the radial centric grade. The resulting tree is congruent with the morphological cladistic analysis of Theriot et al. (2009).

3.2.4. Radial Centrics and Polar Centrics Are Grade Taxa, but the Alternative Cannot Be Rejected

Note that the CMB hypothesis confers nearly the same likelihood upon the molecular data as does the "Thalassiosirales are part of the radial centric grade" hypothesis (Table 2). That is, it is no more or less plausible that there are three clades of diatoms (radial centrics, polar centrics plus Thalassiosirales, pennates with the latter two forming a larger monophyletic group) than it is that radial centrics grade into polar centric which then grade into pennates, with Thalassiosirales in the radial grade. While there is high bootstrap support along the backbone of the radial centrics, there remains weak (bootstrap values less than 50%) support along the polar centric grade even when using three genes (Theriot et al. 2010). In short, the evidence continues to point weakly at the grade hypothesis, but the reality is that it would not take much new evidence to produce a three-clade tree consisting of a radial centric clade, a polar centric clade, and a pennate clade. Equally likely is a tree in which the Thalassiosirales are part of the radial centric grade.

In such cases, it is not hard to imagine that a few morphological characters might overturn a molecular hypothesis if the phylogenetic signal of the morphological characters was strong.

4. An Argument for the Total Evidence Approach in Diatom Systematics

It would be surprising if there were not both points of conflict and points of agreement between traditional hypotheses and molecular data. Given experiences with other taxonomic groups, it is also not surprising that no single dataset is sufficient to obtain a robust phylogeny of a group that is approximately 200 million years old and consists of hundreds of thousands if not millions of species. Because of the noncanonical nature of traditional hypotheses, it is difficult to determine whether the source of the conflict between two traditional hypotheses or between any one traditional hypothesis and a molecular hypothesis is a result of different approaches to phylogeny reconstruction, the data or taxa included, or some other factor. It is also challenging, without formal tests such as those conducted here, to determine if the conflict is strong or weak. However, it is inevitable that some degree of conflict will continue to be uncovered between data (even between various genetic markers) as diatomists add new genes and possibly morphology to formal analysis. Thus, it is important to understand how to measure conflict, what conflict means, and how to resolve conflict.

Our efforts here are limited to measuring conflict and only then measuring one part of conflict. We cannot conduct reciprocal tests. The finding that a traditional tree does not confer worse probability upon a molecular dataset does not, in and of itself, mean that the molecular results can be safely rejected in favor of another hypothesis. It is entirely possible that the optimal molecular tree might confer significantly worse probability upon a morphological dataset. Regardless, it is also possible that the best estimate of phylogeny will be based on combined data and not on either dataset alone.

4.1. SIGNAL IN MOLECULAR AND MORPHOLOGICAL DATA

A practical concern about combining molecular and morphological data is the potential for molecular data to somehow swamp morphological data because of the sheer number of molecular characters. More molecular characters do not necessarily reflect greater phylogenetic signal, however. Lee et al. (2007) presented an example in which a snake morphological dataset of 260 parsimony informative sites (263 total) had nearly the same phylogenetic signal as a concatenated molecular dataset of 1,261 parsimony informative sites (4,161 total). Incidentally, that paper provides an up-to-date approach to assessing relative strength and congruence between datasets. Edgar and Theriot (2004) demonstrated that morphology and molecular data did not conflict so much as each spoke to complementary

areas of the tree (of the diatom *Aulacoseira*), especially when quantitative data were included. Again, relative dataset size was not a gauge of the final result.

A little studied and poorly understood phenomenon called "hidden signal" is relevant to this issue of dataset size and apparent conflict among datasets. Goertzen and Theriot (2003) provided an example from the heterokont algae, the larger group to which diatoms belong. SSU supported one grouping with high bootstrap support. The *rbc*L gene supported another at a bootstrap support of less than 50%. Counter intuitively, when the data were combined, the *rbc*L solution was recovered, not the SSU solution. Moreover, the *rbc*L solution was recovered with high bootstrap support with the addition of SSU data even though the apparently stronger SSU signal ostensibly conflicted with the apparently weaker *rbc*L dataset! This suggested that there was a "hidden signal" in the SSU data which was congruent with the *rbc*L solution. This phenomenon may have been first identified by Olmstead and Sweere (1994) in an analysis of a three-gene dataset for the Solanaceae, and concluded that the best hypothesis was that obtained by a total evidence approach even though there was conflict among the individual datasets. Gatesy et al. (1999) performed the first thorough analysis of hidden signal analyzing a large multigene dataset. However, it has since been shown to be relevant to combined morphological and molecular data as well as combinations of genetic markers alone (Gatesy et al., 2003; Gatesy and Arctander, 2000; Gatesy and Baker, 2005; Gatesy and O'Leary, 2001; Lee et al., 2007; Wahlberg et al., 2005). Every dataset contains some degree of noise. If that noise is uncorrelated among datasets, then one expects that signal across datasets will be added in a total evidence approach, whereas noise is not (Lee et al., 2007). In fact, even when separate morphological and molecular data yield conflicting results, and when the molecular topology results from a total evidence analysis, it can still be the case that the morphological data provide the strongest support for disputed nodes.

4.2. DECIDING WHICH OUTWEIGHS WHICH IS ARBITRARY WHEN DATA ARE SEPARATE

Diatomists actually do allow morphology to speak to phylogenetic inference, often citing morphological evidence as support for molecularly derived clades. In fact, diatomists have even occasionally argued that morphological data outweigh molecular data. However, when doing so, the process seems as noncanonical as that engaged in traditional hypotheses.

Medlin and Kaczmarska (2004, p. 252) wrote: "*Paralia* roots the entire diatom lineage in all analyses, but has strong morphological ties with other centric diatoms in clade 1, so we have placed it [in] clade 1 than in its own clade." Nevertheless, other parts of that paper argued that other morphological similarities for taxa disjunct in the molecular tree are convergent or parallelisms. In the introduction, for example, they argued that a molecular study showed the complex

fibulae of *Nitzschia* and *Surirella* have evolved at least twice based on SSU analysis (Medlin et al., 2000).

Sato et al. (2008) provided another example, one in which raphid pennates were found to be paraphyletic. The araphids *Striatella* and *Pseudostriatella* grouped with the raphid genus *Eunotia* with high Bayesian support. We select this example not for criticism but for pedagogy because it is one in which many diatomists would likely find a major point of agreement: that raphid pennates are monophyletic despite the molecular results. Sato et al. (2008, p. 386) themselves wrote that "Some features of our tree, such as the sister relationship between the *P. oceanica–S. unipunctata* clade and the raphid genus *Eunotia*, have high support but are frankly implausible because of morphological and reproductive evidence." They supported this claim with observations that auxosporulation pattern was different between *Striatella* and *Eunotia* (that of *Striatella* being more like other diatoms with araphid structure), and that there was no evidence of a raphe in *Striatella*. They further argued that the placement of *Striatella* is inconsistent among molecular studies. They effectively concluded that morphological data should be the arbiter of monophyly of the raphid pennates even in the face of strong statistical evidence against this hypothesis from their own analysis. Again, we do not disagree. We only point out that the conclusion was reached without any sort of formal analysis of either alternate hypothesis.

Sato et al. (2008) presented a counterargument in the same paper. They concluded that *Toxarium* and its allies are polar diatoms in spite of similarities of shape with other highly elongate pennates. In fact, the position of *Toxarium* in the diatom tree varies, and it has even been recovered as sister to the pennate diatoms (Chepurnov et al., 2008), which would be consistent with the highly elongate shape as a synapomorphy of pennates. In both cases, the diatom in question (*Toxarium* or *Striatella*) occurs in various places of the diatom tree, with greater or lesser support, and in both cases, the molecular placement appears to be incongruent with some morphological character or characters. Yet, in one example, morphology outweighs a molecular result but does not in the other.

We do not disagree with the particulars of the conclusions reached by Medlin and Kaczmarska (2004) or by Sato et al. (2008). We only use these to demonstrate that, so far, diatomists continue to treat morphological data in noncanonical ways. It is unclear how morphology can be used to reject molecular data in one instance, and then molecular data are used to reject morphological data in another.

The placement of the Thalassiosirales among the polar centrics is considered certain by some diatomists, so much so that Mann and Evans (2007) argued that determining this has been one of the successes of molecular phylogenetics and that it is one of the things we now "know." Indeed, molecular analyses have consistently placed the Thalassiosirales among the polar diatoms, in spite of morphological evidence to the contrary. In this instance, there has even been a formal morphological analysis in support of excluding Thalassiosirales, albeit the analysis was limited in taxonomic and character scope (Theriot et al., 2009). Yet, the

topology implied by morphology does not confer a significantly worse likelihood on the molecular data. It remains to be seen whether a combined approach will reach a different conclusion than that based on molecular data alone.

4.3. DIATOMS ARE EMINENTLY SUITABLE TO FORMAL MORPHOLOGICAL ANALYSIS

The diatom shell alone does not lack for characters suitable to phylogenetic analysis. Theriot and Seriyessol (1994) identified more than 80 characters that could be used in the Thalassiosirales. Many more are available from cellular structure and life history data (Mann and Evans, 2007). There is a wealth of quantitative data which can be transformed into character states (Edgar and Theriot, 2004; Theriot, 1992). So, why are there not more studies utilizing morphological data? There are, to be sure, practical difficulties in obtaining many morphological characters. Characteristics of chloroplast shape require living diatoms. Developmental features may require induction of sexual reproduction, thin sectioning, and observation by transmission electron microscopy. Few labs have been willing to make such a commitment. Consequently, these data are sparsely available.

In spite of such difficulties, formal phylogenetic study of a suite of morphological characters has been conducted at lower taxonomic levels, particularly in certain araphid groups (Williams, 1985, 1990), in gomphonemoid and cymbelloid pennates (Kociolek and Stoermer, 1988, 1989, 1993), in the surirelloid diatoms (Ruck and Kociolek, 2004), in the Chaetocerotales (Rines and Theriot, 2003), in tangentially undulate *Thalassiosira* species (Julius and Tanimura, 2001), and in the *Stephanodiscus niagarae* complex (Theriot, 1992). Cox and Williams (2000) applied cladistic analysis to biraphid pennates and also reviewed the cladistic studies on raphid pennates prior to 2000. In other cases, explicit cladistic principles were applied to one or a handful of characters in discussing evidence for or against certain groups, including *Cyclotella*, *Cyclostephanos*, *Mesodictyon*, and *Stephanodiscus* (Tapia et al., 2004; Theriot, 1990; Theriot and Bradbury, 1987; Theriot et al., 1987).

There is only one diatom study that attempted to formally combine morphological and molecular data in a "total evidence" approach (Kluge, 1989) using the same exemplars (i.e., the same specimens to gather both morphological and molecular data). Edgar and Theriot (2003) combined quantitative and qualitative morphological data with molecular data (in the genus *Aulacoseira*). Jones et al. (2005) conducted separate morphological and molecular analyses of *Petroneis* and related pennates, but did not analyze a combined dataset.

Both studies highlight an important fact. The use of morphological data always has the potential to be more inclusive of diatom diversity than molecular data. Those studies used molecular techniques which required the use of cultured material. We can now extract and amplify DNA from single cells using a variety of methods (Medlin et al., 2008). The chelex method, in particular, shows promise

of making single-cell DNA extractions routine in diatom systematics (Ruck and Theriot, in prep.) However, even then many rare diatoms will be difficult to find and include in molecular analyses. It is unlikely that the great many extinct diatoms will ever be amenable to molecular analysis. Inclusion of morphology will be the only way to formally place these extinct forms into the diatom phylogeny.

4.4. THE IMPORTANCE OF RECIPROCAL TESTS

That a morphology-based tree, whether canonically derived or not, confers a statistically improbable likelihood to molecular data does not mean that the morphology tree is "worse" or "wrong," or simply less probable. A reciprocal test might find each dataset to be improbable against the tree of the other, or just one, or neither. The consequences for interpretation are important. If, for example, the best morphological tree does recover raphid pennate monophyly, but also finds raphid pennate paraphyly to not be significantly worse, then NEITHER dataset strongly support monophyly. If there is disagreement between the two datasets, then this means that the conflict is possibly just a result of noise in both datasets.

5. Conclusions

Our attempt at understanding conflict between traditional hypotheses and molecular results is but a first, small step, toward understanding the relationship between morphology and molecular phylogenies in diatoms. Our molecular dataset is limited, and the traditional hypotheses are difficult to interpret for reasons discussed above. Ergo, we could not perform the necessary reciprocal tests of the relative strengths of the traditional hypotheses. While our analyses were thus necessarily incomplete, they still revealed some surprising results. Araphid monophyly, a result shared by Simonsen (1979) and the Ur-diatom hypothesis (Round and Crawford, 1981, 1984), and supported by a taxonomically limited *cox*I dataset (Ehara et al., 2000) could not be rejected. The Thalassiosirales, a radial centric by traditional and limited cladistic analysis of morphology, is placed among the polar centrics by our three-gene dataset, but that position is not significantly better than solutions in which the Thalassiosirales are constrained to the radial centric grade. Our molecular data are indecisive as to whether or not raphid pennates are monophyletic as evidenced by the fact that small variation in the model used produced different topologies, which were not significantly different. Our three-gene dataset also could not determine if the three major structural groups of the CMB hypothesis (radial centrics, polar centrics, and pennates) are monophyletic or paraphyletic.

Our review of the literature illustrates that systematists working on other taxonomic groups have had success combining morphological and molecular data. In particular, that literature suggests that conflict between any two datasets

(different genetic markers, genetic markers and morphological data, even different kinds of morphological data) is sometimes an artifact of the partitioning of the data. Combined analysis emphasizes the areas of congruence, particularly when there is hidden signal in one or more of the partitions. There is obviously still much work to be done to understand diatom phylogenetic relationships. Nevertheless, the ubiquity of diatoms, their complex morphologies, an extensive fossil record, and the potential to unlock the diatom genome, even from rare and unculturable forms, hold great promise for understanding the diatom phylogeny as well as the relative roles of morphology and molecular data in recovering any phylogenetic hypothesis.

6. Acknowledgments

This paper was supported by NSF EF 0629410 awarded to Theriot and Jansen and by the Jane and Roland Blumberg Centennial Professorship in Molecular Evolution.

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PART 2:
DIATOM BIOLOGY

Mann
Chepurnov
Chaerle
Roef
Van Meirhaeghe
Vanhoutte
Tiffany
Tomaru
Nagasaki
Congestri
Albertano
Gordon
Tiffany
Townley

Biodata of **David G. Mann**, author of "***Size and Sex***."

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 145–166
DOI 10.1007/978-94-007-1327-7_6, © Springer Science+Business Media B.V. 2011

SIZE AND SEX

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1. Introduction

Diatoms are curious in many respects, such as their huge species diversity, their complex silica cell walls, and the method of locomotion in the most species-rich group, which involves channeled secretion along special slits in the cell wall (the raphe system). Of all their features, however, the strangest is surely the life cycle, in which size and sex play special roles and which is remarkably long for a unicellular organism.

The key characteristic of the life cycle in most diatoms is an alternation between (1) a vegetative phase lasting months or years, in which the cells divide mitotically and mean cell size decreases and (2) a rejuvenation phase lasting a few days, usually preceded by sexual reproduction, in which cell size is restored to a maximum through formation of a special cell, the *auxospore* (Fig. 1, redrawn from Mann in Round et al., 1990).

Reduction in cell size during the vegetative phase occurs because of the way diatom cells divide: Cytokinesis and the development of new valves both occur while the dividing cell is still wholly surrounded by the intact wall of the parent cell (Fig. 1, center inset), which is highly rigid in many cases because it is strongly impregnated with silica. Consequently, after each mitotic division, one of the sibling cells produced is the same size as the parent, while the other is slightly smaller. Cell shape also changes during size reduction, except in diatoms with circular valves, because the girdle region (which is where new valves are usually produced) does not flex equally around its circumference. In general, shape becomes simpler during size reduction and, in elongate diatoms, length decreases much more than width. Although these allometric changes have been known for a long time (Geitler, 1932), they still cause problems for taxonomy because auxosporulation and the largest cells of the life cycle are rarely observed, for reasons discussed later.

Diatoms are diplonts, with meiosis occurring during gametogenesis. Meiosis itself exhibits no unusual features, except that two or three of the haploid nuclei degenerate during oogenesis of centric diatoms and during development of the isogametes of pennate diatoms. There has been rapid progress during the last 20 years in our understanding of diatom sex. Especially noteworthy has been the discovery that many pennate diatoms are heterothallic (reviewed Chepurnov et al., 2004, with further reports by Poulíčková et al., 2007; Mann and Chepurnov, 2005;

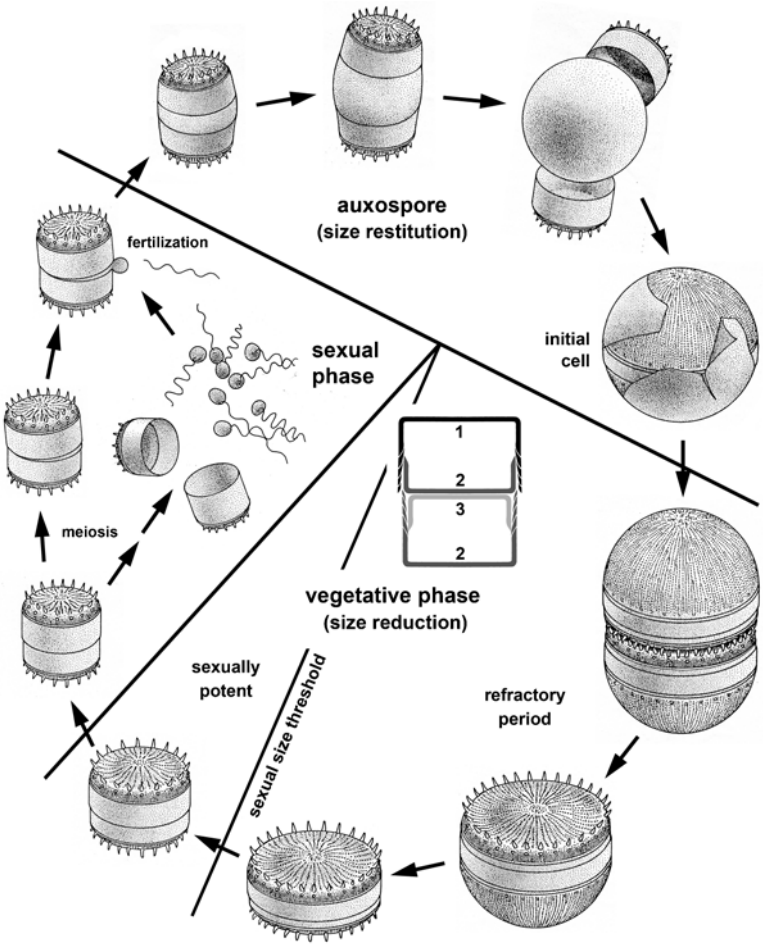


Figure 1. The life cycle of a sexual centric diatom, comprising vegetative, sexual, and auxospore phases. Size reduction occurs during the vegetative phase because of internalized cell division. The inset diagram shows a dividing cell in section, with the two new valves contained within the old cell wall. Three sizes of valve are present, labeled 1–3, largest to smallest: one of the sibling cells produced (*top*) is the same size as the parent cell; the other sibling is smaller. During the first, "refractory" period of the vegetative phase, cells cannot be induced to become sexual whatever the circumstances, but after they pass the sexual size threshold, they are potentially sexual and become sexualized when environmental and other conditions are suitable. Meiosis occurs during the formation of the gametes, which in centric diatoms are differentiated into small male sperm (a series of special mitoses precede meiosis) and large egg cells. The auxospore is derived from the zygote, expands to a maximum size characteristic of the population or species, and then forms an "initial cell."

Amato et al., 2007; Davidovich et al., 2009; Trobajo et al., 2009; Mann and Poulíčková, 2010, and others). Centric diatoms, on the other hand, with few exceptions, are homothallic: they are sequential hermaphrodites, in which a female phase, in which egg cells are produced, generally precedes (but overlaps with) a male phase

producing sperm (Chepurnov et al., 2004). The visible events of meiosis have been described in detail by, for example, Geitler (1927) and Mann and Stickle (1989), and many aspects of sexual reproduction and auxosporulation have been reviewed by Round et al. (1990), Edlund and Stoermer (1997), and Chepurnov et al. (2004). I will not attempt to cover all the same ground as these reviews here. Instead, I will focus on the interrelationship between sexual auxosporulation and size variation in natural populations, and on the special processes and structures found during the sexual phase, up to the point where fusion occurs between the gametes (plasmogamy). "Sexual reproduction" is taken to refer to the process by which two gametes produced by *different* cells (gametangia) are brought together and fuse to produce a zygote and so restore the diploid condition; such diatoms are referred to as "allogamous." Some diatoms are not fully sexual in this sense because the gametes are produced by the same cell. These are "automictic," with fusion either between two nuclei within an undivided cell (autogamy) or between two cells derived from the same gametangium (paedogamy: Geitler, 1973; Chepurnov et al., 2004). Automictic diatoms retain meiosis and recombination is possible, but of course, if automixis is obligate, these organisms are totally inbred.

2. Diatom Life Cycle as a Process of Development

In sexual diatoms, a clone is born as an auxospore, formed through the fusion of two gametes; it dies when all its remaining cells are consumed by conversion into gamete-producing cells or reach the minimum viable size. During its life, a clone changes in many respects. Size and shape changes have already been mentioned and there are also alterations in the surface area: volume ratio, which probably have profound effects on nutrient uptake and other processes mediated by the cell periphery. Locomotion may be affected in raphid diatoms, because of the changing relationship between the length of the raphe and the area of the cell experiencing significant drag close to the substratum (Edgar, 1982). The physiology of diatom clones must therefore change significantly as they grow older (and become smaller celled), and it can be expected that selection will act to improve the fit between life cycle dynamics and variation in environmental conditions. So far, there have been few studies of such interrelationships, but Potapova and Snoeijs (1997) showed in *Diatoma moniliformis* that cells had higher surface area to volume ratios during the period of optimal growth in late spring. On the other hand, in some diatoms, intrinsic growth rates remain constant despite major reduction in cell dimensions (Paasche, 1973).

The best-studied change that occurs during size reduction is in sexual competence. There is a refractory period at the beginning of the life cycle, when sexual reproduction is impossible: the largest cells of a clone are incapable of sex and auxosporulation, regardless of environmental conditions, and it is only when cells have declined in size below a certain critical threshold that they can be sexualized (Fig. 1). The threshold is size, not age, related, as can be shown by artificially altering cell size through microsurgery or nutritional manipulations (von Stosch, 1965;

Chepurnov et al., 2004). It appears, however, that the sexual size threshold is not like an on–off switch: Observations of natural populations suggest that cells become more easily sexualized as they decline further below the threshold (e.g., Mann et al., 1999; Mann and Chepurnov, 2005); an alternative explanation for these observations is that populations are genetically heterogeneous with respect to the sexual size threshold.

Reaching the "sexual size threshold" is a necessary, but not sufficient, condition for sexual reproduction to take place: Cells below the threshold are sexually potent but become sexualized only when external factors, for example, population densities and environmental conditions, permit; indeed, cells smaller than the threshold size frequently dominate natural populations (e.g., Mann et al., 1999). Chepurnov et al. (2004) have reviewed the very few detailed studies made of the influence of external factors on sexualization in diatoms. Species differ in what they require for induction but there is "no significant antagonism between factors promoting vegetative growth and those eliciting gametogenesis" (Drebes, 1977a; see also Geitler, 1932). Non-induced cells of most diatoms usually continue to grow and divide while the induced cells are entering meiosis, forming gametes, performing plasmogamy, and expanding as auxospores: Cessation of vegetative growth is not necessary before the sexual phase can be initiated. However, since sexual reproduction has never been observed in most diatoms, it is possible that our current perception of how sex is induced is biased. Already, some exceptions are known. For example, in the marine benthic diatom *Cocconeis scutellum* var. *ornata*, sexuality is promoted by a combination of low temperatures (10–14°C) and short days, whereas the best conditions for vegetative growth are higher temperatures (14–18°C) and long days (Mizuno and Okuda, 1985). Automictic auxosporulation of *Pinnularia nodosa* can be suppressed by high N (Poulíčková and Mann, 2008). And some freshwater centric diatoms seem to respond not to benign conditions *per se* but to an *amelioration*, such as rising N or P levels (Jewson, 1992a; Pérez-Martinez et al., 1992).

In culture, very small cells of a species sometimes lose the ability to become sexual (in this case, the life cycle is referred to as "closed") and the lineage is then destined to die out unless cells can enlarge vegetatively (Chepurnov et al., 2004). It is unclear whether populations usually or ever exhibit closed cycles in nature.

3. The Tempo and Significance of the Diatom Life Cycle

The course of the diatom life cycle, with its alternation of slow size reduction and rapid rejuvenation, might seem inevitable given the apparent inflexibility of the diatom frustule and the internal formation of new frustule elements. However, some diatoms can divide without getting smaller (Wiedling, 1948) and some can even expand during mitotic cell division (e.g., Geitler, 1932, pp. 97, 98). Clearly, then, size reduction is *not* an inevitable consequence of diatom cell structure and division and it is reasonable, therefore, to ask whether the size reduction–restitution

cycle could itself be adaptive. Lewis (1983) pointed out that sex is expensive for small organisms, simply because meiosis is much longer than mitosis and therefore interrupts the multiplication of cells to a greater extent (unless population growth is already at a standstill because of nutrient or light limitation). There are also the costs of sex that are shared by all organisms, large and small, such as gametes that find no mate or the formation of nonviable new gene combinations. On the other hand, sex brings benefits in reducing mutational load in a population, faster response to selection (by bringing together or recombining genes previously separated in different lineages), and possibly DNA repair (Maynard Smith, 1989; Long and Michod, 1995). Lewis (1984) suggested, therefore, that the size reduction–restitution cycle in diatoms evolved primarily as a "sex clock," to optimize the cost–benefit balance for sexual reproduction. According to this view, the life cycle is a mechanism that allows diatoms to space sex at intervals that are partially or wholly independent of annually or stochastically recurring environmental cues, such as changes in day length, temperature, nutrient status, etc. If so, the life cycles of most diatoms might be expected to last significantly more than, or less than, 1 year.

In laboratory cultures maintained at room temperatures (15–25°C) and subcultured regularly to maintain exponential growth, reduction in average size from the largest to the smallest cells that a species can produce can take place in several months (e.g., Geitler, 1932). In nature, such rapid progression is unlikely ever to take place because of limitation of growth by nutrient availability or light, or because of seasonally unfavorable temperatures. Measurement of life cycle length in nature has rarely been attempted and the data are not easy to interpret, but it is sometimes possible to follow the fate of a particular size class from its origin via auxosporulation to its loss through conversion into gametes. Studies of planktonic freshwater diatoms, based on seasonal sampling (e.g., Round, 1982) and examination of size spectra in the datable laminae of subalpine lakes (e.g., Nipkow, 1927), were reviewed by Mann (1988a), who also presented data for a benthic diatom, *Nitzschia sigmoidea*. Subsequently, Jewson (1992a, b) made detailed studies of *Stephanodiscus* sp. and *Aulacoseira subarctica*, both freshwater planktonic diatoms, and Potapova and Snoeijs (1997) studied the brackish littoral diatom *Diatoma moniliformis*. All these studies concur in suggesting that the longevity of diatom clones in nature is greater than 1 year and may be as much as 8 years or more (but my 1988a estimate of c. 40 years for some *Aulacoseira* has been disputed by Jewson, 1992b, and needs reevaluation). Diatoms are therefore comparable to short-lived perennials of higher plants in the lengths of their life cycles, though they differ profoundly in the numbers of independent but clonal individuals that are produced.

4. Auxosporulation and Size Spectra in Natural Populations

Compared to size reduction, the process of size restitution via an auxospore is transient, usually lasting only a few days (restitution sometimes occurs via vegetative cell enlargement, this too being rapid: Chepurnov et al., 2004). It is perhaps not

surprising, therefore, that auxosporulation has never been seen in the vast majority of diatom species. However, the likelihood that it will be observed depends also on its phenology. For sexual species, Edlund and Stoermer (1997) have made a helpful distinction between "synchronous" and "asynchronous" sexuality. In "synchronous" species, sexual reproduction is restricted to a few days or weeks, separated by extended periods in which cells remain vegetative. This has long been known for freshwater epiphytic diatoms such as *Cymbella*, *Gomphonema*, *Didymosphenia*, or *Eunotia* (e.g., Geitler, 1927, Geitler, 1951a, b; Meyer, 1929), and it has also been recorded in planktonic diatoms such as the marine *Corethron* (Crawford, 1995) and the freshwater *Stephanodiscus* (Jewson, 1992a). Sometimes almost the whole population becomes sexual simultaneously. In "asynchronous" diatoms, on the other hand, auxosporulation is spread over a longer period. Examples are the freshwater planktonic diatoms *Aulacoseira subarctica* (Jewson, 1992b) and *Cyclotella ocellata* (Pérez-Martinez et al., 1992), although in the latter, the auxospores were not demonstrated to have been produced sexually.

However, even in the "asynchronous" *Aulacoseira subarctica*, there are periods (here April–July) when auxosporulation appears to be rare or absent. Hence, if the life cycle lasts more than a year, and if only a proportion of the population becomes sexual each year, populations should exhibit a multimodal size distribution, the series of ever smaller celled modes corresponding to successively earlier periods of auxosporulation. Examples of these are shown in Fig. 2d, e for *Nitzschia sigmoidea* (multimodal) and *Tabellaria fenestrata* (bimodal), from the data of Mann (1988a) and Nipkow (1927). In *N. sigmoidea*, a complex distribution was present throughout, and the fates of the principal size classes were followed for more than 3 years. By contrast, if a population were *fully* synchronous, one would expect a unimodal distribution to be produced and maintained, with the population sometimes being dominated by large cells, which would then gradually decrease in size until the next sexual event. Inspection of some published size spectra suggests unimodal distributions, for example, in *Cocconeis*, *Sellaphora*, and *Neidium* (Mizuno and Okuda, 1985; Mann et al., 1999; Mann and Chepurnov, 2005), often with a positive (right) skew, for example, in *Fragilariopsis kerguelensis* (van der Spoel et al., 1973) and most of the distributions illustrated by Jewson (1992b) for *Aulacoseira subarctica*. However, although unimodal distributions may indeed sometimes arise from fully synchronous auxosporulation, published examples have the unexpected characteristic that the mode is usually of *small* cells. Furthermore, when auxosporulation is observed in a species in which it has never been recorded before, the initial cells are often significantly larger than any previously observed cells. In other words, there is generally a deficit of large cells in diatom populations. Mann (1988a) outlined an explanation for this phenomenon but a longer, illustrated account is needed.

The sequence in Fig. 2a shows the size spectrum of a hypothetical diatom population developing during 1.4 years. The graphs show the percentage of cells of different sizes within the population and the vertical line marks the sexual size threshold. The *environmental* conditions suitable for sexual reproduction are assumed to occur for only a short period at the same time each year and cells are

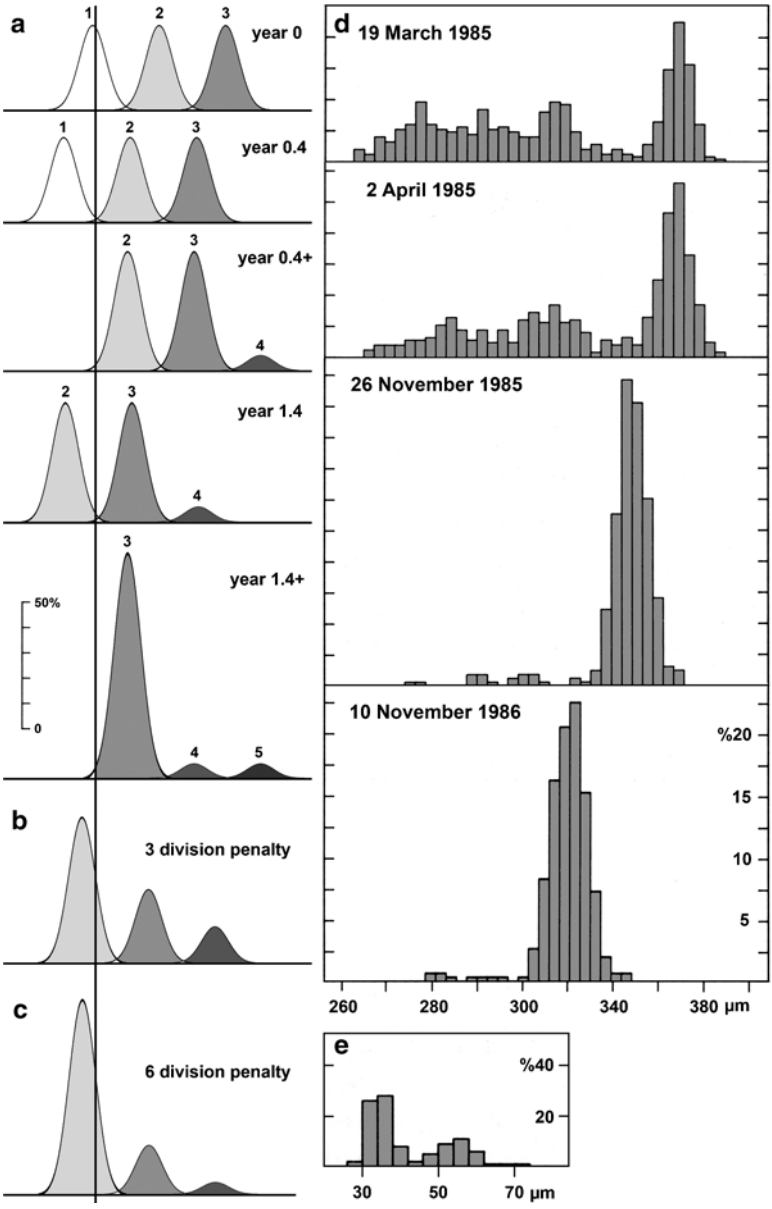


Figure 2. Graphs plotting percentage frequency against cell length ("size spectra") for various theoretical and natural diatom populations. (**a**)–(**c**) Theoretical populations, showing the effect of a "sex penalty" (or auxosporulation penalty): see text for explanation. (**d**) Selected size spectra of *Nitzschia sigmoidea* in Blackford Pond, Scotland, in 1985 and 1986, showing complex multimodal size distributions; individual size classes can be tracked in time (e.g., the excellent correspondence between March and April 1985), allowing the length of the life cycle to be estimated as up to c. 8 years (redrawn from Mann, 1988a). (**d**) Size spectrum of *Tabellaria fenestrata* in 1916 sediment of the Zürichsee (Data from Nipkow, 1927: see Mann, 1988a).

assumed to divide mitotically throughout the year, with accompanying reduction in size. At the beginning (year 0), the population of the species consists of three size classes, each derived from short bursts of auxosporulation at yearly intervals. The three classes are initially made equal. After a few months (year 0.4), the size spectrum has shifted to the left (to smaller cell sizes), and all the cells of class 1 pass the sexual size threshold and enter the phase when they are potentially sexual. Within a short interval (year 0.4+), these cells reproduce sexually, form auxospores and expand, forming a new cohort of large cells, class 4. However, class 4 is not equal to classes 2 or 3, which are still above the sexual size threshold, because of the penalties incurred by class 1 as it auxosporulates. During meiosis and auxospore expansion, no new cells of class 1 are produced (and, as noted previously in relation to the sex clock, some gametes may not find a mate), whereas classes 2 and 3 continue to divide mitotically. In this hypothetical example (Fig. 2a), a "sex penalty" of three mitotic divisions is applied, reflecting interruption of synthesis during meiosis, plasmogamy, and auxospore development. The initial class frequencies of 0.33 therefore change to 0.47, 0.47, and 0.06 for classes 2, 3, and 4, respectively. After another year (year 1.4), almost all cells of class 2 have declined below the sex size threshold, but classes 3 and 4 are still in the refractory phase. When class 2 auxosporulates, again with a three-division penalty, the class frequencies shift to 0.8, 0.1, and 0.1 for classes 3, 4, and 5 (year 1.4+), respectively. Of course, during the following year of this example (not illustrated), the frequencies of the three year classes would return to being equal and they would then cycle between the three states shown (years 0.4, 1.4, 1.4+), but this means that for most of the time, small and medium cells will predominate over large cells. With a three-division penalty and 3-year life cycle as in the example, the size spectrum of year classes would be stable with relative frequencies of 4: 2: 1 (year 1:year 2:year 3). With a six-division penalty and 3-year life cycle, the stable ratio would be 16: 4: 1 (Fig. 2b, c).

This example is oversimplified. For example, it ignores the fact that variance of cell size must increase during size reduction according to both of the schemes of cell division known to exist in diatoms, namely the MacDonald–Pfitzer model (in which the length of the cell cycle is the same in both daughter cells at each division, so that a binomial distribution of cell lengths results) and the Müller model (in which the smaller daughter cell has twice the cell-cycle length as the other: see Fritsch, 1935, or Rao and Desikachary, 1970); the increase in variance means that size classes will "collapse" and merge as they age and even produce the semblance of a skewed unimodal distribution. Also, real size spectra may contain more or less than three classes (Fig. 2; Mann, 1988a). And because passing the sexual size threshold makes cells only potentially, not actually sexual, two or more year classes may sometimes be present below the threshold if environmental or other conditions (e.g., cell densities) have remained noninductive. Finally, as already noted, the sexual size threshold may not act like an on–off switch, contrary to the assumption in Fig. 2a. Nevertheless, the general principle seems secure: if some cells in a population are above the threshold when auxosporulation occurs, these cells will increase in frequency relative to newly enlarging cells.

The three-division penalty assumed in Fig. 2a, b is probably minimal. In *Seminavis robusta*, pairing, sexual reproduction, and the restitution of cell size via auxospores requires 45 h, while the mitotic cell cycle in similar conditions is c. 12 h (Chepurnov et al., 2008). Thus, there is a 3.75-division penalty from this source alone, to which must be added further penalties due to mismatched gametangia (e.g., in triplet pairing) and aborted gametes and zygotes. Furthermore, most oogamous diatoms produce only one egg cell per female cell and so there can never be as many auxospores as gametangia (because some gametangia are males) and in some pennate diatoms, two gametangia habitually yield only one auxospore (Geitler, 1973), automatically doubling the other penalties of auxosporulation. It appears too that the larger cells of the life cycle may decrease in size more rapidly (per division) than smaller cells (e.g., Jewson, 1992b; Potapova and Snoeijs, 1997). Much further research is certainly needed.

Direct demonstration of the sex penalty seems to have been made only once, by Koester et al. (2007) in *Ditylum*. Pouíčková and Mann's (2008) study of *Pinnularia nodosa*, on the other hand, indicates a check to vegetative growth during induction of auxosporulation and meiosis, which would reduce the penalty caused by interruption of synthesis (but this was an automictic clone, and extrapolation to sexual species may be unwise). Overall, however, the combination of the sex (and auxosporulation) penalty and more rapid size reduction during the early part of the life cycle makes it almost inevitable that large cells will be rare, unless there is only ever one size class present.

5. The Morphology and Mechanics of Sex

Textbooks (e.g., van den Hoek et al., 1995; Graham et al., 2008) state that the lineages of centric diatoms are oogamous, producing small anteriorly flagellate sperm (e.g., Jensen et al., 2003) and large immobile egg cells (Fig. 1), whereas pennate diatoms are morphologically isogamous. These generalizations remain true. No pennate diatom has yet been found to produce sperm, though the non-flagellate "male" gametes of *Rhabdonema* gametangia are like spermatia, being active and much smaller (because of special depauperating mitoses) than the "females" (von Stosch, 1958). However, many lineages of pennate diatoms and polar centric diatoms have not yet been investigated in detail and it is unknown whether the change from oogamy to isogamy preceded, accompanied, or followed the evolution of true pennate structure, in which transapical ribs develop from a longitudinal sternum to produce a feather-like valve.

The link between auxosporulation and sexual reproduction is not absolute, as sometimes assumed. Thus, whereas no instance is yet known where sexual reproduction does not result in the formation of an auxospore, it is certainly not the case that size restitution implies the occurrence beforehand of meiosis and fertilization. For example, *Ditylum brightwellii* clones from southwest Australia and the east and west coasts of the USA reproduce sexually, whereas most clones

from the North Sea appear to restore size through vegetative cell enlargement (von Stosch, 1965, 1987), and Chepurnov et al. (2004) list several diatoms in which auxospores are formed asexually or after automixis. Even the demonstration of gametogenesis in cultured clones does not prove that size restitution is sexual: auxospores seem to expand without prior fertilization in some *Melosira* and *Cyclotella* clones that are also capable of producing sperm (Chepurnov et al., 2004, p. 109). Indeed, because the origin of an auxospore cannot be established without evidence of fertilization and because plasmogamy (fusion of the gametes) is transient and inconspicuous in oogamous diatoms (there are very few reports that demonstrate plasmogamy in supposedly oogamous diatoms: op. cit., p. 126), it is quite possible that many centric diatoms do in fact, sometimes or always, form their auxospores asexually. Some araphid pennate diatoms may also form their auxospores without sex (e.g., Sato et al., 2008). In raphid diatoms, the link between sexual reproduction and auxosporulation is usually obvious because copulation occurs between apparently undifferentiated vegetative cells, which then remain very closely associated with each other during meiosis, gamete formation, and the development of the auxospores.

The details of sexual reproduction and auxosporulation vary considerably between different genera and species. Geitler (1973) provided a succinct summary of auxosporulation in pennate diatoms, in which he classified the different types first according to whether auxosporulation was allo-, auto- or apomictic and the number of auxospores produced per gametangium or mother cell, then by whether reproduction was isogamous or anisogamous, and finally by the relative orientations of the gametangia and auxospores, production of special copulation structures, and other special features. Mizuno (2006, 2008) has provided a similar summary classification for centric diatoms, concentrating on oogenesis and spermatogenesis. Introductions to auxosporulation diversity have also been provided by Round et al. (1990) and Chepurnov et al. (2004). Here, I concentrate on special activities and structures in diatoms undergoing sexual reproduction, which must add further to the costs of this process; the counterbalancing advantages are not understood and need investigation.

5.1. PRE-FERTILIZATION DEVELOPMENT IN CENTRIC DIATOMS

The most obvious sign of sexual reproduction in centric diatoms is spermatogenesis, involving first a series of depauperating mitoses (which are decoupled from cell growth and mostly unaccompanied by formation of silica valves) and then meiosis, during which flagella are formed (for a particularly complete account, see von Stosch et al., 1973). No centrioles or basal bodies have ever been reported from vegetative diatom cells (Pickett-Heaps et al., 1990), and the development of the single basal body and flagellar axoneme of the sperm occurs *de novo* during meiosis (Manton et al., 1970). The formation of the flagellum, the functioning of the unusual 9 + 0 flagellum, the mechanism of chemotaxis (which must surely be

involved in guiding movement of sperm toward the egg cells), flagellum excision or resorption, and the loss of the basal body following fertilization, have not been investigated in detail.

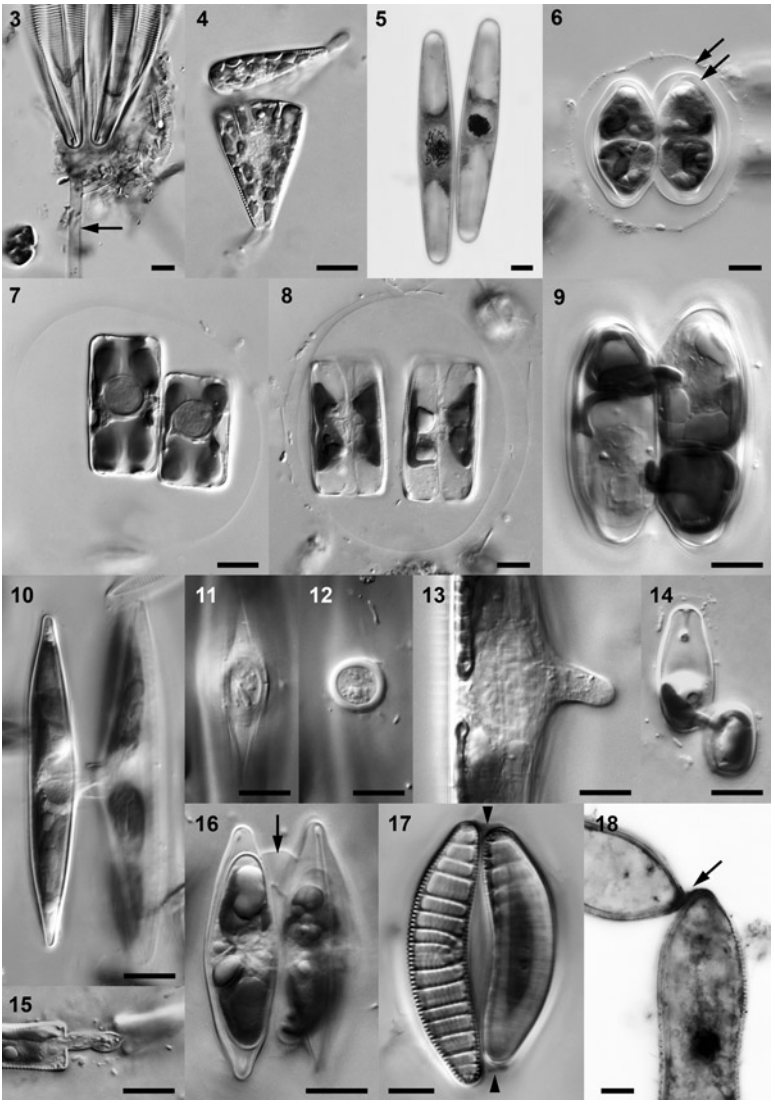
Differentiation of the egg cells is less obvious than spermatogenesis, but often involves unusual elongation of the girdle and accumulation of extra or larger plastids. Only one egg is formed per oogonium (all except one meiotic product abort), except in *Attheya* and some *Odontella* species where two are formed (von Stosch, 1954; Drebes, 1977b; Mizuno, 2006, 2008). No special structural secretions by the oogonium or egg have been reported before fertilization by sperm.

5.2. COPULATION IN PENNATE DIATOMS

The change from oogamy to isogamy during the evolution of pennate diatoms, with total loss of flagella and basal bodies, was accompanied by a change in sexual behavior from the attraction, recognition, and fusion of gametes, to copulation between the cells producing the gametes (the gametangia; Figs. 3–18). This required the evolution of new mechanisms to bring the gametangia together and hold them close to each other during meiosis, gametogenesis (producing one or two large gametes per cell), and fertilization.

In all pennate diatoms, sexual reproduction seems to require compatible cells to touch or be in very close proximity before gametogenesis is initiated (Chepurnov et al., 2004). Thus, whereas clones of centric diatoms will produce sperm even when there are no eggs to be fertilized, pennate diatoms will remain vegetative and continue to divide mitotically if compatible partners are absent. In *both* centrics and pennates, however, there must be a lower limit to population density, below which sexual reproduction will be impossible because insufficient gametes or gametangia will find a mate. The limit will be higher in heterothallic diatoms but it exists for all allogamous diatoms and means that extreme rarity is impossible, because rare diatoms will be unable to complete their life cycle and will become locally extinct (which *inter alia* weakens one of the key assumptions of the ubiquitous dispersal hypothesis of, e.g., Finlay et al., 2004).

Raphid diatoms use their motility to bring sexualized cells into contact, probably guided by chemotaxis (Chepurnov et al., 2004). However, the change from oogamy to isogamy seems to have preceded the evolution of the raphe (Sims et al., 2006) and even though some rapheless pennate diatoms are capable of sustained unidirectional movements (Sato and Medlin, 2006; also some elongate centric diatoms: Pickett-Heaps et al., 1991; Kooistra et al., 2003), most raphe-less pennate diatoms probably depend on water movements or growth together in dense swards to bring cells close enough to initiate sex. No special structures seem to be formed by the pairing cells in araphid pennate diatoms (Fig. 4), except that there may be extremely diffuse mucilage enveloping both gametangia (e.g., Tschermak-Woess, 1973a, p. 25). In raphid diatoms, on the other hand, it is



Figures 3–18. Pairing and copulation structures in pennate diatoms (living cells, unless stated otherwise). Figure 3 *Cymbella lanceolata*: the stalk (*arrow*) bearing the paired cells is produced by only one gametangium (*left*). Figure 4 Paired stalked Licmophora cells. Figure 5 Navicula oblonga cells in meiotic prophase (hematoxylin stained). Figure 6 *Placoneis gastrum* gametangia with two gametes, surrounded by a structured capsule of two layers (*arrows*). Figure 7 *Dickieia ulvacea* gametangia, surrounded by unstructured capsule. Figure 8 *Caloneis* gametangia during dehiscence of gametangia, surrounded by structured capsule. Figure 9 *Neidium*: gametes beginning to move through copulation apertures to effect plasmogamy. Figure 10 *Nitzschia recta*: gametangia connected by a single central copulation tube. Figures 11–13 *Nitzschia sigmoidea*: transverse and longitudinal (13) optical sections through a forming copulation tube. Figure 14 Plasmogamy in *Sellaphora capitata*: one gamete is formed by each gametangium; the active gamete squeezes through the copulation aperture to fertilize the passive gamete. Figure 15 *Eunotia*: the copulation tube is formed from close to the cell apices. Figure 16 *Frustulia*: two copulation tubes are formed, one near each cell apex (e.g., *arrow*). Figure 17 *Epithemia* gametangia attached to each other by pads of mucilage produced from the apices (*arrowheads*: safranin-stained preparation of L. Geitler). Figure 18 *Cymatopleura*: the gametangia bond to each other via a single pad of organic material (*arrow*) produced from the synaptic poles (hematoxylin stained). Scale bars = 10 μm.

common for copulating cells to produce special secretions not seen at any other stage of the life cycle (e.g., Figs. 6–8 and 10–18).

In some raphid diatoms, movement is restricted to only one of the two copulating cells. For example, paired *Cymbella*, *Gomphonema,* and *Rhoicosphenia* cells are almost always attached to the substratum via the stalk of one partner (e.g., Geitler, 1927, 1932, 1952a), requiring that this cell remained still while the other became detached from its own stalk and moved (Fig. 3). Geitler (1932) reported this kind of behavior in homothallic clones of *Gomphonema parvulum*, showing that, here at least, the differences in motility are not genotypic. It is possible that clonal differences in motility exist in some attached raphid diatoms and that it is associated with heterothallism (one mating type providing active cells, the other passive), analogous to the male–female differentiation of the gametangia in araphid pennate diatoms (Chepurnov and Mann, 2004), but there is little relevant evidence. In *Achnanthes longipes*, clones capable of intraclonal reproduction grow vegetatively in tufts, whereas non-inbreeding (unisexual or bisexual) clones disperse themselves more effectively across the substratum, but no differences have been reported between these kinds of clones with respect to which cell remains attached during copulation. In heterothallic raphid diatoms that produce structures to aid plasmogamy, such as mucilage sheaths or copulation tubes, both partners contribute to their formation (Mann et al., 2003; Poulíčková et al., 2007; Trobajo et al., 2009).

In pennate diatoms, as in centrics, fertilization can only occur if the cell membranes of the gametes come into contact and so the gametangia must dehisce, partially or completely. Though dehiscence has often been observed, it is not understood. In vegetative cells, the thecae are bound tightly together, never exposing areas of naked membrane, except pathologically. Waterkeyn and Bienfait (1987) demonstrated the presence of a callose strip at the edge of the hypotheca in *Pinnularia*, which apparently functions as a "gasket" linking the epitheca and hypotheca and disappears when the thecae separate following cell division. Local or complete degradation of such a gasket must presumably also occur during dehiscence of gametangia. However, pennate diatom gametes are large and would surely be very vulnerable to grazers and parasites if not protected (the effectiveness of the frustule as armor has been tested experimentally: see Hamm and Smetacek, 2007). Freshwater diatoms have the extra problem of osmoregulation of the naked gametes (some, including some *Craticula* and *Navicula* species, possess contractile vacuoles: Subrahmanyan, 1947; my unpublished observations). Not surprisingly, therefore, copulating raphid diatoms usually develop special structures, which hold the cells together and (often in combination with the gametangial frustules themselves) seem to provide a protective environment for plasmogamy. My classification refers only to structures produced during pairing and gametogenesis; further secretions are frequently made by the zygotes and auxospores.

5.2.1. No Copulation Devices

In raphid diatoms, it is rare for there to be no detectable structures or mechanisms to hold the gametangia together. Examples are *Navicula oblonga* (Fig. 5; Mann and

Stickle, 1989) and *Seminavis robusta* (Chepurnov et al., 2002), and *Pseudo-nitzschia* species (Davidovich and Bates, 1988; Amato et al., 2005), though it is likely that extremely fine adhesive material is present in all of these. In *N. cryptocephala*, all that can be found linking the gametangia is sparse material secreted from the girdle region (Poulíčková and Mann, 2006). Despite the virtual absence of protection, the gametangia dehisce fully before plasmogamy.

5.2.2. Diffuse Copulation Envelopes

Use of Indian ink sometimes reveals the presence of a watery, mucilaginous sheath around copulating cells, whose boundary is almost undetectable in the light microscope (e.g., in *Craticula*: Subrahmanyan, 1947). Diffuse sheaths can also sometimes be detected by exclusion of debris, or colonization of the sheath's periphery by bacteria. The gametangia dehisce fully and the gametes come into contact through expansion within the copulation envelope and/or autonomous movement (e.g., Mann, 1988b; Mann and Stickle, 1991, 1995).

5.2.3. Unstructured Capsules

Here a capsule of apparently homogeneous mucilage surrounds the copulating cells, with a boundary that is clearly detectable without outlining by extraneous surface material (though this may also be present). Examples include *Dickieia* (Fig. 7; Mann, 1994), which also produces capsules around the vegetative cells, *Berkeleya* (Tschermak-Woess, 1973b), and probably *Gomphonema parvulum* (Geitler, 1932). The gametangia dehisce fully and their thecae separate in *Dickieia* and *Berkeleya*, allowing the expanding gametes to move and come into contact, but in *Gomphonema* dehiscence seems to be partial, so that the thecae separate mainly on the side where they face each other, creating a confined space in which the gametes move. The distinction between unstructured capsules and diffuse envelopes is possibly only quantitative, and the study by Cohn et al. (1989) suggests that their population of *Craticula* produced capsules rather than the diffuse envelopes present in Mann and Stickle's (1991) material.

5.2.4. Structured Capsules

Capsules differentiated into inner and outer layers are present in *Caloneis*, *Lyrella* and *Placoneis* (Figs. 6–8; Mann, 1989b; Mann and Stickle, 1993, 1995), and probably also in *Didymosphenia* (Meyer, 1929, e.g. Figs. 14 and 15). In *Caloneis*, the inner layer is sufficiently fluid to allow the young gametangia to move within the capsule during early meiosis. Dehiscence of the gametangia is complete or partial.

5.2.5. Copulation Apertures (Canals)

Sexualized *Sellaphora* cells bond firmly to each other via their girdles and often move around as pairs or larger groupings before beginning meiosis, when they become stationary. Although extracellular material must be present linking the cells together, no envelope or capsule can be detected around the cells during meiosis. Toward the end of gametogenesis, the thecae separate slightly on the

side where the cells adjoin and an aperture is formed between them, allowing the (single) gamete from the "male" gametangium to squeeze through (Fig. 14; Mann, 1989a). Similar apertures occur in *Neidium*, except that here there are two apertures, one for each pair of fusing gametes (Fig. 9; Mann, 1984); again, dehiscence is only partial and the gametangial thecae protect the gametes during development and fertilization.

5.2.6. Copulation Tubes

In *Eunotia* (Fig. 15; Mann et al., 2003) and some *Nitzschia* species (Figs. 10–13; Geitler, 1928; Mann, 1986), the girdles of the gametangia split apart locally, at the poles or center, and papillae with organic walls grow out and fuse to create a single copulation tube. Pairs of short copulation tubes are produced in *Frustulia* and *Amphipleura* (Fig. 16; Geitler, 1949, 1952b), and *Nitzschia palea* (Trobajo et al., 2009).

5.2.7. Attachment Pads

In all of the categories listed above, cells seem to have to touch before recognition is complete and so there are probably localized or general changes to the cell surface during sexualization, possibly involving the secretion of new material. In addition to this, however, some diatoms secrete pads of mucilage that seem to glue the pairing cells together in the right configuration for gametogenesis and fertilization. Examples include *Cymatopleura* (Fig. 18; Mann, 1987) and *Surirella* (Thaler, 1972; Mann, 2000), where a single pad links the cells at one pole, and *Epithemia* (Fig. 17; Geitler, 1932), where the cells are held together by two pads produced close to the cell poles.

5.3. PLASMOGAMY

In oogamous diatoms, fertilization is facilitated by temporary exposure of part of the egg by flexing of the oogonium frustule (e.g., *Melosira moniliformis*: Idei and Chihara, 1992), or dehiscence of the oogonium (e.g., *Attheya decora*: Drebes, 1977b), or full release of the egg (e.g., *Lithodesmium*, *Streptotheca*: von Stosch, 1954) (see also Chepurnov et al., 2004). This allows the anteriorly flagellate sperm (which appear to be attracted to the eggs chemotactically) to contact the egg via the flagellar tips and fuse with it (e.g., Drebes, 1977b).

Plasmogamy in pennate diatoms is usually achieved by simple swelling or, more often, by amoeboid movement or unilateral contractions of the gametes, following partial or complete dehiscence of the gametangia. Taxa vary in whether fusing gametes behave similarly (= true isogamy) or differently, one being active, the other passive (= behavioral or physiological anisogamy); in cases where two gametes are produced per gametangium, there is a further division, according to whether both active gametes are produced by the same gametangium or not. The distribution of different kinds of behavior among genera has been summarized

by Geitler (1932, 1973). Behavioral anisogamy is generally associated with the more sophisticated kinds of copulation apparatus, such as structured capsules, copulation apertures, and copulation tubes, but the correlation is not exact: *Eunotia* is isogamous and the auxospore develops within the copulation tube (e.g., Mann et al., 2003). In some cases, the gametes act independently during plasmogamy, but elsewhere they act as coordinate pairs (e.g., Mann, 1984).

6. Sex and Species

Successful sex completes the life cycle in many diatom species. That sex is an expensive process in diatoms is evident from the interruption of synthesis by meiosis and auxospore expansion; the extra potential for mortality because of the delicacy of the gametes and young zygotes; the cost of wasted gametangia and gametes; and the cost of copulatory movement and special copulation structures where present (in raphid diatoms, cells must cooperate to create the conditions suitable for plasmogamy and so must be well matched developmentally). In addition, in all sexual organisms, some of the new genetic combinations are likely to have low viability, the proportion being higher if the parents have adapted to different environments that are not bridged by intermediates. It is likely, therefore, that if differently adapted but compatible diatoms come into contact and their hybrids have reduced viability, there will be strong selection for reproductive isolation at the earliest possible prezygotic stage. In centric diatoms, the earliest known stage at which reproductive isolation can occur is fertilization, by which time many of the costs of sex have already been incurred. In pennates, on the other hand, recognition seems to take place before cells become committed to sexual reproduction, offering the possibility of avoiding any costs associated with "illegitimate" mating. This difference may help explain why the centric diatoms, despite having arisen and diversified much earlier than the pennates (e.g., Sims et al., 2006), are much less speciose.

7. Acknowledgments

I am most grateful to all those who have worked with me on the life cycles of diatoms or taken the time to discuss their or my results with me. These include Alberto Amato, Susan Brawley, Eileen Cox, Domenico d'Alelio, Masahiko Idei, Julie Koester, Shigeki Mayama, Marina Montresor, Alka Poulíčková, Shinya Sato, Alan Stickle, Rosa Trobajo, Pieter Vanormelingen, and especially Victor Chepurnov. I also give Anna-Marie Schmid sincere thanks for introducing me, many years ago, to the doyen of diatom sex, Professor Dr Lothar Geitler, and warm thanks to Rosa Trobajo and a reviewer for reading through this chapter and giving many helpful comments, which I hope I have addressed.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 167–194
DOI 10.1007/978-94-007-1327-7_7, © Springer Science+Business Media B.V. 2011

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CLASSICAL BREEDING IN DIATOMS: SCIENTIFIC BACKGROUND AND PRACTICAL PERSPECTIVES

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"Cross the best with the best, select the best, and hope for the best!"

John W. Snape (2004)

1. Introduction

Nowadays, tiny aquatic photosynthetic organisms commonly referred to as "microalgae" are becoming increasingly important as subjects for the development of economically efficient and simultaneously environment-friendly multipurpose industrial technologies (e.g., Benemann, 2003; Spolaore et al., 2006) including biofuel production (Chisti, 2007, 2008; Rosenberg et al., 2008; Hu et al., 2008; Mascarelli, 2009). In the economic exploitation of microalgae, the diatoms (Bacillariophyta) are expected to play a significant role. This group of unicellular phototrophs is enormously diverse (perhaps 200,000 extant species, see Mann, 1999), remarkably abundant and highly productive. They carry out c. 20% of the net primary production of the planet and consequently produce every fifth molecule of O_2; thus, the "creativity" of diatoms in the scale of biosphere is more significant than that of all the world's tropical rainforests called sometimes "the lungs of the Earth." They form the foundation of the trophic pyramid in many parts of the world ocean playing "a particularly important part in sustaining fisheries, as the major producers of 'new' phytoplankton biomass" (Mann, 1999). Some diatom species grow very fast; their cells can divide 2–5 times per day (e.g., Furnas, 1991). The biochemical composition of these microalgae is of high quality for various industrial applications (Lebeau and Robert, 2003) and, as diatoms can store neutral lipids in their cells (Fig. 1), there is a growing belief that they may become a sustainable source of renewable biofuel (e.g., Sheehan et al., 1998; Hu et al., 2008; Bozarth et al., 2009; Ramachandra et al., 2009). Simultaneously, there is a growing body of evidence indicating that the development of microalgae technologies including the demandable selection of appropriate strategies and methods in choosing the suitable algae and then their sustainable improvement

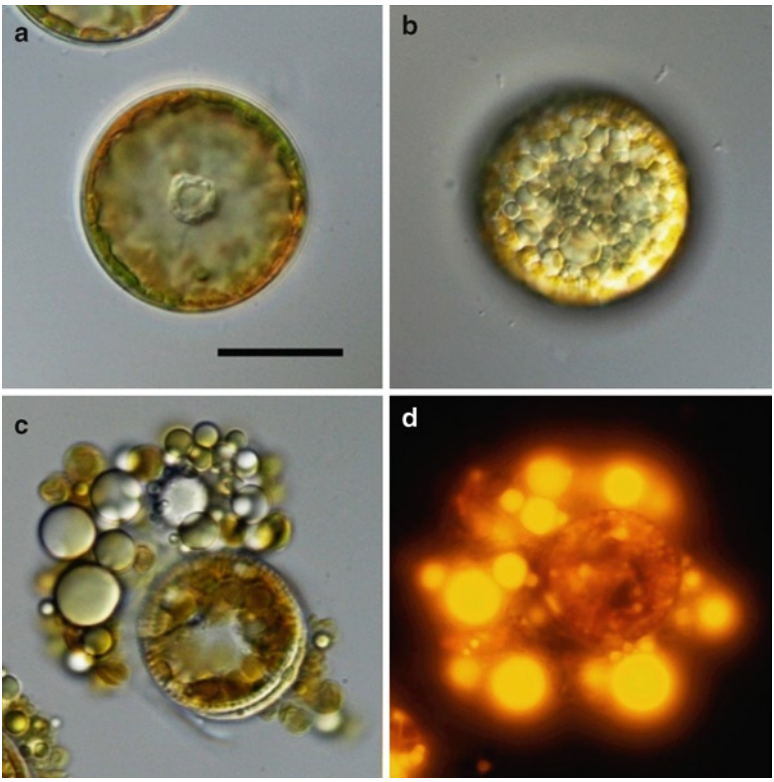

Figure 1. Accumulation of lipids by diatom cells. (**a**) Cell taken from intensively growing culture contains no visible droplets of oil. (**b**–**d**) Cells triggered for oil production. (**b**) Intact cell containing numerous oil droplets. (**c** and **d**) The cell content has been released. (**d**) Staining with a fluorescent dye (Nile Red, Sigma-Aldrich, Bornem, Belgium) has visualized the presence of neutral lipids in the droplets. Scale bar, 20 μm.

may largely depend on the utilization of the c. 10,000 years experience accumulated by traditional plant agriculture and agriscience in particular. More precisely, this will primarily concern the approaches and the procedures well known as "plant breeding programs" [under "plant," angiosperms are born in mind, since, with a few exceptions, almost all agricultural and horticultural crops and all hardwood trees belong to this group (Forbes and Watson, 1992)]. "A broad definition of plant breeding usually refers to the purposeful manipulation of genetic material through hybridization, mutation, or genetic engineering to produce new genotypes followed by selection of outstanding individuals to establish cultivars which are populations of related plants with economic value" (Bliss, 2007). Here we shall try to illustrate that, due to striking similarity between flowering plants and diatoms in a number of biological attributes including sexuality, the knowledge on

plant reproductive systems and the experience of manipulating them for practical purposes can potentially help to identify some guiding principles for the development of future diatom breeding programs.

2. Plant Breeding

In plant breeding, five principal groups of procedures are usually recognized, namely, domestication, induced mutations, classical breeding, genetic engineering, and tissue culture technologies (e.g., Murphy, 2007). In the present contribution, we shall focus on the first four groups. Domestication (bringing the plants under human management), with further selection of those specimens that possess desired phenotypic traits and/or are better adapted to new environments, has played a crucial role at the earliest stages in the evolution of human civilization. Mutation breeding (increasing mutation frequency using chemical mutagens and ionizing radiation) was extensively applied during the past few decades but eventually did not become a broadly accepted procedure, since "it is not possible to direct the mutation process so that a specific type of mutation can be produced" and "the results from mutation breeding in cultivar development have been rather meager in relation to the effort expended" (Sleper and Poehlman, 2006; see also Murphy, 2007). Since the rediscovery of Mendel's laws of inheritance at the beginning of the twentieth century and recognition of genetics as important scientific discipline, the classical breeding approach (also "conventional" breeding or "traditional" breeding and sometimes "hybridization") in which genetic variability is identified, increased, and exploited through sexual reproduction (Sleper and Poehlman, 2006) had began to contribute the most in improvement of cultivars that eventually "gave birth to a revolution in plant and animal breeding which produced the spectacular twentieth century agricultural process and made it possible to feed the exploding population of the Earth" (Roll-Hansen, 2000). "Classical breeders improve crops simply by crossing plants with desired traits, and selecting the best offspring over multiple generations … In some ways, breeding is like accelerated, targeted evolution, and as long as test crops and seed banks are maintained, the possibilities can never be fully exhausted" (Knight, 2003). Genetic engineering (Gepts, 2002; Sharma et al., 2005) or transgenesis (Murphy, 2007) represents a relatively new, advanced procedure of genetic modifications of plants by transferring genes between sexually incompatible backgrounds. From the very beginning, transgenic technologies hold great promise for improvement of commercial plants and currently receive most of the attention and, consequently, investment, often at the expense of development of traditional breeding (e.g., Knight, 2003; Gepts and Hancock, 2006; Gurian-Sherman, 2009). A comparison between two principal approaches of genetic modification, i.e., classical breeding versus genetic engineering, is a topic of continuous analysis and discussion (e.g., Gepts, 2002; Bradford et al., 2005; Sharma et al., 2005; Murphy, 2007; Lemaux, 2008; Ulukan, 2009). Despite a broad variation in existing opinions and

controversies that surround this subject, most of the critical analysts seem to agree that the methods are different but complementary and genetic engineering cannot be considered as an actual or potential substitution of the classical methods. Moreover, it is sometimes emphasized that "… genetic engineering is presented erroneously as a speedier, more precise plant breeding alternative, whereas genetic engineering is simply another way of generating genetic diversity at the onset of a plant breeding cycle (Gepts, 2002). In short, improved cultivars are still generated through conventional approaches" (Gepts and Hancock, 2006; see also Knight, 2003; Goodman, 2004; Gurian-Sherman, 2009).

3. Algae Breeding

By now, in various algal groups, the four principal breeding approaches briefly considered in the previous section have already been employed, but so far mostly on a modest scale. Typically, all the activities were of preliminary character and were more useful in basic research but practical aspects of the results, e.g., higher productivity and "better" (from commercial point of view) biochemical quality of the algae modified, were always kept in mind. With a dramatic increase of economical significance of microalgae, development of programs for strain selection and improvement are expected to be accelerated. Parallel to "algae domestication," i.e., extensive isolation of well-performing algal strains in nature, deposition of them in sustainable algal culture collections and their appropriate characterization, some attempts were periodically undertaken to apply random mutagenesis by UV and chemicals for obtaining "improved" strains (e.g., Lopez Alonso and Segura del Castillo, 1999; Chaturvedi and Fujita, 2006; Huesemann et al., 2009). In recent years, since the successful development of lab-scale transgenic methods for various microalgae, genetic engineering has been promoted as the most promising strategy in the improvement of algal productivity and quality (León-Bañares et al., 2004; Chisti, 2007, 2008; Hallmann, 2007; Rosenberg et al., 2008); in this respect, the diatoms are not an exception (Kroth, 2007; Bozarth et al., 2009; but see Pulz and Gross, 2004, highlighting potential problems and restrictions). In turn, classical breeding approach involving genetic manipulations via sexual reproduction – that unambiguously proved its effectiveness in plant breeding – remains undoubtedly the most underexplored in microalgae. Such a situation can easily be explained by the fact that, in many industrially interesting groups and species, there is no information on sexual reproduction (some of such microalgae may simply be entirely asexual) or the data on sex are still very limited, restricting the opportunities for developing efficient methods of strain modification via sexual breeding. However, we believe that present-day knowledge on sexuality of diatoms and accumulated experience in its experimental manipulations are quite sufficient and reliable enough to make an attempt to evaluate the potential and the prospects for the development of classical breeding procedures for this group of microalgae.

4. The Diatoms: Sexual Reproduction in the Life Cycle

Among the major groups of microalgae, our knowledge on principal attributes of life cycles in diatoms can apparently be considered as comparatively rich and well structured. Although life cycle traits have been studied in only a tiny minority of extant diatom species, these however represent many genera and almost all of the principal diatom lineages (Mann, 1999; Chepurnov et al., 2004). In addition, successful and sustainable progress in gathering this type of biological information could largely be explained by the fact that, unlike other groups of algae, diatoms are highly uniform with respect to the principal traits of their life history (e.g., Mann, 1993; Chepurnov et al., 2008). It is also well established that most of diatoms are sexual organisms and "sex is not facultative, as it can be in other algae" (Mann, 1999). Examples of asexuality in diatoms are also known but these were reported to occur occasionally in what are otherwise predominantly sexual lineages (e.g., Chepurnov et al., 2004). A series of life cycle attributes are very peculiar and unique to diatoms; and the place of sex in the life cycle and control over sexuality are unique to this group too. Diatoms are highly unusual among algae in having a diplontic life cycle. The only haploid cells are the gametes, which are short lived. Vegetative multiplication is accompanied by gradual reduction of cells in size; this principal is well known as the MacDonald– Pfitzer rule and the mechanism by which cells decline in size during the vegetative phase was repeatedly described elsewhere (e.g., Round, 1972; Crawford, 1981; Pickett-Heaps et al., 1990). Cell size is restored through the development of a specialized cell called an auxospore, and formation of auxospores results from sexual reproduction. Auxosporulation thus involves linkage of two important processes – genetic recombination and cell size restitution – into a single chain of events. Cells that fail to undergo sexual reproduction and auxosporulation continue dividing mitotically until they become critically small and finally die: "in most diatom species, after one to several years of size reduction, every surviving individual of every lineage must reproduce sexually or die" (Mann, 1999). The capacity of cells to become sexualized is size dependent: only comparatively small cells can be triggered to switch from mitotic cycles to meiosis. Thus, according to an opinion of W.M. Lewis, Jr. (1984), "the diatoms stand apart from other taxa of unicellular algae in their virtually universal retention of sexual capacity" and the phenomenon described by the MacDonald–Pfitzer rule (couple with size-dependent control over sexuality) "is probably a unique evolutionary solution to the problem of clocking sex over many generations …, and simultaneously allows evolutionary control over the degree of investment in sex when sex does occur." The maximal size of initial cells (those formed by developed auxospores), the size of largest cells capable of sexual reproduction and the minimum viable size, are fairly strict, species-specific characteristics; these values (typically cell diameter or length) are referred to as "cardinal points" in the diatom life cycle (e.g., Geitler, 1932; Drebes, 1977; Mann and Chepurnov, 2004).

5. Sexuality as a Controlled Procedure: Opinions and Options

A progress in studying various aspects of diatom biology and evolution can hardly be separated from the improvement of our knowledge on their reproductive behavior: "Diatoms are some of the most sexual organisms on earth; our problem in understanding them is that we do not invade their privacy often enough" (Mann, 1999). Since recently, the interest in diatoms has started to grow rapidly, often with a focus on global ecological questions and economically relevant issues. Consequently, more and more research groups and biotech companies select the diatoms as a subject of rigorous investigations. The efforts of researchers who study diatoms using primarily modern molecular approaches and technologies look nowadays the most conspicuous; they are also expected to contribute the most to the economic evaluation and exploitation of diatoms (e.g., Lopez et al., 2005; Kroth, 2007; Bozarth et al., 2009). However, there is a danger that disproportionally fast (e.g., "gold rush for algae," see Mascarelli, 2009) and insufficiently coordinated efforts (e.g., typical problems in managing "conflict of interest," see Maurissen et al., 2005) may also lead to unintentional biases and controversies over some important aspects of diatom biology. Sexuality of diatoms seems to have become one of such controversial themes. Two aspects of this issue appear to be both important and relevant to the context of the present paper. First, since the diatoms had attracted attention of molecular biologists who were motivated to reveal their "molecular secrets" (Falciatore and Bowler, 2002) for scientific and biotechnological purposes, it was clearly realized that experimental control over sex in diatoms and performance of classical genetic manipulations with their clones are very important but simultaneously problematic because "no one has been successful in controlling a sexual cycle in culture" (Apt et al., 1996). During the last decade, a tremendous progress has been achieved in molecular-based studies of diatoms including availability of two complete genome sequences, various profound findings based on this genomic information (Armbrust, 2009), and successful applications of transgenic methods (Walker et al., 2005; Poulsen et al., 2006). However, what has not changed yet at this time is a belief that sex in diatoms is still unmanageable experimentally (e.g., Walker et al., 2005; Grossman, 2005, 2007). "It may take a strong concerted effort to understand the factors that control the diatom life cycle and to be able to control those factors" (Grossman, 2007), therefore "… genetic manipulation of diatoms has relied primarily on the addition of new versions of genes (transformation) or on reduced expression of targeted genes (RNA interference)" (Armbrust, 2009). In contrast to such opinions, there exists a different view on the same topic. This alternative view is based on the assumption that the experiences of previous researches (e.g., Geitler, 1932; von Stosch, 1965; Roshchin, 1994) clearly illustrate how cultures can be manipulated experimentally in life cycle studies of sexual diatoms. Moreover, apparently most of crucial discoveries related to diatom sexuality were made based on culture investigations and, importantly, it was repeatedly proved that the practical experience of previous authors is reproducible (e.g., Mann and Chepurnov, 2004;

Chepurnov et al., 2004) and can serve as an excellent foundation for controlled manipulations of sexual reproduction (Chepurnov et al., 2008). Second, by now, two diatoms have been selected and advertised as principal diatom model systems, centric *Thalassiosira pseudonana* and pennate *Phaeodactylum tricornutum*; their genomes have been wholly sequenced (e.g., Armbrust et al., 2004; Lopez et al., 2005; Bowler et al., 2008). Despite their obvious advantages for much current diatom research, both diatoms (more precisely, all the strains involved in the experimental work) apparently lack sexuality (although numerous attempts have been made to find it) and therefore deny the possibility of manipulation through classical genetic methods (Chepurnov et al., 2008). Taking into account the crucial significance of sexual reproduction in evolution and diversification of diatoms (e.g., Lewis, 1984; Mann, 1999) and its unique integration in the life cycle, the lack of opportunity to induce sexual reproduction in these two diatoms essentially reduces their value as representative model systems. Consequently, to complement the existing diatom experimental models and to realize the full potential of the molecular approaches, new model species for diatom research will be required, in which the broader context of sexuality is taken into account and eventually experimental control of self-fertilization and sexual crosses are firmly established. Hopefully, the selection and development of experimental systems in diatoms, using species with expression of unique diatom features and allowing conventional genetic analysis, can already be considered as shifted from the intention to the action stage (Chepurnov et al., 2008).

6. The Diatoms: Mating Systems

A study of evolution in plant species and evolutionary dynamics of their populations is closely tied to a deep understanding of their mating (breeding) systems which are "probably a major factor controlling molecular diversity and genome evolution" (Glémin et al., 2006; see also Barrett, 2002; Charlesworth, 2006). Although in plant science and agricultural research, there is variation (often inconsistent) in the use of the terms "breeding system" and "mating system" (Neal and Anderson, 2005), here we shall consider these as synonyms due to still fairly restricted information on how sexual systems function in diatoms and limited opportunities to interpret the data available. In a general sense, the mating system can be defined as "the mode of transmission of genes from one generation to the next through sexual reproduction" (Barrett, 1998) and "research on plant mating attempts to determine who mates with whom in plant populations and how and why mating patterns become evolutionarily modified" (Barrett and Harder, 1996). It is also obvious that the same basic knowledge on reproductive systems is profoundly important for the development of practical plant breeding programs and procedures. "Most of the staple crops of the world, wheat, maize, rice, sorghum, millet, beans, bananas, coconut, and many others, are fruits or seeds, the yield of which is a direct production of the breeding systems of the plants and their

efficiencies. The better we understand these systems, the better shall we all be fed" (Richards, 1997).

To focus attention on mating systems in diatoms makes obvious sense, at least, because most diatoms are biparental sexual organisms: a zygote results from fusion of two gametes produced by different individuals (gametangia) (e.g., Drebes, 1977; Mann, 1993; Chepurnov et al., 2004). Although mating systems in diatoms remain largely a neglected aspect of their biology, over the last c. 80 years, the relevant information on this issue was gradually accumulated from various diatom lineages, and eventually has started to contribute substantially to the improvement of our understanding of evolution and diversification of diatoms (e.g., Mann, 1999; Casteleyn et al., 2008; Evans et al., 2008) and other aspects of their biology (e.g., Mann and Chepurnov, 2004; Chepurnov et al., 2008). Consequently, the knowledge and experience available allow, in our opinion, to conclude that mating systems in diatoms are immensely diverse and, remarkably, almost all principal research aspects and practical issues attributable to plant mating systems can also be extrapolated to diatoms. In addition, attractive patterns of variation in plant reproductive systems as, for instance, those specified by S.C.H. Barrett (1998), i.e., (a) "analysis of any plant community reveals a variety of pollination and mating systems that coexist under apparently similar ecological conditions," (b) "close relatives can reproduce in different ways just as unrelated taxa often share similar floral adaptations," and (c) "an attractive feature of flowering plants is that many taxonomic groups display considerable inter- and intraspecific variation encompassing several reproductive systems" – are expected to be found among the diatoms as well. In continuation of drawing the analogy between plants and diatoms, apparently it would seem convenient to characterize breeding systems in diatoms by "borrowing" one of the approaches used in the field of plant reproductive biology, e.g., "mating systems descriptions involve three important aspects: first, whether sexual reproduction occurs at all; second, whether individuals have both sex functions ("cosexual," including hermaphroditic and monoecious plants) or whether some or all are unisexual males or females (dioecy) …; and third, whether cosexual individuals are self-compatible or not …" (Charlesworth, 2006). As regards the first aspect, there have been several confident reports on diatoms which lack a sexual phase in their life cycles (reviewed in Chepurnov et al., 2004). However, "no family or genus, or even a species-rich section of a genus, is known in which all the species are asexual or parthenogenetic" (Mann, 1999). In most asexual diatoms, auxospore formation has been documented. Here, however, auxosporulation was preceded not by meiosis but by a single mitotic division and can apparently be considered as a secondary modification of a basically sexual pathway of development. Hence, asexual auxosporulation is probably best referred to as apomixis, by analogy with higher plants, where the term generally means asexual reproduction through seeds (as opposed to purely vegetative propagation), with meiosis and fertilization being bypassed (e.g., Sadivan et al., 2001). The second aspect, i.e., cosexuality versus unisexuality, is definitely attributable to diatoms. Some diatom species have

been reported in which all the strains isolated were capable of intraclonal allogamous reproduction. Traditionally, they were referred to as monoecious (e.g., Wiese, 1969; Drebes, 1977; von Stosch, 1982). In contrast, many others belonging to various diatom lineages are dioecious (sexually polymorphic), having separate males and females (or opposite mating types if the sexual reproduction pattern is isogamous); some experimental data indicate that here the sex might be determined genetically. In diatoms, the terms "monoecy" and "dioecy" are also interchangeable with "homothally" and "heterothally" (Chepurnov et al., 2004). The third element to consider is "whether cosexual individuals are self-compatible or not" and this can be addressed to diatoms which are capable of monoecious (homothallic) reproduction. In all experimental works known to us, where the experimentalists succeeded to induce (or to observe) the simultaneous production of opposite sex gametes intraclonally, barriers to self-fertilization have never been documented. Finally, it is worth mentioning that in plant science, it is clearly realized that "the simple dichotomy in mating systems between selfing and outcrossing, which is often used as a heuristic tool, is clearly an oversimplification. As originally pointed out by Herbert Baker (1959, p. 178): between the extremes represented by habitual outbreeders and inbreeders lies the probable majority of flowering plants which show varying degrees of outcrossing" (Barrett, 2003). In other words, "most plants have evolved a mixed reproduction strategy" (Richards, 1997). Since current information on mating systems in diatoms is still restricted to descriptive reports on combined versus separate sexes (based largely on morphological criteria), there exists a negligible chance for reliable interpretations of these data in the frame of "the outcrossing–selfing paradigm" (see Barrett, 2003) or the functional gender, i.e., "the extent to which an individual passes genes to the next generation via pollen or seeds" (Charnov, 1982). But, even with this limited data, it is becoming increasingly obvious that many diatom species exhibit more complex "sex types" (sensu Charnov, 1982, p. 254) than uniformly homothallic or strictly heterothallic.

7. Classical Breeding in Diatoms: Provisional Practical Approaches and Methods

Before discussing the methods, we find it particularly relevant to call the reader's attention to the fact that there is overwhelming evidence that sexual systems are significantly different between two major diatom groups, centrics and pennates (Chepurnov et al., 2008). In centric diatoms, only one pattern of allogamous sexual reproduction is known so far and this is oogamy, with production of large "eggs" (one or two per oogonium) and small uniflagellate spermatozoids (often many spermatocytes per gametangium, each spermatocyte dividing to give four sperm). Given suitable environmental conditions, gamete formation occurs spontaneously in cells that have passed a critical size threshold and cells of the opposite sex do not have to be present for sexualization to occur. Both types of gametes were found to be produced in monoclonal cultures in a wide variety of

centric lineages. All of these, then, are homothallic. The opposite type of mating system, heterothally (strict dioecy), has never been found in centric diatoms. In contrast, many pennate species are heterothallic, with presumably genetic sex determination, and here gametogenesis is triggered by cell–cell interaction of compatible cells. There is a wide diversity of isogamous–anisogamous patterns of sexual reproduction among the pennate taxa but flagellate gametes have never been reported.

7.1. CENTRIC DIATOMS

The hermaphroditic nature of centric diatoms and compatibility of opposite sex gametes produced intraclonally makes it easy to induce sexual reproduction in clonal cultures. At first view, it creates an obvious opportunity for obtaining the homozygous (pure) lines considered as important in molecular genetic studies of diatoms (e.g., Walker et al., 2005; Grossman, 2005, 2007). In practice, pure-line selection methods are vital to breeding of self-pollinated crops (Sleper and Poehlman, 2006). Previously, several experimental works performed on strains of various centric diatoms dealt with observations on the offspring obtained via self-fertilization. Although production of inbred F1 generation proved to be possible, it was noticed that the inbred progeny often had a significantly reduced fitness if compared to their parental clones (Sect. 7.4). If the task is to perform controlled sexual crosses, the principal difficulty the experimentalist will typically face is how to discriminate between F1 cells which, in the mixed cultures of homothallic clones, can be produced simultaneously via self- and cross-fertilization. Unfortunately, no appropriate markers are available so far, which would facilitate a reliable selection among the cells of the new generation. Nevertheless, by now, there is a reasonable chance to achieve a sufficiently acceptable control over interclonal crosses between the homothallic clones of centric diatoms if cultures would be manipulatable for serving as only one sex at a time. Here, to obtain female-functioning or male-functioning subcultures, two principal approaches could be proposed. First, it was illustrated that monoclonal cultures of many oogamous centrics exhibited partially temporal segregation of sex function. The size ranges for oogenesis and spermatogenesis were generally different, although they overlap: oogenesis started first, then both eggs and sperms were produced, and finally, when the cells were small, only sperms were formed (e.g., von Stosch, 1954, 1956; Mizuno, 1977; Drebes, 1977). Second, some diatoms were reported to alter their allocation to male versus female function in response to alternations in external conditions. Among the conditions investigated, manipulations with light regimes (intensity and photoperiod) are apparently the most promising and easiest practically to focus on (reviewed in Drebes, 1977; Chepurnov et al., 2004). Finally, efficient microscopic recognition of gametangia and the development of methods for their isolation may create the opportunity for selective transfer of opposite sex gametangia from different cultures into a single small-volume vessel (e.g., a well of 96-well plate) to ensure cross-fertilization.

7.2. PENNATE DIATOMS

Unisexuality of clones in many pennate species and inducibility of gametogenesis via interaction between the cells of opposite sexes (mating types) largely simplifies the development of protocols and procedures to perform controlled sexual crosses. In order to trigger sexual reproduction in a mixture of two opposite mating-type clones, both the clones should be in the sexually inducible cell size range. Usually, external conditions that are favorable for vegetative growth are suitable for successfully triggering sex and obtaining viable F1. To determine "who mates with whom" in a mixed culture, the clones crossed should preferably be visibly different in size as illustrated, for instance, in *Seminavis* (Chepurnov et al., 2008, fig. 3), *Neidium* (Mann and Chepurnov, 2005, figs. 34 and 35), *Sellaphora* (Mann et al., 1999, figs. 36–44), and *Pseudo-nitzschia* (Chepurnov et al., 2005, figs 23–32). In some raphid pennate diatoms, it was shown that small-celled strains approaching the critically minimal size limit became almost or completely incapable of reproducing sexually. Apparently, this is linked with the fact that the small cells typically exhibit reduced motility that, consequently, restricts their capacity for pairing. Undoubtedly, more rigorous attention should be paid to experimentation with pennate diatoms in which mating systems are more complex than strictly heterothallic. In some of the cases reported, two opposite mating types were still recognized but, simultaneously, episodic intraclonal sex also occurred. By analogy with higher plants, this pattern of variation in reproductive system could be tentatively interpreted as "sex inconstancy" (e.g., Dorken and Barrett, 2004). Sporadic appearance of cells which have sex opposite to the rest of the cells in the clone can be illustrated unambiguously in those anisogamous pennate diatoms in which the gametangia are clearly differentiated into "males" (producing migratory gametes) and "females" (producing stationary gametes), e.g., *Sellaphora blackfordensis* (D.G. Mann and V.A. Chepurnov, unpublished) and *Nitzschia longissima* (V.A. Chepurnov, unpublished). In *N. longissima*, we observed occasional appearance of female gametangia in numerous male clones isolated from various locations (Black Sea, North Sea, Mediterranean Sea, U.S. Atlantic coast), but we never found confirmation of intraclonal sexual interaction between two male cells as this was reported (but not documented photographically) by Davidovich et al. (2004). Sometimes, sex inconstancy is a characteristic feature of both mating-type clones, e.g., *Nitzschia lanceolata* (Roshchin, 1994), or it can only be restricted to one mating type, e.g., *Neidium ampliatum* "major" (Mann and Chepurnov, 2005) and *Nitzschia longissima*. Situations when clones are uniformly homothallic and the intraclonal reproduction is vigorous, can also take place among the pennate series, e.g., *Sellaphora bisexualis* (Mann et al., 2009).

7.3. SELECTION METHODS

In conventional plant breeding, numerous methods have been developed and it is always of crucial significance to choose a suitable one for a particular cultivar.

In sexually reproducing plants, the choice is largely based on the knowledge about their natural mating systems, i.e., whether the cultivar is a habitual inbreeder (self-pollinated) or outbreeder (cross-pollinated) (e.g., Allard, 1999; Sleper and Poehlman, 2006). In essence, this is because of "the contrasting effects of outcrossing versus selfing on plant fitness through heterosis and inbreeding depression" (Barrett, 2003). In other words, according to Sleper and Poehlman (2006), in breeding self-pollinated plant, the homozygous nature of the individual plant is exploited while in cross-pollinated – heterozygous. Once the appropriate breeding method has been chosen, the next deciding step is selection, i.e., which of the specimens will be picked out from a diversity of genetic constitutions for further workout and utilization.

Numerous selection procedures exist and, again, their efficient application is largely based on natural mating system mechanisms, e.g., pure-line selection for selfers and sib selection for outbreeders. The knowledge on reproductive systems in diatoms remains very limited but there is enough evidence that future classical breeding programs and population biology studies of sexual diatoms should be analyzed and characterized in the frame of the "outcrossing–selfing paradigm" (Barrett, 2003). The experience with controlled manipulations over diatom sexuality is still fragmentary and hence restricts the opportunity to consider a broad variety of potential selection methods. However, two principal approaches, pedigree selection and bulk population selection, deserve a critical examination. In a very broad context, the pedigree method in progeny selection largely relies on carefully controlled sexual crosses; parents–progeny relationships are reliably recorded. By now, only a few attempts have been undertaken to obtain diatom strains experimentally via homothallic reproduction or interclonal (often full-sib) crosses and then maintain the lineage in a series of successive generations. The principal goal of these investigations was to study the effect of inbreeding, e.g., in pennate diatoms *Synedra*, *Fragilaria*, *Nitzschia* (Roshchin, 1994), and *Achnanthes* (Chepurnov and Roshchin, 1995; Chepurnov and Mann, 1999, 2000). This experience proved to be an excellent foundation for establishing the first carefully documented and well-preserved multigeneration pedigree of a heterothallic pennate diatom *Seminavis robusta*. This laboratory lineage is currently kept at Ghent University, Belgium (http://www.pae.ugent.be/collection). There are many reasons and arguments in favor of selecting *Seminavis* as a model experimental system (Chepurnov et al., 2008). The life cycle is "typical" of diatoms: there are cell size reduction–restitution changes and restoration of size takes place via sexual auxosporulation (Chepurnov et al., 2002). The mating system of *Seminavis* is heterothallic that allows induction of sex to be controlled reliably, via mixing compatible clones. No other diatom examined so far in experiment has approached the same success in mating and F1 development (Chepurnov et al., 2008). Finally, availability of protocols for cryopreservation, reliable control over cell cycle synchronization, and tolerance to inbreeding also contribute essentially to the value of *Seminavis* as promising experimental organism. From a practical point of

view, this example may prove to be instructive for the future development of breeding techniques in various diatoms. Simultaneously, *Seminavis* could be utilized as a good training system to learn practically how sex can be induced and how its results can be controlled including selection and isolation of the offspring.

In comparison with pedigree selection, a bulk-population approach is applied more often in plant breeding, because it is simpler, less effort consuming, and requires modest professional skills. In addition, it allows the breeder to handle very large numbers of individuals inexpensively. Furthermore, during the period of bulk propagation, natural selection tends to eliminate plants having poor survival value. At maturity, the cultivar is harvested in mass, and the seeds are used to establish the next generation in a similar plot. No record of ancestry is kept. Presumably, a similar approach can be applied to mass algae production occurring in a large set of photobioreactors or open pond installations. To our knowledge, no information related to this issue has ever been published nor did come up for discussion before. However, the reason why we have decided to turn to this theme is our experience of mass cultivation of the centric diatom *Cyclotella meneghiniana*. To grow this particular alga, we designed an open installation, filled with c. 70,000 l of water, in which we maintained the bulk continuously during 2 years. Initially, the system was started from a small inoculum which was collected in nature and contained *Cyclotella* cells. Quickly, we succeeded in creating the suitable environment and selecting the necessary nutrient regime and CO_2 supply that resulted in sustainably high productivity of the system over most of the annual cycle. Within a few weeks after inoculation, the algal community became completely dominated by *Cyclotella* which proved to be the most productive and the most adapted to the conditions created. Since then, the absolute numerical dominance of *Cyclotella* remained constant. In addition, rigorous monitoring of various parameters was established including regular microscopic examination of the biomass produced.

Soon it was noticed that the average size of cells in the *Cyclotella* population declined gradually, approaching the critical size limit (Fig. 2a), c. 5–8 μm in cell diameter, known to us from our previous experience (e.g., Håkansson and Chepurnov, 1999). We reacted immediately and quickly developed a procedure where we induced massive size restitution in a large number of cells collected from the installation. Then, the sample containing large *Cyclotella* cells was reintroduced into the main system (Fig. 2e). This approach helped us to prevent loss of the cultivated population due to the size-dependant reason. Simultaneously, under lab conditions, we examined the process of size restitution in detail. By then, the information on the life cycle of *Cyclotella meneghiniana* was incomplete and, in respect of the cell size restitution mechanism, largely controversial (Iyengar and Subrahmanyan, 1944; Schultz and Trainor, 1968; Rao, 1970, 1971, 1996). In addition, according to Beszteri et al. (2005), the diatom identified morphologically as *C. meneghiniana* may actually represent a complex of cryptic

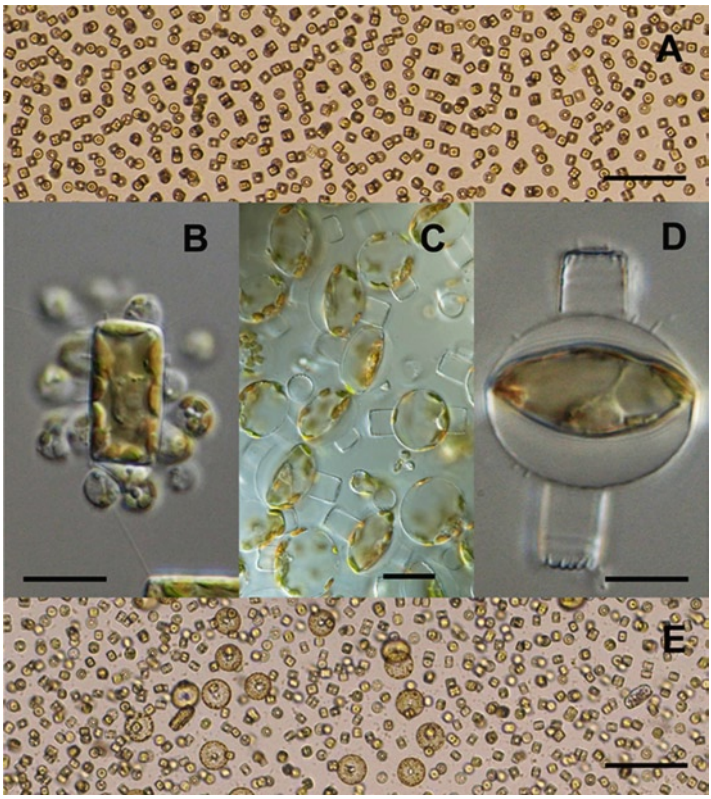

Figure 2. Cell size restitution preceded by oogamous reproduction in *Cyclotella meneghiniana*. (**a**) Small-celled culture. Gamete formation has not been triggered yet. (**b**) Gametogenesis has been induced. Oogonium and numerous spermatozoids attracted by the female gametangium are illustrated. (**c**) Vigorous auxosporulation. (**d**) Cell size restitution has occurred. Large initial cell is enclosed in the auxospore cell wall (thecae of parental oogonium are still attached to the wall). (**e**) The culture containing both "old" (small-celled) and "new" (large-celled) generations. Scale bars, 100 μm (**a** and **e**), 10 μm (**b** and **d**), and 25 μm (**c**).

species; thus, there is no guarantee that the previous authors investigated the life cycle attributes of a single species. In our case, we have confidently identified that the size restitution occurred via auxospore formation preceded by oogamous sexual reproduction (Fig. 2b–d). From this, we can conclude that while reintroducing the large-celled inoculum in the production system, we simultaneously bring a great variety of new genotypes resulting from sexual recombination within the initial gene pool. Currently, we focus on the optimization of this method for *Cyclotella* and expect that this approach will eventually be transformed into a technological procedure based largely on the same principles as bulk-population selection utilized in plant breeding.

7.4. INBREEDING AND ITS CONSEQUENCES

The effect of inbreeding, i.e., self-fertilization or crosses between closely related individuals, is of special interest in plant science and practical plant breeding. More precisely, it usually concerns a phenomenon known as inbreeding depression. The debilitating effect of inbreeding, e.g., the reduction in viability, fertility, and increased susceptibility to disease pathogens, was commonly detected in inbred offspring of various naturally cross-pollinated plants. It is well known that inbreeding increases homozygosity; for instance, selfing (an extreme form of inbreeding) reduces heterozygosity by 50% each generation. The harmful effect of inbreeding results primarily from the manifestation of deleterious recessive alleles that are normally hidden from selection in the heterozygous condition in outcrossing populations. Broader spectrum of hypotheses explaining the phenomenon of inbreeding depression can be found elsewhere (e.g., Charlesworth and Charlesworth, 1999; Carr and Dudash, 2003).

Deleterious consequences of inbreeding have been repeatedly reported in various diatoms and, hence, for further exploration of diatom breeding programs, this must definitely be taken into account. In centrics, the severe negative effect of inbreeding was evident already in F1 progeny generated after intraclonal sexual reproduction in *Stephanopyxis* (von Stosch, 1965) and *Chaetoceros* (von Stosch et al., 1973). In a simple manner, the level of inbreeding depression could be determined by comparing the fitness of selfed vs. outcrossed progeny. For centric diatoms, such information is absent in scientific literature. In one instance, however, both selfed and outcrossed progeny were simultaneously obtained in a centric diatom and this was *Melosira moniliformis* from the Black Sea (A.M. Roshchin and V.A. Chepurnov, unpublished). The outcrossed progeny (6 clones) outperformed self-progeny (12 clones). The differences were evident with selfed F1 clones, in general, having lower viability and fertility than outcrossed ones. The data on inbreeding depression suggest that many homothallic centric species are habitual outbreeders in nature. In some vascular plant, monoecy may have evolved as a mechanism to promote outcrossing (e.g., Charlesworth and Charlesworth, 1978), especially when male and female organs of a single flower are mature at different time (dichogamy). Dichogamy is a widespread floral strategy and "has been almost universally interpreted as an anti-selfing mechanism" (Barrett, 2003). Similarly, an ability of homothallic clones of many centrics to separate their sex functions temporally can also illustrate that homothally is not in contradiction with the situation when the realized mating system of populations might be predominantly outcrossing. In addition, it should also be taken into account that most centric diatoms are planktonic organisms, i.e., they live in the water column where a degree of mixing is typically fairly high. This significantly increases the chance of spatial separation of opposite sex gametangia produced by a single strain. Thus, homothally could still be an effective breeding strategy promoting outcrossing and the incidence of selfing is apparently very low in natural populations.

A negative influence of inbreeding was also repeatedly reported in obligatory or predominantly heterothallic pennate species. This effect was mentioned in inbred clones obtained after both sib-crosses and intraclonal reproduction (where it occurred). Typically, inbreeding led to general loss of vigor and abortion of cells at the stage of developing auxospores and initial cell formation, e.g., *Achnanthes longipes* (Chepurnov and Mann, 1999, 2000) and *Nitzschia lanceolata* (Roshchin, 1994). In addition, in *Tabularia* (as *Synedra tabulata* in Roshchin, 1994), inbreeding also resulted in selective elimination of one of the sexes during auxospore formation. *Seminavis robusta*, in turn, proved to be almost unique among the pennates (see also *Fragilaria* in Roshchin, 1994) in exhibiting no signs of inbreeding depression in a series of subsequent sub crosses (Chepurnov et al., 2008, fig. 4). This attractive feature of the *Seminavis* experimental system offers a realistic opportunity for establishing homozygous lineages required for molecular genetic studies of diatoms (e.g., Grossman, 2007).

If inbreeding depression takes place and this is mainly due to recessive deleterious alleles manifested in the phenotype when in a homozygous state, the theory and practice of plant science show that the genetic load may potentially be "purged" in a number of successive inbred generations that, consequently, may result in a rebound in fitness (e.g., Crnokrak and Barrett, 2002). Apparently, it would be worth trying to apply the same approach to diatoms which exhibit a drop in values of fitness components in the first inbred generations. It can't be excluded that, at least in some of these diatoms, further continued production of inbred progeny in subsequent generations might eventually lead to a recovery of fitness to levels comparable to originally selected strains. If restoration of heterozygosity is required, the purged inbred lines can be outcrossed.

Hypothetically, one should expect that no inbreeding depression must occur in diatoms which are strict inbreeders. Pennate *Nitzschia fonticola* is apparently such a species. The mode of sexual reproduction in this *Nitzschia* is obligatory automictic: the sexual process occurs within a single gamatengia and no cell pairing is involved in induction of meiosis, both intraclonally and in mixed cultures (Trobajo et al., 2006). The offspring was examined in a series of subsequent generations. Not surprisingly, the experimentally obtained strains grew and reproduced as good as the original isolates (V.A. Chepurnov, unpublished observations). Natural populations of this diatom are expected to be highly homozygous.

7.5. POTENTIAL GENETIC RESOURCES

Once classical genetic manipulations with various diatom species are practiced routinely in various research laboratories worldwide and this approach finds applications in "strain improvement" programs, an access to a diverse source of genetic material will inevitably become a crucial prerequisite. In agriculture, assembly of a wide assortment of germplasm is often considered as "the initial step in a breeding program" (Sleper and Poehlman, 2006). The strategy and methods for collection,

characterization, and conservation of domesticated plant species, including their wide relatives, and utilization of these genetic resources (still mainly via sexual recombination) are based on tremendous practical experience and have also received considerable scientific attention (e.g., Damania, 2008; Sachs, 2009). Coordinated efforts between basic research activities and industrial applications are yet to be developed for diatoms, but perhaps it is time to initiate a discussion on the exploration and utilization of potential diatom genetic resources and to define important priorities in this field. First, we believe that the way how germplasm collections of famous plant cultivars are preserved and exploited may prove to be a very rich source of useful information and relevant experience. For instance, a review by Holbrook and Stalker (2003) on peanut breeding and its genetic resources could be, in our opinion, very illustrative and instructive. The article shows how different scientific disciplines (e.g., taxonomy, phytogeography, reproductive biology, and genetics) can substantially contribute to the evaluation and management of the genetic resources. This multidisciplinary knowledge also provides a solid foundation for the practical activities including application of different conventional breeding procedures. From another example (maize), we can learn about important factors responsible for efficient utilization of genetic resources to extend genetic variability in breeding programs and to secure continuous genetic improvement (Nass and Paterniani, 2000). Undoubtedly, from data of basic diatom research works, it is already possible to extract a considerable amount of information which is "breeding relevant." This information is "hidden" in and scattered over numerous publications devoted to the study of taxonomy, speciation, population biology, and biogeography using approaches of different classical disciplines (e.g., morphology, cytology, reproductive biology) in combination with advanced molecular methods. In general, these contributions clearly illustrate that populations of various diatoms are highly diverse genetically. Molecular microsatellite-based studies have documented an extensive clonal genetic diversity within a single population in both marine diatoms, e.g., centric *Ditylum brightwellii* (Rynearson and Armbrust, 2005) and pennate *Pseudo-nitzschia pungens* (Evans et al., 2005), and freshwater diatoms, e.g., pennate *Sellaphora capitata* (Evans et al., 2009). In addition, it was also reported that there can be significant differentiation between populations belonging to a single diatom species (e.g., Rynearson et al., 2006; Evans et al., 2009). Moreover, further investigations of mating systems involving controlled crossing experiments are expected to be of increasing significance for revealing the potential of classical breeding approach for the exploitation of genetic diversity accumulated in natural diatom populations. For various diatoms found in different habitats, it was already repeatedly illustrated that clones, which were isolated from different (often far distant) geographical locations and identified adequately as belonging to a single species, typically exhibited sexual compatibility producing viable F1 progeny, e.g., in *Sellaphora* (Behnke et al., 2004; Evans et al., 2009), *Nitzschia* (Trobajo et al., 2009), *Pseudo-nitzschia* (Casteleyn et al., 2008), and *Seminavis* and *Achnanthes* (V.A. Chepurnov, unpublished data). Thus, the extensive genetic diversity of natu-

ral populations and the evidence that even intercontinental (for freshwater forms) and interoceanic (for marine) hybridization between the isolates is possible in experiment imply great potential for the classical breeding approach in diatoms. It can't be excluded that the growing demand in practical utilization of diatoms will result in an increasing number of germplasm banks focusing on their industrial applications. Today, however, it seems important to critically evaluate the "genetic resources" of diatoms which are currently available in general algal collection and already have economic value (e.g., those used in aquaculture). Then, the priorities for their further development should be defined. There is little doubt that appropriate characterization of both biologically significant and economically useful traits, i.e., the information underpinning the development of realistic breeding approaches, must be among these priorities. Ironically, there are still no satisfactory descriptions of the life cycle in the world's most studied diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, even though their genomes are available (Armbrust et al., 2004; Bowler et al., 2008), and they have found practical applications (e.g., Lebeau and Robert, 2003).

8. Some Future Prospects

Once we have acknowledged that achievements of and advances in plant reproductive biology and plant breeding are indispensible for efficient progress in fundamental and applied studies of diatoms, it would also be logic to suggest that the analysis of future trends and challenges in plant sciences and biotechnologies will equally prove relevant to the future of diatom research. A fortiori, since increasingly more researchers who initially have molecular-based experience in plant sciences reveal their growing interest in diatoms, which is largely due to the tremendous ecological importance of this algal group and its promising industrial applications. Thus, among the literature available, a personal view of J.W. Snape (2004) on "challenges of integrating conventional breeding and biotechnology" was chosen by us for consideration in detail. Most of the challenges and problems this author discussed in his article can be well extrapolated to what already exists or is expected to occur in "diatom breeding" as soon as the classical genetic methods have also found their practical applications. Before discussing the challenges, the author has specified plant breeders' needs. "Plant breeders need sources of genetic variation; tools for its manipulation; and tools for validating that they have achieved their objectives in putting together and identifying new adapted gene complexes. Added to this are techniques to speed up the whole process." In accordance with these requirements, the principal challenges were formulated as follows:

– "The major challenge is to translate the enormous advances in understanding into technologies that plant breeders can apply routinely. But "we have created a 'technology gap' where our understanding and resource development capacity in plant genomics and biological understanding has exceeded our

ability to apply it to practical plant breeding problems and situations" (see also Sasaki, 2009).
- "We have an administrative challenge in creating the right mix and balance between investment into fundamental discovery into plant processes and the market-driven needs of crop improvement. However, the 'success percentage' of scientific discovery in models, 'faster, easier, tools there' attitude, often drives awards from peer-review granting bodies, rather than drivers for solving a plant breeding problem or developing new paradigms for plant breeding" (see also Knight, 2003).
- "The challenge is also one of restraint in not overexaggerating the promise and the speed of living up to that promise. The history of biotechnology in plant breeding research is arguably littered with 'over-egged' research."
- "The current most obvious and pertinent use of genetics and genomics information in conventional plant breeding is its application for marker-assisted selection" (see also Collard and Mackill, 2008).
- "There is also a recognized problem in this respect in having trained personnel able to recognize phenotypes! This may sound trivial, but is a real problem in European research Institutes which have a surfeit of molecular biologists, but a huge deficit of phenotypers!"

Despite the obvious utility and advantages, "today, … less resource is put into the development of 'steam biotechnology' … that do not require complex equipment, protocols, consumables or highly trained personnel" (e.g., tissue culture systems or cytogenetics). Consequently, "there is a serious risk that the skills will be lost to plant breeding as the major practitioners of 'traditional' science' retire, and the lab, glasshouse, and field skills passed down in laboratories for much of the last century will be lost to the corporate memory… We need to maintain the infrastructures and training to maintain these so that the 'intellectual space' between fundamental plant science and plant breeding application is populated."

If classical diatom breeding has been accepted as having a perspective for practical applications, clearly this approach should be ranked among the "low-tech" systems; it would not require much investment to develop this. It is quite likely that controlled manipulations over sex in diatoms will quickly find direct applications (e.g., in various "strain improvement" programs) and contribute significantly to exploration of diatoms using advanced scientific technologies. However, the risk of losing much of the lab experience and the skills in manipulating sex in diatoms is high. Unfortunately, the diatom models used for much current diatom research do not offer the opportunity to sharpen and develop these skills: sex has never been documented confidently in their cultures. New experimental systems are definitely required. Concrete practical steps for selecting such model systems were recently performed (Chepurnov et al., 2008), that could help lay the foundation for both practical training in classical breeding methods and obtaining the relevant molecular genetic information.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 195–209
DOI 10.1007/978-94-007-1327-7_8, © Springer Science+Business Media B.V. 2011

EPIZOIC AND EPIPHYTIC DIATOMS

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1. Introduction

Diatoms live in many habitats; their primary requirements are adequate water, light, and nutrients, especially silica for their frustules. One major habitat is that of adherence to living substrata, both plants or algae (epiphytic diatoms) and animals (epizoic diatoms). Species that inhabit this habitat differ from planktonic and freely moving benthic forms in several ways. Many of them are highly silicified and sessile (e.g., *Arachnoidiscus*, *Isthmia*) and could not remain floating long in the plankton or could become buried in sediments. Most produce copious amounts of mucilage for attachment, although this is not limited to epibionts (e.g., the plankonic *Thalassiosira* and *Cyclotella* attach in chains using mucilage threads). Most aquatic vegetation, saline or freshwater, hosts diatoms (Round et al., 1990; Round, 1992). Diatoms are even epiphytic upon other diatoms (Tiffany and Lange, 2002). They are not parasitic, rather attaching to the external plant tissues for a "spot in the sun." Currents flowing past the hosts as well as swaying of their fronds provides constant access to nutrients. Less commonly reported are epizoic diatoms that have been reported from a number of species varying from ciliates and copepods (Fig. 1) to whales.

Diatoms attach to these substrata in several ways. The material used is a mucilaginous material (extracellular polymeric substances) (Hoagland et al., 1993). Adnate diatoms (e.g., *Cocconeis* spp.) appress tightly to surfaces. Monoraphid diatoms in this category attach by mucilage secreted by the valve possessing the raphe, the non-raphid valve being unable to extrude mucilage. Thus, all valves lacking the raphe face outward (Fig. 2).

A number of diatom species attach by a mucilage pad (Fig. 2). Other diatoms attach by a mucilage stalk (e.g., Fig. 1). The structure of these stalks is quite complex (Wustman et al., 1997, 1998; Wang et al., 1997; Gebeshuber et al., 2002; Chiovitti et al., 2007).

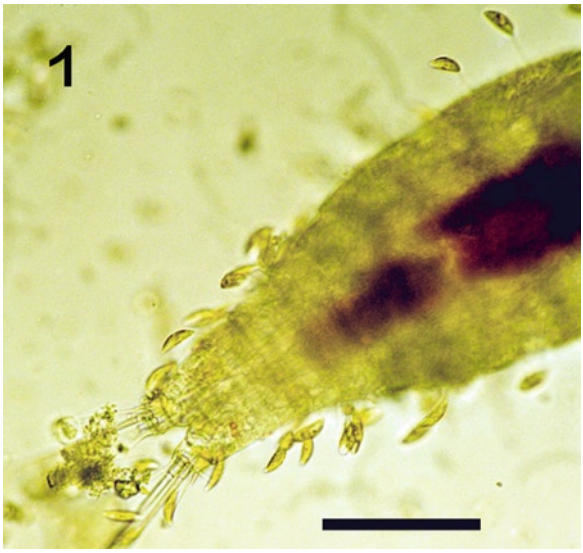


Figure 1. LM (light microscopy). *Pseudohimantidium pacificum* on the harpacticoid copepod, *Euterpina acutifrons* (from Mission Bay, California). Scale bar = 500 μm.

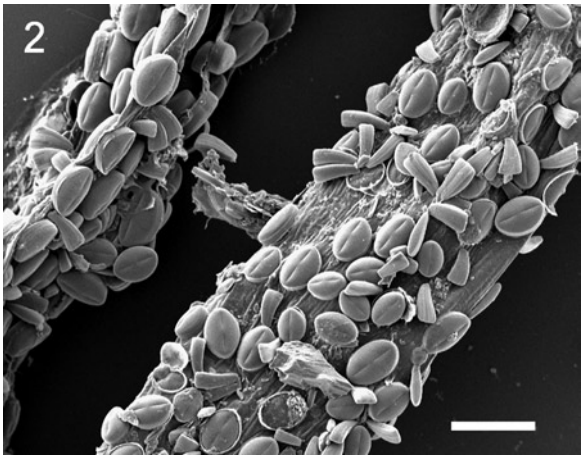


Figure 2. SEM (scanning electron microscopy). *Cocconeis placentula* and *Rhiocosphenia* sp. on *Chaetomorpha linum*. *Cocconeis* is an adnate species, *Rhoicosphenia* attaches via a mucilage pad. Scale bar = 50 μm.

2. Epizoic Diatoms

Diatoms are found attached to a number of diverse animal groups such as ciliates (Gárate-Lizárraga and Muñetón-Gómez, 2009), copepods (Fee and Drum, 1965; Takano, 1983; Gibson, 1978; Hiromi et al., 1985; Winemiller and Winsborough, 1990;

Gárate-Lizárraga and Muñetón-Gómez, 2009; fig. 1), cladocera (Gaiser and Bachmann, 1993), barnacles (Bigelow and Alexander, 2000), hydroids (Round et al., 1961; Round et al., 1990; Romagnoli et al., 2007), krill (McClatchie et al., 1990), bryozoans (Wuchter et al., 2003), whales and porpoises (Hart, 1935; Holmes, 1985), and diving birds (Croll and Holmes, 1982; Holmes and Croll, 1984). The diatoms may be adnate or attach by a stalk. In most cases, there is a high degree of specificity among the epibionts. The question of why is yet to be answered by future research. Perhaps, it is the nature of the surface of the host's skin or cuticle. To illustrate, in Fig. 1, only a single species (*Pseudohimantidium pacificum* Hustedt & Krasske) is attached to the copepod, *Euterpina acutifrons*, from Mission Bay, California. Some of the most studied diatom–animal associations are described below. Whether the diatoms are harmful to the host or not is unknown.

2.1. WHALES AND PORPOISES

A *Cocconeis*-like diatom was observed on the hairs of sei whales as long ago as 1934 (Hausman, 1934, fig. 1). Holmes (1985) described several new species (*Bennettella ceticola* and *Epipellis oiketis*) found only on the skin of cetaceans including killer whales and Dall's porpoises. A Japanese study of the pattern of diatom (probably *Bennettella ceticola*) attachment on several species of whales captured in 1967–1968 found that diatoms have a patchy distribution on the skins of whales (Kawamura, 1992). Kawamura speculated that the diatoms may be partially saprophytic. In sei whales, diatoms were located primarily around the eyes whereas in fin whales, they were mostly in patches along the back. He attributed this to a difference in the structure of the skin and/or the past history of migration seen between species of whales. Denys (1997) found *Epiphalaina* and *Tursiocola* on a stranded sperm whale. Morejohn (1980) found a number of species of diatoms on the skin surface of Dall's porpoise, harbor porpoise, and Pacific white-sided dolphins. Populations of diatoms can be dense enough to cause yellow coloration of the skin (Feinholz and Atkinson, 2000). Nagasawa et al. (1989) found diatoms in the sediments presumably shed by Dall's porpoises in coastal waters of Japan. Contact between individuals is the likely way diatoms are transferred between individual cetaceans (Holmes et al., 1993).

2.2. SLOTHS

Along with various filamentous green algae and cyanobacteria, the diatom *Melosira* sp. was reported living on the sloth (*Bradypus*) (Thompson, 1972). Their fur is frequently green tinged. Apparently, these animals use algae as a form of camouflage (Aiello, 1985). In the wet season, when vegetation is green, their fur is greenish, in the dry season the algae desiccates and becomes brown.

2.3. BIRDS

Diving birds such as the red-throated loon, arctic loon, and common murre can have dense populations of diatoms on their feathers (Croll and Holmes, 1982; Holmes and Croll, 1984). These can be a source for the dispersal of diatoms from one water body to another.

2.4. CRUSTACEA

Copepods are very common small crustaceans in the zooplankton of fresh- and saltwaters and are important members of aquatic food webs. Copepods can be free living or parasitic, and sometimes are infested by epizoic ciliates and diatoms such as *Falcula* and *Pseudohimantidium* (Stoermer, 1964; Fee and Drum, 1965; Hiromi et al., 1985; Prasad et al., 1989; Winemiller and Winsborough, 1990). Prasad et al. (1989) found *Falcula hyalina* on copepods in Florida and *Pseudohimantidium* appears to be common in the Pacific (Hiromi et al., 1985; Gárate-Lizárraga and Muñetón-Gómez, 2009) but is also found in Florida (Gibson, 1978). Typically, only one species of diatom is attached to an individual (as in Fig. 1), why this should be so is unknown (Hiromi et al., 1985). It is also unclear whether this epizoic load hinders the copepod in some way such as slowing down its avoidance of predators.

Cladocerans (water fleas such as *Daphnia*) are common in freshwaters and diatoms have rarely been reported living on them (Gaiser and Bachmann, 1993, 1994). Gaiser and Bachmann (1993) reported that *Synedra cycloporum* was the dominant diatom living on large *Daphnia* in lakes in Iowa but many other species were also found on them.

Diatoms have even been reported living abundantly on the cirri of barnacles (Bigelow and Alexander, 2000).

2.5. MOLLUSKS

A rich flora may be found on bivalve shells (Round, 1981). The surface of shells of mollusks resembles rocks in the intertidal. Diatoms growing on shells are typical of those growing nearby on other surfaces, indicating there may be nothing special about the shells as a substratum for diatoms (Pantazidou et al., 2006).

2.6. HYDROIDS

A rich diatom community associated with hydroids (sessile invertebrates) and dominated by *Cocconeis* has been illustrated by Round et al. (1990, fig.76e).

The load of epiphytes can be so heavy as to color the hydroids brown. Interestingly, they are found only on the perisarc (branches) not on the hydranth (portion with tentacles) (Romagnoli et al., 2007). Romagnoli et al. (2007) found a variety of diatoms attached to *Eudendrium racemosum*, including adnate forms such as *Amphora* and *Cocconeis* on the base and central parts of the hydroid colonies, erect forms such as *Tabularia* and *Licmophora* on the apical portions, and, rarely, tube-dwelling species such and *Berkeleya* and *Paribellus*.

3. Epiphytic Diatoms

In shallow fresh and saline waters, vegetation may be attached or free floating. Flowering plants abound in freshwater lakes and streams, and a few flowering plants have invaded the sea (seagrasses). Macroalgae is also common nearshore as deep as light permits (seaweeds and filamentous algae). All these can provide habitat for epiphytic diatoms. *Cladophora* and *Chaetomorpha*, for example, can be heavily encrusted with diatoms, especially *Cocconeis pediculis* (Fig. 2, Dodds, 1991 and references therein). One primary difference between planktonic and epiphytic diatoms is the degree of silicification. Planktonic diatoms must contend with staying in the upper euphotic zone, so they tend to be lightly silicified (Round et al., 1990). Epiphytic diatoms, on the other hand, remain attached to their host and can use thicker, stronger valves. Heavier silicification may protect them from grazing as the frustules are harder to crack open (Hamm et al., 2003). Being attached to aquatic vegetation permits access to light and nutrients, some of which might be leachates from the host plants themselves.

3.1. SEAWEEDS

Seaweeds are usually grouped into three categories, green, red, and brown, and all can host diatoms (Tanaka, 1986). For instance, *Hyalodiscus stelliger* is attached to a brown seaweed in Fig. 3, the diatoms in Figs. 4–9, 10 and 11 are on red seaweeds and those of Figs. 2, 12 and 13 are on a green filamentous seaweed. They adhere quite tightly to the surface of the seaweed and are unlikely to be removed by water turbulence or wave action (Tanaka, 1986).

Seaweeds are common hosts for epiphytic diatoms (e.g., Tanaka et al., 1984; Al-Handal and Wulff, 2008; Totti et al., 2009). Macroalgae can be so infested by diatoms as to negatively impact the seaweed. This is attributed to the interception of light and nutrients by the diatom limiting growth of the seaweed (Ruesink, 1998). Unicellular diatoms reproduce more rapidly than multicellular seaweed and can rapidly smother the larger organism (e.g., Figs. 2 and 12), changing their hue from red or green to the golden color of the diatom epiphytes.

Figure 3. Light microscopy. *Hyalodiscus stelliger* attached by a mucilage pad to *Ectocarpus* sp. Scale bar = 100 μm.

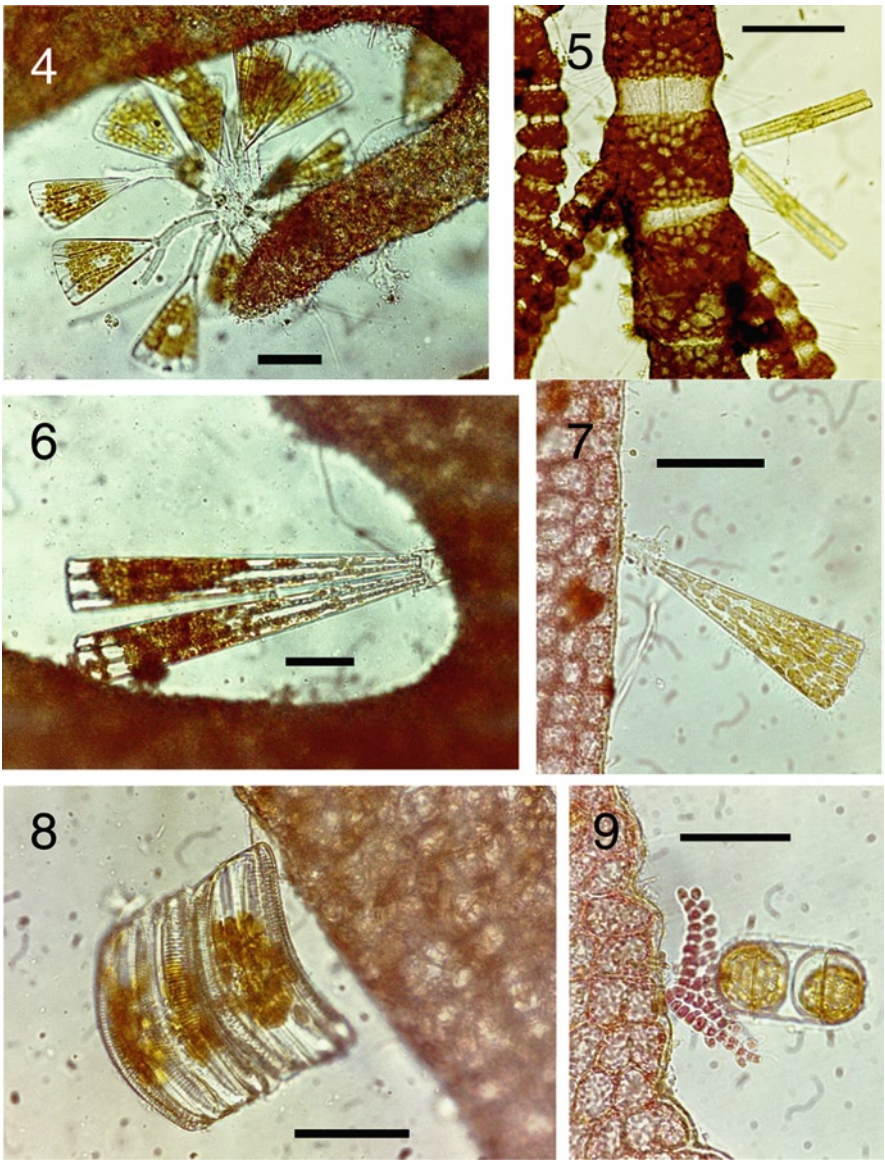
Grazers (herbivores, including amphipods, crabs, isopods, and polychaete worms) feed on the diatom epiphytes (Ruesink, 2000). This may lead to a benefit to the host as it is cleared of its epiphyte load. Many seaweeds also produce compounds containing iodine and bromine to deter grazing, protecting them from the same herbivores (Westlund et al., 1981).

In Fig. 4, a colony of *Licmophora* is attached to a red seaweed. Its branching stalks are clearly seen. *Ardissonea*, *Climacosphenia*, *Licmophora*, and *Gephyria* are connected by mucilage pads in Figs. 5–8, respectively. The *Melosira* in Fig. 9 is epiphytic upon an epiphytic red seaweed, reminiscent of the old saying about "big fleas have little fleas upon their backs to bite them." Figures 10 and 11 illustrate *Arachnoidiscus* on a red seaweed. At times, this large species can be so abundant that it can be seen on the seaweed with the naked eye (Round et al., 1990). *Tabularia* encrusts the green seaweed in Fig. 12 and the mucilage stalks of *Achnanthes brevipes* are visible in Fig. 13.

3.2. SEAGRASSES AND SURFGRASS

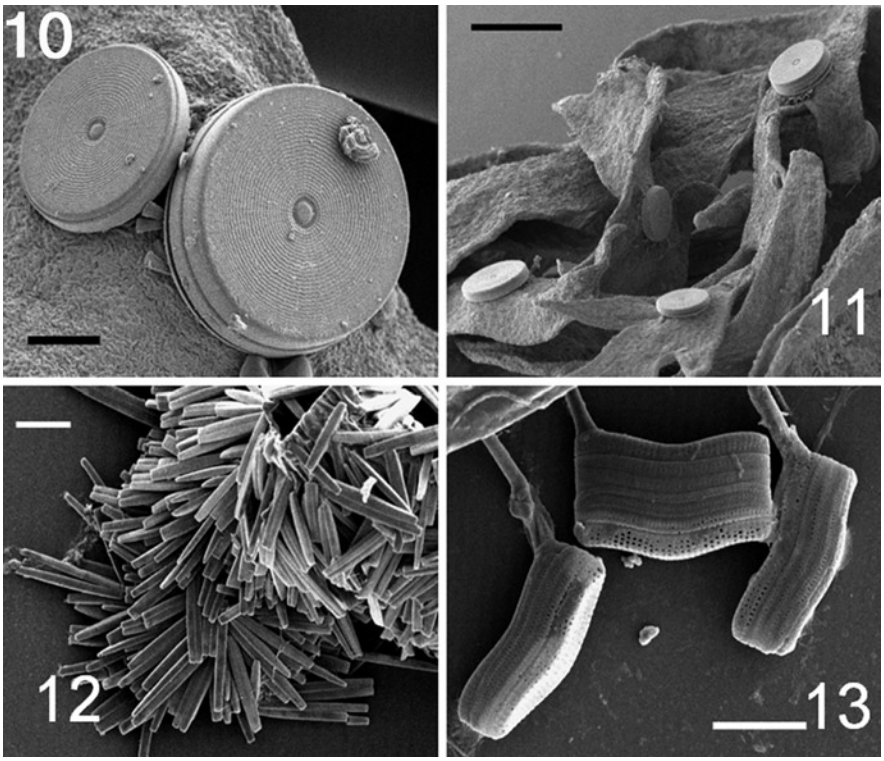
Seagrass (marine angiosperms) meadows grow in shallow waters around the world. They are important nurseries for fish and invertebrates. Their flat, broad leaves are often epiphytized by diatoms and other algae (Harlin, 1980). Seagrasses have a seasonal growth pattern with greatest growth in spring and summer (Green and Short, 2003). Thus, the available substrate varies through the growing season.

Diatom epiphytes on *Ruppia maritima* (widgeon grass) were studied in New Jersey by Sullivan (1977) and in Brazil by Ferreira and Seeliger (1985). Sullivan



Figures 4–9. Light microscopy. Figure 4 *Licmophora* sp. on a red seaweed. Figure 5 *Ardissonea* sp. on *Ceramium* sp. Figure 6 *Climacosphenia monilegera* on a red seaweed. Figure 7 *Licmophora* sp. on a red seaweed. Figure 8 *Gephyria media* on a red seaweed. Figure 9 *Melosira* sp. on *Acrochaete* sp, itself epiphytic on a red seaweed. All scale bars=50 µm.

(1977) felt that the origin of these diatom colonies was from the sediments as the plants grew. Ferreira and Seeliger (1985) followed the sequence of diatoms colonization on artificial substrates resembling *Ruppia* leaves within the bed and



Figures 10–13. Scanning electron microscopy. Figure 10 *Arachnoidiscus ehrenbergii* on *Odonthalia floccosa*. Scale bar=100 µm. Figure 11 *Arachnoidiscus ehrenbergii* on *Odonthalia floccosa*. Scale bar=500 µm. Figure 12 *Tabularia parva* on *Chaetomorpha linum*. Scale bar=50 µm. Figure 13 *Achnanthes brevipes* on *Chaetomorpha linum*. Scale bar=20 µm.

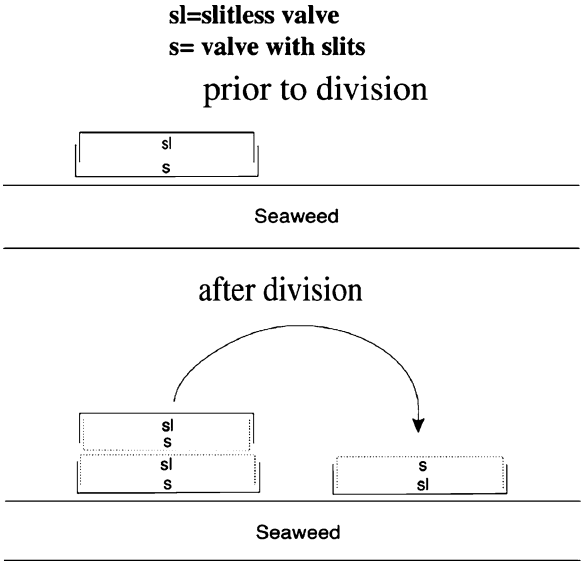


Figure 14. Proposed method of recruitment of upper daughter cell of newly divided *Arachnoidiscus ehrenbergii* to open surface of a red seaweed in order for epivalve to be the attached valve.

found that adnate species such as *Cocconeis* were the first to grow, followed by *Synedra* (now *Tabularia*) *fasciculata* attached by pads and finally by stalk-forming forms such as *Biddulphia*.

Diatoms adhering to *Zostera marina* (eelgrass) have recently been studied by Hasegawa et al. (2007), Jaschinski and Sommer (2008), and Lebetron et al. (2009). Hasegawa et al. (2007) found a clear seasonality in the growth of epiphytes with *Cocconeis* again the pioneer genus followed by more upright forms.

Posidonia oceanica is an important seagrass found in the Mediterranean. Its meadows are presently being endangered by an exotic seaweed, *Caulerpa taxifolia* (Pergent et al., 2008). De Stefano et al. (2000, 2003) and De Stefano and Romero (2005) studied adnate forms such as *Cocconeis* and *Campyloneis* commonly found on *Posidonia*. Most of their studies were taxonomical in nature.

Turtle grass (*Thalassia testudinum*) is a tropical seagrass. It supports a lush community of diatoms (Armitage et al., 2006; Frankovich et al., 2006, 2009) similar to that growing on the prop roots of the mangrove, *Rhizophora mangle* (Frankovich et al., 2006) and dominated by species in the genus *Mastogloia*.

Surfgrass (*Phyllospadix*) can also have attached diatoms (Stewart and Myers, 1980; Harlin, 1975).

3.3. OTHER FLOWERING PLANTS: SUBMERGED AND FLOATING

In marine waters, mangrove prop roots and pneumatophores provide excellent substratum for diatom communities (Siqueiros-Beltrones et al., 2005). In freshwaters, duckweed, *Lemna* spp., commonly supports populations of *Lemnicola hungarica* along with *Achnanthes*, *Gomphonema*, and *Fragilaria* (Goldsborough, 1993; Buczkó, 2007). Epiphytic diatoms were found on *Najas* and *Chara* in farm ponds in Oklahoma (Troeger, 1978). Sullivan (1982) studied epiphytic diatoms on *Spartina alterniflora* (cord-grass). Other aquatic vegetation known to host diatoms are *Potomogeton*, *Ceratophyllum*, *Eichornia*, and *Echinochloa* (Abo El-lil, 2003).

3.4. BRYOPHYTES AND AQUATIC FERNS

Diatoms are often associated with bryophytes, some of which are epiphytic. Knapp and Lowe (2009) found abundant diatoms growing attached to various mosses and liverworts in the Great Smoky Mountains National Park. The most abundant of these were in the genera *Eunotia*, *Planothidium*, and *Achnanthidium*. They found that the greatest densities of diatoms were found within whorls of leaves (protected from grazers). Also, the adaxial leaf surfaces (top) with greater exposure to the sunlight generally had much higher densities than the abaxial (bottom). Bryophytes, of course, inhabit moist habitats. Aquatic ferns such as *Salvina rotundifolia* (Tesolin and Tell, 1996) and *Azolla* (Abo El-lil, 2003) can also act as hosts for diatoms.

4. Conclusions

It is obvious from the above examples that diatoms can grow attached to many living aquatic organisms. Some creatures do not allow diatoms to grow on their outer surfaces. Many turtles, for example, spend periods of time basking in the sun, and this would desiccate any potential algal epiphytes. Likewise, the surface of some creatures (such as frogs) might not be adequate for attachment or is constantly renewed or shed frequently. Some seaweeds produce allelopathic substances that inhibit the growth of diatoms (Tanaka and Asakawa, 1988). This might explain why some seaweeds have fewer epiphytes than others. Also being attached to aquatic vegetation or fauna avoids the danger of being buried by sediments.

Limitations for diatom growth include silica, nutrients, and solar insolation. Thus, diatoms cannot grow below certain water depths (depending on water turbidity). As long as light, moisture, nutrients, and an appropriate surface are available, attached diatoms will probably be present.

Future research could include quantitative studies on the importance of attached diatoms to overall primary productivity in various habitats. Because of their rapid growth, they play an important role for invertebrate grazers and thus the entire ecosystem. The role of epibionts in many communities may have been underestimated. The interaction of host and epiphytic and epizoic diatoms is an avenue for future exploration. Another potential direction for investigation is determining how epiphytic and epizoic diatoms recruit to newly formed surfaces (e.g., fresh growth of plant hosts or newborn animals). Seaweeds often grow seasonally (Lüning, 1993), and new surfaces for potential attachment by diatoms are continually being produced. For a specific example, *Arachnoidiscus ehrenbergii* inevitably attaches by its epivalve (either slitted or slitless) in a monolayer on red seaweeds (personal observations, Fig. 10). When a cell divides vegetatively, the upper daughter must detach and somehow flip over and attach to an available spot on the host (possibly encountering it by chance) (Fig. 14). Or is there a method for locating the host without loss of the new daughter cell? *Cocconeis* seems to have a similar quandary (see Fig. 2).

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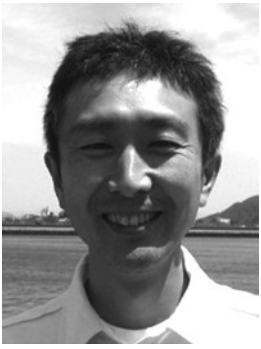

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 211–225
DOI 10.1007/978-94-007-1327-7_9, © Springer Science+Business Media B.V. 2011

DIATOM VIRUSES

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1. Viruses in Marine Environments

Since the first reports of large numbers of virus-like particles (VLPs) in natural seawater, the aquatic viruses have been intensively studied (Bergh et al., 1989; Wommack and Colwell, 2000). Currently, the viruses are regarded as one of the major biological factors that regulate carbon cycling, microbial biomass, and the genetic diversity of protists and algae (Fuhrman, 1999; Brussaard, 2004; Suttle, 2005; Brussaard et al., 2008). The number of viruses in natural waters is estimated to be *ca.* 10^6 particles ml^{-1} in oligotrophic waters and *ca.* 10^8 particles ml^{-1} in higher productive areas; however, this may be underestimated due to insufficiencies in the detection methods and instruments (Suttle, 2007; Tomaru and Nagasaki, 2007).

Most of the virus particles in aquatic environments are considered to be bacteriophages because of the high abundance of their hosts in natural waters. And the viruses infecting eukaryotic phytoplankton may rank second in abundance. At least 29 viruses infecting eukaryotic microalgae have been identified and reported (Suttle, 2007; Nagasaki, 2008). Many of these viruses harbor a large double-stranded DNA (dsDNA) genome and thus are classified into the family Phycodnaviridae based on the deduced amino acid sequences of the DNA polymerase domain (Wilson et al., 2005). Other than phycodnaviruses, recent studies show diverse microalgal virus species harboring single-stranded DNA (ssDNA), single-stranded RNA (ssRNA), and double-stranded RNA (dsRNA) genomes (Brussaard and Martinez, 2008). Although the basic biological characters of these viruses have been intensively studied, their taxonomic position is not sufficiently understood due to the few marine viruses in the databases. This indicates the study of marine viruses is still one of the frontier fields within the aquatic sciences.

Several microalgal host–virus systems in natural environments have been studied, e.g., *Emiliania huxleyi* (Prymnesiophyceae) (Schroeder et al., 2003; Allen et al., 2007), *Phaeocystis globosa* (Prymnesiophyceae) (Baudoux and Brussaard, 2005), *Micromonas pusilla* (Prasinophyceae) (Zingone et al., 1999, 2006), *Heterosigma akashiwo* (Raphidophyceae) (Nagasaki and Yamaguchi, 1997; Tomaru et al., 2008a), and *Heterocapsa circularisquama* (Dinophyceae) (Nagasaki et al., 2004b; Tomaru and Nagasaki, 2004) and their respective viruses. In these

Table 1. Viruses infecting diatoms.

Virus	Host	Size (nm)	Genome	Reference
RsetRNAV	*Rhizosolenia setigera*	32	ssRNA	Nagasaki et al. (2004a)
CtenRNAV	*Chaetoceros tenuissimus*	31	ssRNA	Shirai et al. (2008)
CsfrRNAV	*Chaetoceros socialis* f. *radians*	22	ssRNA	Tomaru et al. (2009)
CsalDNAV	*Chaetoceros salsugineum*	38	ssDNA	Nagasaki et al. (2005b)
CdebDNAV	*Chaetoceros debilis*	32	ssDNA	Tomaru et al. (2008b)
CspNIV	*Chaetoceros* cf. *gracilis*	25	nd	Bettarel et al. (2005)

relationships, the viruses contribute to the disintegration of the host blooms and the succession of host clonal composition (Nagasaki et al., 2004b; Tomaru et al., 2004b). Therefore, the roles of viruses in natural environments are important from the viewpoint of the ecological dynamics of microalgal host populations.

Although the importance of diatoms as key players in the oceanic carbon cycle has been recognized (Smetacek, 1999), the existence of diatom viruses has been scarcely known until recently. Transmission electron microscopy showed VLPs were occasionally found in unidentified diatom cells that were involved in phytoplankton aggregations in sediment trap samples collected from the north-eastern Pacific Ocean (Proctor and Fuhrman, 1991); however, until recently, no isolation of diatom viruses has been reported. The first diatom virus was reported in 2004, an ssRNA virus infecting *Rhizosolenia setigera* (Table 1) (Nagasaki et al., 2004a). After the initial discovery, several *Chaetoceros* viruses have been successfully isolated and characterized. These discoveries are very important to further understand diatom ecology, the carbon cycle related to diatom production and evolution of diatoms. In the following sections, we summarize the basic ecology, physiology, and genetic features of diatom viruses isolated thus far.

2. Diatom Viruses

2.1. SINGLE-STRANDED RNA DIATOM VIRUSES

2.1.1. Rhizosolenia setigera RNA Virus

Rhizosolenia setigera RNA Virus (RsetRNAV) is an icosahedral virus (32 nm in diameter) lacking a tail (reported as RsRNAV in Nagasaki et al., 2004a). Virus particles accumulate in the host cytoplasm. This virus was first isolated from water samples of Ariake Sound in western Japan in April 2002. The latent period and burst size of RsetRNAV are 48 h and 1,100–3,000 infectious units per host cell, respectively. The infection specificity of this virus is strain specific rather than species specific (see Sect. 3.1). The major structural proteins of RsetRNAV are 41.5, 41.0, and 29.5 kDa. The RsetRNAV genome is an ssRNA which is 8,877 nt long, polyadenylated, lacking a cap structure, and has two major open reading frames (ORFs): ORF-1 (4,818 nt) and ORF-2 (2,883 nt) (Shirai et al., 2006).

2.1.2. Chaetoceros tenuissimus RNA Virus

Chaetoceros tenuissimus RNA Virus (CtenRNAV) causes the lysis of the bloom forming marine diatom *Chaetoceros tenuissimus* Meunier (Shirai et al., 2008). CtenRNAV was first isolated from water samples of Ariake Sound in western Japan during June 2004. This virus is an icosahedral virus (31 nm in diameter) and lacks a tail (Fig. 1).

Virus particles accumulate in the host cytoplasm in a crystalline array formations (Fig. 2). The latent period and burst size of CtenRNAV are <24 h

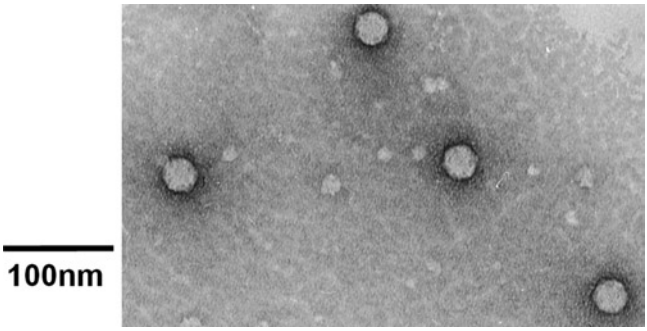

Figure 1. Negatively stained CtenRNAV particles.

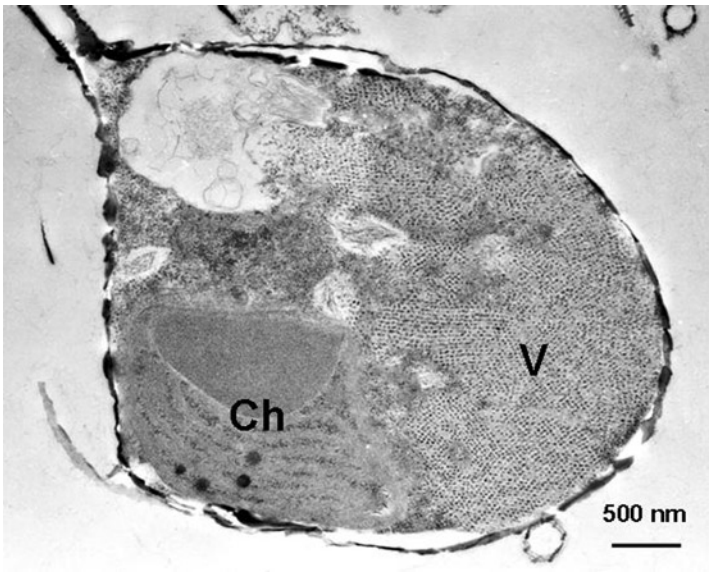

Figure 2. Transmission electron micrograph of a thin section of a CtenRNAV-infected *Chaetoceros tenuissimus* cell at 48 h post-virus inoculation. Virus-like particles (VLPs) accumulate in the host cytoplasm. Ch and V indicate chloroplast and VLP, respectively.

and ~10^4 infectious units per host cell, respectively. One of the unique features of this virus is its exceptionally high yields at ~10^{10} infectious units ml^{-1}; this is much higher than those for any other microalgal viruses previously characterized. This is advantageous for investigators to conduct future characterizations where large numbers of virions are necessary. Another noted character is the virus sensitivity of the host culture is different from other host–virus systems. Microalgal cultures are generally more sensitive to virus infections in logarithmic growth phase than stationary growth phase, whereas *C. tenuissimus* cultures show opposite responses to CtenRNAV, i.e., cultures in stationary growth phase show faster lysis than in logarithmic phase cultures. This phenomenon may be a key to understanding the host–virus relationship in natural marine environments; however, the reason has not been determined.

CtenRNAV harbors a ssRNA genome that is 9,431 nt (excluding a poly-A tail region) that includes two ORFs: ORF-1 (5,211 nt) and ORF-2 (2,646 nt). The major structural proteins are 33.5, 31.5, and 30.0 kDa.

2.1.3. Chaetoceros socialis f. radians RNA Virus

Chaetoceros socialis f. *radians* RNA Virus (CsfrRNAV) causes lysis of the bloom-forming diatom species, *Chaetoceros socialis* Lauder f. *radians* (Schütt) Proschkina-Lavrenko (Tomaru et al., 2009). CsfrRNAV was first isolated from water samples of Hiroshima Bay in western Japan in April 2005. CsfrRNAV is a very small polyhedral virus (22 nm in diameter) that lacks a tail. The virus particles accumulate in the host cytoplasm. The latent period and burst size of CsfrRNAV are <48 h and 66 infectious units per host cell, respectively. CsfrRNAV harbors an ssRNA genome that encodes at least three polypeptides of 32.0, 28.5, and 25.0 kDa. Using a RNA sequencing analysis, the genome was shown to be 9,467 nt (excluding a poly-A tail) that has two ORFs: ORF-1 (5,070 nt) and ORF-2 (2,688 nt).

2.1.4. Bacillarnavirus

Five different ssRNA viruses infecting marine stramenopiles are recognized: HaRNAV infecting a bloom-forming raphidophyte *Heterosigma akashiwo* (Tai et al., 2003; Lang et al., 2004), SssRNAV infecting a fungoid protist *Aurantiochytrium* sp. (Takao et al., 2005, 2006) and the three ssRNA diatom viruses described above. Their phylogenetic analysis was conducted where molecular biological features were compared and they were phylogenetically analyzed by Tomaru et al. (2009). The AU ratios of the three ssRNA diatom viruses were from 60.4% to 63.7%, while the HaRNAV and SssRNAV were much lower at 53.1% and 50.2%, respectively. The ssRNA diatom viruses, RsetRNAV, CtenRNAV, and CsfrRNAV, harbor an ssRNA genome with two ORFs (Fig. 3) that encode putative replication-related proteins and capsid proteins. In contrast, HaRNAV and SssRNAV genomes include one and three ORFs, respectively. A BLASTP analysis showed the amino acid sequences of ssRNA diatom viruses are highly similar to each other (E value = 0 ~ $2E-108$), while they were less similar to the HaRNAV and SssRNAV (E value = $3E-72$ ~ $5E-22$) (Tomaru et al., 2009). The basic genome structures of the ssRNA diatom viruses, therefore,

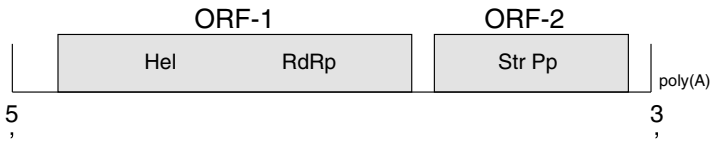

Figure 3. Schematic genome structure of single-stranded RNA diatom viruses, RsetRNAV, CtenRNAV, and CsfrRNAV. The genome size is *ca*. 9 kb, excluding a poly-A tail region and includes two open reading frames (ORFs). ORF-1, *ca*. 5k nt, encodes a putative RNA helicase (Hel) and a RNA-dependent RNA polymerase (RdRp) and ORF-2, *ca.* 2.6k nt, encodes a structural polyprotein (Str Pp) (Shirai et al., 2006, 2008; Tomaru et al., 2009).

are considered to be different from the two other stramenopile-infecting viruses. Further, the phylogenetic relationships based on the deduced amino acid sequence of the RNA-dependent RNA polymerase (RdRp) domains among positive-sense ssRNA viruses were analyzed. The result strongly supported the monophyly of RsetRNAV, CtenRNAV, and CsfrRNAV with a bootstrap value of 100% using both the neighbor-joining method and maximum likelihood method. Based on this data, this virus group is approved by the International Committee on Taxonomy of Viruses (ICTV) as a new genus, *Bacillarnavirus*, in 2010.

2.2. SINGLE-STRANDED DNA DIATOM VIRUSES

2.2.1. Chaetoceros salsugineum DNA Virus

Chaetoceros salsugineum DNA Virus (CsalDNAV) is a 38-nm icosahedral virus accumulating in the nucleus of *C. salsugineum* (reported as CsNIV in Nagasaki et al., 2005b). CsalDNAV was first isolated from water samples of Ariake Sound in western Japan in April 2003. The latent period and burst size are <24 h and ~300 infectious units per host cell, respectively. The CsalDNAV genome structure is unique among those of previously reported viruses. It consists of a single molecule of covalently closed, circular single-stranded DNA (ssDNA; 6,000 nt) as well as a segment of linear ssDNA (997 nt) (Fig. 4). The linear segment is complementary to a portion of the closed circle creating a partial double-stranded region. Six ORFs are found in the genome.

2.2.2. Chaetoceros debilis DNA Virus

Chaetoceros debilis DNA Virus (CdebDNAV) is a polyhedral virus (30 nm in diameter) lacking a tail and infects the cosmopolitan marine diatom *Chaetoceros debilis* Cleve (Tomaru et al., 2008b). This virus was first isolated from water samples of Ariake Sound in western Japan in March 2003. The virus particles accumulate primarily in the cytoplasm of *C. debilis* (Fig. 5); however, accumulations are also observed in the host's nucleus.

Host specificity of CdebDNAV is strain specific as shown with RsetRNAV. The latent period is <24 h. Agarose gel electrophoresis of the extracted CdebDNAV genome shows four bands at *ca.* 7, 5, 1.4, and 0.8 kb; they are

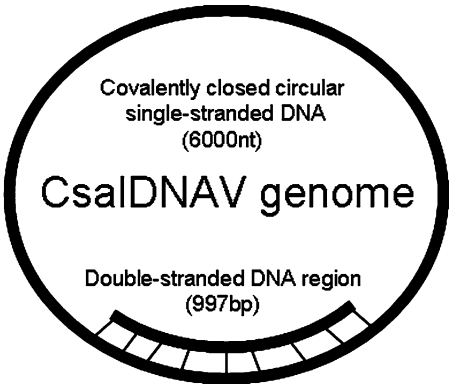

Figure 4. Schematic genome structure of CsalDNAV (Nagasaki et al., 2005b).

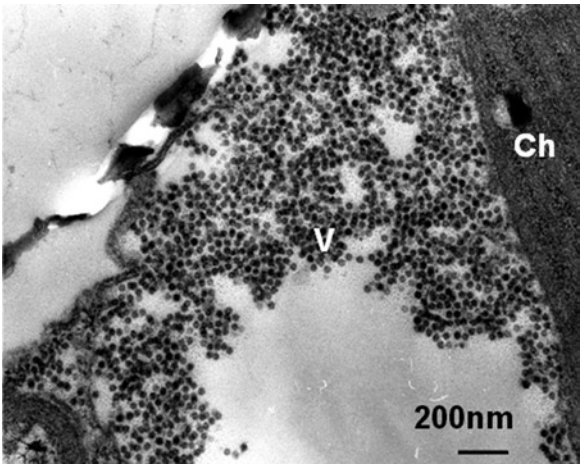

Figure 5. Transmission electron micrograph of a thin section of a CdebDNAV-infected *Chaetoceros debilis* cell at 48 h post-virus inoculation. Virus-like particles (VLPs) accumulate in the host cytoplasm. Ch and V indicate chloroplast and VLP, respectively.

completely digested using a S1 nuclease. This indicates the genome type of CdebDNAV is ssDNA. Its structure, however, is not understood. Sequence analysis shows the existence of at least one large contiguous segment at *ca*. 7 kb (unpublished data) that includes several ORFs.

The burst size of CdebDNAV was estimated to be ~50 infectious units per host cell based on a growth test where we calculated the decrease in host cell number and increase in virus number. This value, however, may be underestimated by comparing this data and the particle numbers found in a thin section view of the infected cells, e.g., Fig. 5. Such a discrepancy between the burst size data and the thin section view has been observed with CsfrRNAV and

CsalDNAV. Possible explanations for the small burst size may be aggregation of virus particles that causes an underestimation of the most probable number measuring the infectious virus units, difficulties in distinguishing dead cells and living cells using optical microscopy, or a dominance of defective particles lacking infectivity.

2.2.3. Bacilladnavirus

Two different ssDNA viruses infecting *Chaetoceros* species, CsalDNAV and CdebDNAV, have been isolated. The deduced amino acid sequences of their putative replicase gene are highly similar (*E* value = 2*E* – 69), and also they have a low similarity (*E* value > 2E − 4) to bird-infecting circoviruses which harbor a similar single-stranded circular genomic DNA (Tomaru et al., 2008b). These two viruses are considered to be similar based on the data, however, the genome structure of CdebDNAV is not enough understood. The phylogenetic relationship and taxonomic position of the ssDNA diatom viruses have not been fully understood due to the insufficient number of ssDNA viruses in the data bases. Therefore, only CsalDNAV have been approved as a sole member of a new genus, *Bacilladnavirus*, by ICTV in 2010. Characterizations of the new ssDNA diatom viruses, are in progress, e.g., morphologic, physiologic, and genomic characters of ssDNA viruses infecting several *Chaetoceros* sp. strains are highly similar to that of the previously reported ssDNA diatom viruses (unpublished data). Future studies may show further characteristics of the new genus "*Bacilladnavirus*."

2.3. OTHER DIATOM VIRUSES

2.3.1. Chaetoceros Nuclear Inclusion Virus

Chaetoceros Nuclear Inclusion Virus (CspNIV) is a lytic virus infecting *Chaetoceros* cf. *gracilis* isolated from Chesapeake Bay, USA, in April 2003 (Bettarel et al., 2005). The virus particles are *ca.* 25 nm in diameter and accumulate in the nucleus of *C.* cf. *gracilis* forming paracrystalline arrays. The latent period of CspNIV is <24 h. The genome structure of CspNIV has not been reported. Another small virus (*ca.* 30 nm in diameter) infecting *C.* cf. *wighamii* and accumulating in the host nucleus is also reported (Eissler et al., 2009) its genome characters are also unrevealed. Further study is expected to reveal genome structure for these viruses.

3. Ecology of Diatom Viruses

Ecological relationships between microalgal hosts and viruses in natural environments have been extensively studied during the last two decades (Brussaard and Martinez, 2008; Nagasaki, 2008). The effects of microalgal viruses on its host populations are roughly divided into two aspects: (1) quantitative effects that

cause a rapid decrease or restriction of the population abundance and (2) qualitative effects that change the clonal composition of the host populations that have a variety of virus sensitivities. The relationships between diatoms and their viruses *in situ* are relatively unknown; however, some authors have indicated their ecological interactions in nature based on field surveys and physiological studies (Shirai et al., 2008; Tomaru et al., 2008b).

3.1. INFECTION SPECIFICITY OF DIATOM VIRUSES

The infection of RsetRNAV and CdebDNAV is strain specific rather than species specific, and further, the specificity is diverse among virus clones. This indicates the virus sensitivities of diatom host clones are diverse among host clones (e.g., schematic diagram Fig. 6).

Tomaru et al. (2008b) conducted a cross reactivity test between 19 *C. debilis* strains and 29 virus clones infecting *C. debilis* where both were isolated from Ariake Sound in western Japan during blooms in 2005. The results show the intraspecies host specificity is highly diverse among the virus clones tested; therefore, they concluded that a natural *C. debilis* population is composed of highly diverse host clones that differ in virus sensitivity spectra.

The complex relationships between microalgal host–virus systems shown in the schematic relationship of Fig. 6 are generally observed in other microalgal host–virus relationships, e.g., *M. pusilla* and MpV (Sahlsten, 1998), *H. akashiwo* and HaNIV or HaV (Lawrence et al., 2001; Tomaru et al., 2004b), and *H. circularisquama* and HcRNAV (Tomaru et al., 2004a; Nagasaki et al., 2005a; Mizumoto et al., 2007); this is considered to be significant in preventing the complete extinction of microalgal host species due to viral infection.

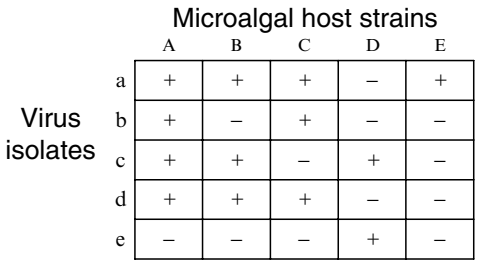

Figure 6. Schematic relationship between microalgal host clones ("A" to "E") and virus clones ("a" to "e") having different virus sensitivity and host specificity, respectively. "+" and "−" indicate lytic and not infectious, respectively.

3.2. INFECTION MECHANISMS

The infection mechanism of diatom viruses is unknown. The diatom's silica wall may restrict access of viruses to the cell membrane; however, most diatoms have a number of pores in the frustule. The particle sizes of RsetRNAV (32 nm) and CsalDNAV (38 nm) are smaller than that of *R. setigera* frustule pores (ca. 80 nm in diameter; Nagasaki et al., 2004a) and *C. salsugineum* setae pores (Nagasaki et al., 2005b), respectively; this may be the possible route of viral infection.

3.3. QUANTITATIVE EFFECTS ON DIATOM POPULATIONS

Bettarel et al. (2005) reported the most widespread occurrence of viruses infecting *Chaetoceros* cf. *gracilis* in Chesapeake Bay was recorded in April 2003 *ca.* 1 month after the winter-spring *Chaetoceros* bloom. The results suggest the importance of diatom viruses in the crash of their host *Chaetoceros* blooms (i.e., quantitative effects of virus infection) because the increase of virus numbers in the water column are considered likely to be the results of its host cells' death due to viral infection.

3.4. STABILITY OF INFECTIVITY

The results given by Bettarel et al. (2005) indicate that the viruses infecting *C.* cf. *gracilis* remain infectious in water columns of the bay at least 1 month after the disappearance of its host diatom. The high stability seems to be a general character of the diatom viruses isolated, e.g., infectious titers of CsfrRNAV suspension after 50 days of storage at 20°C, 10°C, and 4°C in the dark were 25%, 66%, and 145% of the initial titer, respectively (Tomaru et al., 2009); similar results were reported for RsetRNAV, CsalDNAV, CtenRNAV, and CdebDNAV. This feature may support the persistence of diatom viruses in natural environments.

The decay or elimination rate of diatom viruses from natural waters should be far higher due to exposure to irradiation with ultraviolet light, adsorption to various particles, external enzymes from bacteria, ingestion by heterotrophic microorganisms, and other unknown factors (Bitton and Mitchell, 1974; Kapuscinski and Mitchell, 1980; Suttle and Chen, 1992; Noble and Fuhrman, 1997). Some percentage of the viruses which successfully propagate during the host bloom, however, may be preserved in natural environments, enabling their revival in successive host blooms. One of the possible reservoirs of microalgal viruses is likely to be in sediments (Lawrence et al., 2002; Tomaru et al., 2005, 2007) where viruses infecting diatoms have been isolated frequently from bottom sediment samples (Nagasaki et al., 2005b; Tomaru et al., 2008b).

3.5. RESTING SPORES AND VIRUSES

Formations of resting spores by diatoms are essential in their survival strategies in various environments, and they are buried in sediments in many cases. Several studies predicted the possibilities of the diatom resting spores escaping from viral infections. Tomaru et al. (2009) found resting spores of *C. socialis* f. *radians* remaining in CsfrRNAV-inoculated cultures after 23 days postinoculation. The resting spores had chlorophyll fluorescence indicating viability in spite of the existence of numerous ambient virus particles; it is unknown whether formation of resting spores is enhanced by viral inoculation. Similar results were reported in the relationship between *C.* cf. *gracilis* and CspNIV (Bettarel et al., 2005) and between *C. debilis* and CdebDNAV (Tomaru et al., 2008b).

Possibly resting spores are significant for protecting diatom populations against viral attack. The lytic viruses infecting diatoms are frequently isolated from sediments (see above); therefore, the hatching of the resting spores from the sediments without viral infection may be essential for bloom formation. The relationships between the resting spores and the viral infection should be the focus of future studies to reveal the survival strategies of diatoms in natural environments.

3.6. DYNAMICS OF HOST AND VIRUS

Previous studies indicated the significant effects of viruses on their host population dynamics (Brussaard, 2004). Whereas, field research on diatom viruses have been scarcely reported. One of the principal reasons is the difficulty in identifying and counting diatom cells at the species level using light microscopy. The genus *Chaetoceros* includes more than 400 species (Rines and Hargraves, 1988), and differential identification of these species in natural water samples is almost impossible, especially for the small cells (e.g., *C. tenuissimus*) using the light microscope. Therefore, to estimate the impact of diatom viruses on their hosts' dynamics, the development of quantitative detection methods for diatoms is needed. This distinction at the species level is possible using real-time PCR.

4. Proposal for Diatom Virus Nomenclature

There have been no universal codes of algal virus nomenclature. The nomenclature used previously (e.g., *Heterosigma akashiwo* virus = HaV, *Emiliania huxleyi* virus = EhV) is no longer available due to the recent increase of algal viruses in culture. One remedy is to include more host information in the virus abbreviation name, e.g., using four letters out of the host scientific name composed of the initial letter from the host genus name and three letters from the species name, *i.e., Chaetoceros tenuissimus* is represented by "Cten" and *Chaetoceros socialis* f. *radians* is "Csfr." And, including the genome type (DNA or RNA) is meaningful

as we already know one host alga can be infected by various genome types of virus (ex. Brussaard and Martinez, 2008; Nagasaki, 2008; Tomaru et al., 2008a). Here we do not recommend using "NI (nuclear inclusion)" representing the virus replication site when the virus genome type is clear as in the case of CsNIV (Nagasaki et al., 2005b). Hence, following the above rule, RsRNAV (Nagasaki et al. 2004) and CsNIV (Nagasaki et al., 2005b) should be respectively, renamed as RsetRNAV and CsalDNAV, and this has been approved by ICTV in 2010. Of course, this rule may be changed appropriately when there is an increase in the variety of cultured algal viruses.

5. Conclusions

The discovery and successful isolation of diatom viruses imply their potential importance for controlling the quantity (biomass) and quality (clonal composition) of diatom populations in natural environments. Studies about diatom viruses have just began. There are numerous questions concerning diatom viruses, e.g., the effects of biogeochemical cycles in controlling diatom populations, unfound diatom viruses distinct from ssRNA and ssDNA viruses, coevolutions between diatoms and their viruses, and viruses infecting pennate diatoms and freshwater species. Further studies on various diatom host–virus systems should provide answers to these and other questions.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 227–243
DOI 10.1007/978-94-007-1327-7_10, © Springer Science+Business Media B.V. 2011

BENTHIC DIATOMS IN BIOFILM CULTURE

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1. Introduction

Biofilms are heterogeneous, surface-associated microbial communities colonising (living and nonliving substrata) in a range of terrestrial and aquatic environments. Biofilms constitute the prevailing mode of microbial life in most natural habitats. Biofilm microorganisms have crucial roles in earth system process, they possess metabolic and physiological versatility and are integral in virtually all biogeochemical cycling processes and ecosystem functioning (Battin et al., 2003). Phototrophic biofilms have been defined as interfacial microbial consortia driven by light as an energy source. They can develop three-dimensional (3D) structured, multilayered communities which secrete an exopolymeric matrix (EPS, extracellular polymeric substances) that holds the biofilm together, mediates adhesion to the exposed surfaces and serves a suite of other functions: surface-associated motility; protection against UV radiation, desiccation and grazing; immobilisation and accumulation of noxious compounds and cell to cell communication (Stal, 2010).

In natural aquatic ecosystems, phototrophic biofilms are comprised of cyanobacteria and microalgae in association with heterotrophs, such as bacteria, fungi and protozoa. Oxygenic phototrophic microorganisms generate energy and reduce carbon dioxide, providing organic substrates and oxygen for the heterotrophic component of the biofilms. In these laminated and dynamic communities, structural and functional diversification is possible along physico-chemical gradients and in response to alternate diurnal patterns, enabling multiple species with various growth habits, successional appearance and metabolic activities to coexist and interact. Thus, phototrophic biofilms represent unique, self-structured systems that optimise mass transfer from external sources in which complex and coordinated organisation allows efficient internal recycling of nutrients.

2. Diatoms in Aquatic Phototrophic Biofilms

Among eukaryotic organisms, diatoms initiate and promote biofilm formation on a variety of new surfaces when immersed in water; they are important primary

producers and dominant members in biofilms of the littoral zone (Bahulikar and Kroth, 2008 and references therein; Molino and Wetherbee, 2008).

Epipelic diatoms dominate on fine, soft sediments and silt habitats (Admiraal, 1984; Thornton et al., 2002; Ribeiro et al., 2003) where they can significantly contribute, up to 50%, to carbon budgets forming biofilms that secrete EPS, thus transporting primary production to higher trophic levels (Underwood and Kromkamp, 1999; Serôdio and Catarino, 2000; Stal, 2010). Through EPS exudation, biofilm diatoms are also involved in the stabilisation of muddy sediments in marine and brackish environments (Paterson, 1989; Smith and Underwood, 1998; Blanchard et al., 2000; Decho, 2000; Stal, 2010) as in lotic systems (Gerbersdorf et al., 2009). As erosion, deposition and transport of sediment in these ecosystems have great ecological and economic impact, diatom biofilms have gained increased scientific interest in the last decade, leading to unravel diurnal, tidal and nutritional patterns of production and extrusion of these carbohydrate-rich heteropolymers, primarily linked to cell motility (Smith and Underwood, 2000; Staats et al., 2000; Yallop et al., 2000; de Brouwer and Stal, 2002; Underwood et al., 2004; Stal, 2010).

Benthic diatoms, filamentous green algae and cyanobacteria are the most common organisms in stream habitats (Biggs, 1996). Both in lotic and lentic environments, phototrophic biofilms are ecologically important, representing the primary source of fixed carbon, providing an essential source of food for grazers forming a crucial link at the base of the food web (Stevenson, 1996; Sekar et al., 2002; Sabater et al., 2006; Besemer et al., 2007), and sequestering nutrients, such as nitrogen and phosphorus. In particular, by trapping nutrients from the water column, they contribute to nutrient cycling (Wetzel, 1996), and play a key role in the self-purification processes that occur in rivers (Sabater et al., 2002). However, they can also proliferate in nutrient-enriched, stable-flowing streams, causing water management problems. Streams are also exposed to large variations in water quality, due to their hydrology and anthropogenic activities. Benthic organisms can show rapid response to such changes, and therefore, phototrophic biofilms can be considered as potential biological monitoring tools to determine changes in water quality (Larson and Passy, 2005). In artificial environments, such as wastewater treatment plants, indigenous phototrophic biofilms, dominated by raphid diatoms in spring and summer, have been shown to possess a high productivity potential and the ability to grow on a variety of artificial substrata. This coupled to the production of high amounts of negatively charged heteropolysaccharides in the matrix proved particularly suitable for the removal of residual nutrients and noxious cations in wastewaters (Albertano et al., 1999; Guzzon et al., 2005; Guzzon and Albertano, 2009; Congestri et al., 2003, 2005, 2006).

Different to heterotrophic biofilms, for which a growing body of research has been pooled and progressed our understanding of their structure, growth dynamics and physiology in single or multi-species experiments in flow chambers or *in situ* (Hall-Stoodley et al., 2004; Battin et al., 2007; Palmer and Stoodley, 2007), phototrophic biofilms have received relatively little attention until recently. The recent increase of interest in the structure and functioning of phototrophic

biofilms is related to their ecological importance and their high potential for biotechnological applications, such as wastewater treatment (Craggs et al., 1996; Schumacher and Sekoulov, 2003; Roeselers et al., 2008; Guzzon et al., 2008), bioremediation and aquaculture (Bender and Phillips, 2004) and fouling control (Bhadury and Wright, 2004; Patil and Anil, 2005; Molino and Wetherbee, 2008).

3. Microcosm Approach to Study Phototrophic Biofilm Development

In this fragmented scenario, a comprehensive study on phototrophic biofilms was performed in the frame of a EU project, PHOBIA, PHOtotrophic BIofilm and their potential Applications: towards the development of a unifying concept (2002–2006), conceived as a concerted action of six different laboratories aimed to unveil various aspects of phototrophic biofilm biology and development in specially designed incubator prototypes.

The use of a microcosm approach, with the possibility to simultaneously control environmental conditions and coculturing biofilm organisms on a set of artificial substrata in a closed photobioreactor, was considered essential for a realistic assessment of biofilm processes. It is often difficult to investigate phototrophic biofilms in nature because of the large range of biotic and abiotic interactions that occur, the consequent ambiguity of experimental conditions and the impossibility to maintain biofilm integrity during sampling procedures. Nevertheless, cultivation devices for complex environmental biofilms have not received considerable attention in the past, except for flume microcosms designed to study hydrodynamic effects on stream biofilms (Singer et al., 2006; Besemer et al., 2007).

The microcosms developed in PHOBIA met, for the first time, the requirement for appropriate cultivation techniques to address phototrophic biofilm structure–function interactions and also ecosystem implications thanks to their large scale. The PHOBIA experimental design provided an experimental area to study the effect of disturbance, environmental variation and the role of key species in biofilm development starting from freshwater and marine inocula, cultured on polycarbonate slides, in a moving film of medium under controlled temperature and light conditions (Wolf et al., 2007; Zippel et al., 2007).

Here, we report on compositional features of diatom assemblages in the experiments performed in the Laboratory of Biology of Algae, focussing on diatom species accrual and succession during biofilm development in the incubator runs.

4. Incubator Prototype

The flow-lane incubator system used was developed by the Department of Inland Water Research, UFZ Centre for Environmental Research, Magdeburg, Germany (for a detailed description of the system, see Zippel and Neu, 2005; Zippel et al., 2007).

The incubator contained four separate flow light chambers (LCs, 120 × 10 cm each) with integrated inlet and outlet devices at the beginning and the end of

each lane. A total of 47 polycarbonate slides (microscope slides), chosen as adhesion substrata, were inserted flat into the flow lanes.

From the medium beakers, placed directly below the inlet device, the medium was pumped into the light chamber thanks to a submersible aquarium pump, allowing the continuous circulation of medium, and the flow rate was regulated by a valve under the flow meter that allowed the control of medium velocity during the experimental time. Homogeneous flow conditions in each lane were created by a turbulence reducer placed in the inlet device, where a thermosensor was fixed to the turbulence reducer to measure the temperature.

Light source used during the experiments was represented by fluorescent lamps (TrueLight 36 W, Auralight, Sweden) connected to a timer to switch them on and off according to 16-h light and 8-h dark cycle. Each LC was featured by different photon flux densities: 120 (abbreviated as LC120), 60 (LC60), 30 (LC30), and 15 μmol photon m^{-2} s^{-1} (LC15), adjusted with a potentiometer and measured by means of incident light sensors positioned under the lamp in each chamber. Subsurface light sensors were located under three slides in fixed positions along the LC to measure the transmittance, the percentage of incident light attenuation through biofilm thickness. Each light sensor was equipped with three photodiodes. All sensors were connected to a computer through a serial port for continuous data monitoring and acquisition.

5. Experiments with Marine Biofilm

Marine inocula were collected on two occasions (February and March 2005) from biofilms growing on continuously submerged surfaces in flowing Oosterschelde water, the Netherlands. The sampled biofilms were mechanically homogenised and then frozen in order to reduce abundance of protozoa and metazoa. Four aliquots of 100 ml homogeneous suspension were poured into four bottles each containing 3.9 l of marine medium prepared with commercially available aquarium sea salt (HW Sea Salt professional, Wiegandt GmbH, Germany) with additional phosphate (16 mg l^{-1}), silicate (57 mg l^{-1}) and nitrate (150 mg l^{-1}). The inoculum suspension was then circulated through the incubator for 72 h at 100 l h^{-1}. Thereafter, this solution was replaced with fresh medium and the flow rate was set at 25 l h^{-1} (Run1) and 100 l h^{-1} (Run2). The cultures were kept for 3 days under nutrient replete conditions by changing the medium twice a week. Biofilms were cultured at 120 (LC120), 60 (LC60), 30 (LC30) and 15 μmol m^{-2} s^{-1} (LC15). At each irradiance condition, two medium flow rates were tested at 25°C resulting in two experimental runs. Each run lasted 50 days. The development of the biofilm phototrophic mass was followed by a continuous recording of the light attenuation. Biofilms were sampled at three stages of development given as a function of incident light absorption by the biofilm: (1) initial stage, when the absorption A averaged over the three sensors in each lane, equalled 10%; (2) active stage, when

A = 50% and (3) mature stage, at A = 90–95%. On the last day of the run, biofilm was collected even if the active or mature stage had not been reached.

5.1. BIOFILM MASS ACCUMULATION

Non-destructive, real-time monitoring of biomass accumulation was obtained by recordings of the subsurface light sensors (Fig. 1). Plots of biomass *versus* time indicated that after inoculation, during which microorganisms settled and adhered on the slides, a lag phase followed. At the highest irradiances (LC120 and LC60), lag phases lasted about 10 and 18 days, respectively, but were longer at higher flow condition (Run2). Lag phases were also longer at intermediate light (LC30) irrespective of flow rate, and growth was very slow, corresponding to an initial phase of mass accumulation, at 15 μmol m^{-2} s^{-1} in both experiments. Then, biomass increased within a period of 2–5 weeks, depending on the light intensity. After a period of exponential increase, curves assumed an S-shape and reached a plateau only in LC120 and LC60 biofilms in both runs, while approximately 70% absorbance was registered in LC30 on day 50. Biofilm mass accumulation clearly depended on light condition, and 90% absorbance level (mature stage) was only reached at the highest light conditions in Run1 (day 27, LC1; day 43, LC2) and Run2 (day 32, LC1; day 43, LC2). After reaching a mature stage, biofilm sloughing was observed at LC120 and LC60 in the last week of run duration, probably indicating diffusion limitation due to biofilm thickness and variation in adhesion properties of EPS matrix. This phase led to heterogeneity in biofilm spatial distribution, that after appearing as series of large patches, different in pigmentation,

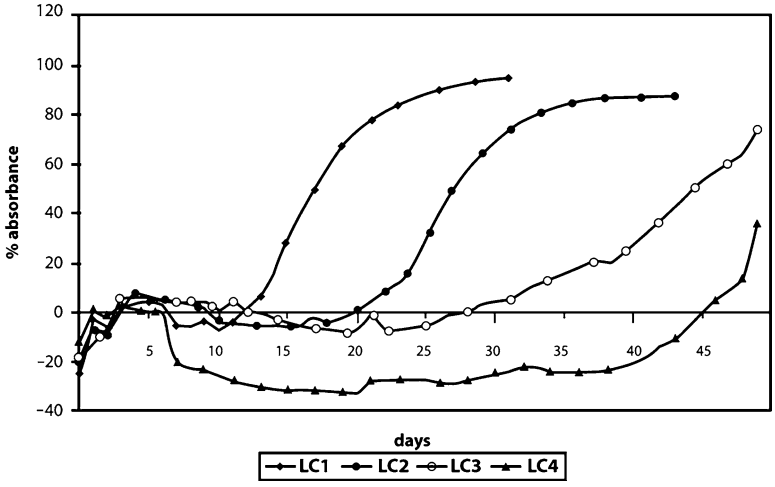

Figure 1. Plot of biomass *versus* time obtained in Run 1.

in the initial phases, became more coherent and multilayered during maturation. Occasionally, loosely attached filamentous phototrophs, streamers were observed floating in flow direction in biofilms at the mature stage.

Thus, light conditioned biofilm mass accumulation on surfaces from inoculation to biofilm maturation also affecting the biofilm structure and physiognomy, as the gross aspect of the photosynthetic biomass visibly changed over experimental time.

5.2. DIATOM COMPOSITIONAL PATTERNS

Epipelic diatoms dominated in taxon numbers and biomass of the phototrophic assemblages inoculated during the experiments. Taxon richness was high, but relatively low numbers of dominant species were observed in inocula, diatoms prevailed in association with few members of cyanobacteria, both coccal and filamentous forms (*Leptolyngbya* and *Pseudanabaena* spp.) along with few unicellular green algae. Tidal patterns in salinity and water level typical of estuarine systems are known to exert strong selective pressure that together with the mode of inoculum formation could have probably been responsible for the biofilm physiognomy and their relatively low taxonomic diversity.

Assessment of diatom diversity was conducted both on fixed material (2.5% formaldehyde), using a Zeiss Axioskop light microscope (LM) equipped with differential interference contrast (DIC) and 40× and 100× objectives, to distinguish between empty frustules and living cells, and on acid cleaned samples, for more accurate identification, at the scanning electron microscope (SEM, Leo Stereoscan 440). A variety of raphid diatoms, around 30 taxa, ranging from 10 to 80 – 100 μm in length, prevailed within inocula, although members of large-celled or colony-forming centrics were obvious within the aggregates (Table 1 for a list of most representative diatom taxa observed).

Cells belonging to *Amphora* spp., *Nitzschia* spp., *Hantzschia* spp., *Craspedostauros britannicus*, *C.* cf. *australis* and a number of naviculoid forms (*Berkeleya* spp. and *Navicula* spp.) were the most frequent within biofilms grown on submerged surfaces in Oosterschelde, and species composition of the two inocula was, as expected, very similar (Fig. 2d–i). Most of the diatoms observed are reported as benthic marine or brackish species living on a variety of substrates and distributed in coastal areas of Atlantic European and Mediterranean regions (Witwoski et al., 2000) as in estuarine habitats (Forster et al., 2006; Sahan et al., 2007). Some were tube-dwelling forms as *Parlibellus delognei*.

Due to the presence of copious mucilage in cultured biofilms, that prevented application of the Utermhöl method (no uniform sedimentation of individual specimen), a semi-quantitative analysis of dominant biofilm members and associated phototrophs was carried out on biofilm portions scraped off slides taken at each stage of biofilm development. Microscope slides provided a suitable sampling unit. The design of slide fixation allowed non-invasive collection of the attached communities, without destruction of biofilms growing next. Thus, a relative

Table 1. Major taxa in biofilm inocula.

Marine	Freshwater
Actinoptychus senarius	*Cyclotella meneghiniana*
Auliscus sculptus	*Fragilaria ulna*
Melosira moniliformis	*Staurosira pinnata*
Melosira nummuloides	*Planothidium lanceolatum*
Odontella aurita	*Cymbella minuta*
Paralia sulcata	*Gomphonema parvulum*
Thalassiosira angulata	*Gomphonema* sp.
Triceratium reticulum	*Craticula accomoda*
Ardissonia cf. *crystallina*	*Craticula cuspidata*
Licmophora sp.	*Diadesmis confervacea*
Opephora spp.	*Eolimna* spp.
Berkeleya spp.	*Fallacia pygmea*
Craspedostauros britannicus	*Luticola mutica*
Craspedostauros cf. *australis*	*Navicula gregaria*
Mastogloia sp.	*Navicula radiosa*
Navicula perminuta	*Navicula salinarum*
Navicula arenaria	*Pinnularia gibba*
Navicula spp.	*Sellaphora pupula*
Parlibellus delognei	*Amphora coffeaformis*
Amphora hyalina	*Bacillaria paxillifera*
Amphora spp.	*Nitzschia amphibia*
Bacillaria paxillifera	*Nitzschia palea* var. *minuta*
Cylindrotheca closterium	*Nitzschia palea* var. *debilis*
Entomoneis sp.	*Nitzschia umbonata*
Hantzschia spp.	
Nitzschia laevis	
Nitzschia cf. *tryblionella*	
Nitzschia spp.	

occurrence score (3 = dominant, 2 = common, 1 = rare) was assigned to each species or morphotype during LM analyses of disaggregated biofilms in randomly selected optical fields.

Patches of *Melosira nummuloides* and *Odontella aurita*, among centrics, and more numerous raphids as *Craspedostauros* spp., various morphotypes belonging to the genera *Amphora*, *Entomoneis* and *Nitzschia* together with *Parlibellus delognei* in conspicuous mucous tubes, generally composed diatom assemblages within biofilms at initial stages (Fig. 2b, c). Cell morphology and filament formation could have favoured recruitment of centric diatoms from the cell suspensions during inoculation although there was no visible growth of these forms on the substrata during experimental time. Clusters of raphids, especially *Amphora* spp. and *Craspedostauros* spp., successful fouling species (Molino and Wetherbee, 2008), adhered and spread on polycarbonate surfaces after the initial phase, with cells actively dividing and forming dense aggregates immersed in mucous material. Cyanobacteria also settled and grow enmeshed with diatoms at this stage. Coccal morphotypes of varied pigmentation, tentatively attributed

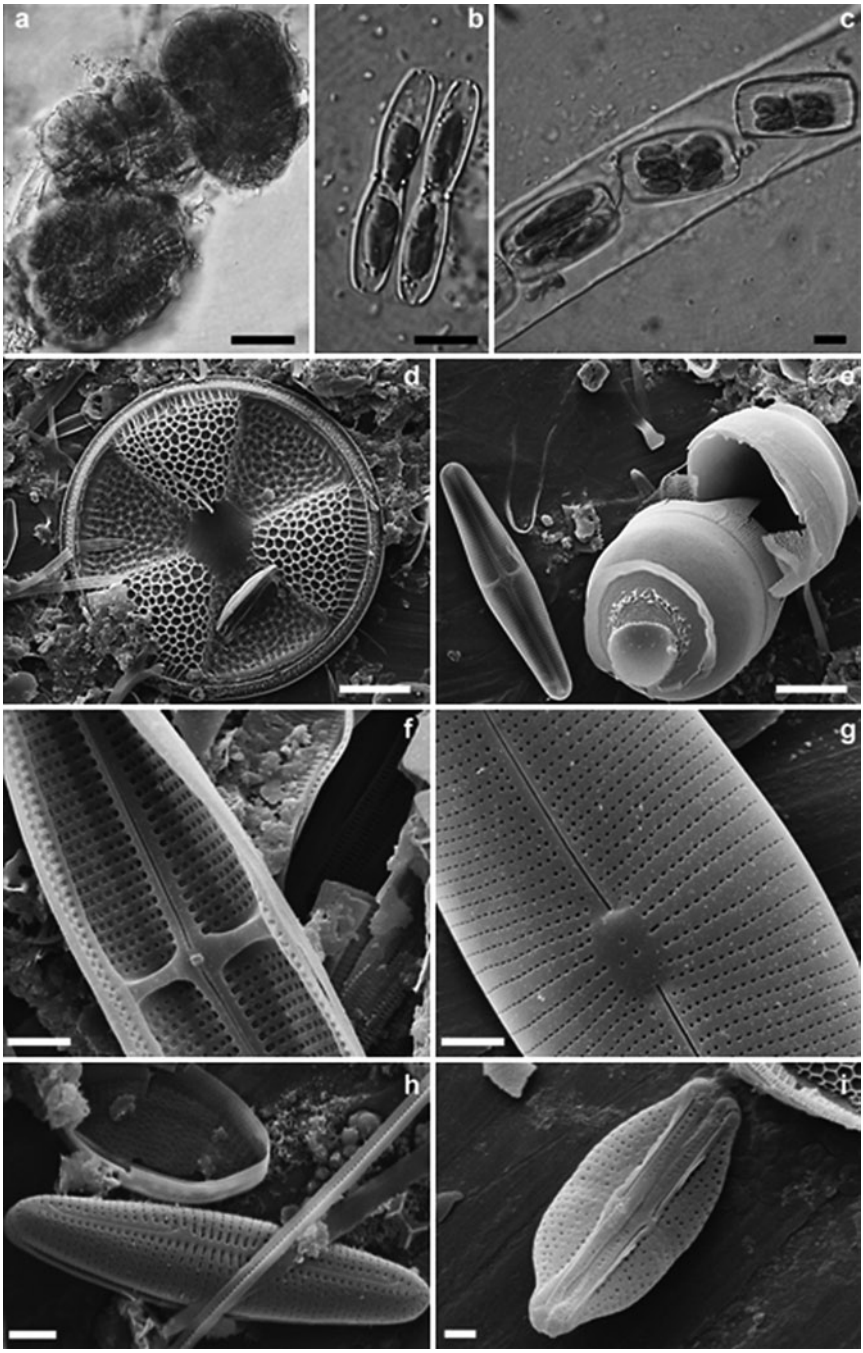

Figure 2. LM micrographs of biofilm members in culture, *Chroococcidiopsis*-like colonies (**a**), *Craspedostauros britannicus* (**b**) and *Parlibellus delognei* in mucous tube (**c**). Diatoms in inocula at SEM: *Actinoptychus senarius* (**d**), *Craspedostauros britannicus*, note elictoglossae at central raphe endings on the inner valve face and the hemispherical valve of *Melosira nummuloides* with prominent collar and pericentral carina (**e**). *Craspedostauros* cf. *australis* with a central knob at internal raphe endings (**f**), *Parlibellus delognei*, central area of the outer valve surface (**g**), *Berkeleya* sp. (**h**) and *Amphora* sp. (**i**). Bars = 2 (**f**–**i**) and 10 μm (**a**–**e**).

to the genus *Chroococcidiopsis* for cell dimensions and three-dimensional colony formation (Fig. 2a), and very thin oscillatorialean forms constituted main representatives of cyanobacterial mass. Ellipsoidal and rounded unicellular greens were also found at this phase in both runs.

A marked shift in phototroph composition followed with a sharp decrease in diatom diversity in all cultures, over the experimental time, especially in LC1-3 biofilms. An inversion of dominance was observed at all tested conditions with colonies of coccal cyanobacteria largely prevailing (or codominating with green algae) in all LCs but LC4, where a degree of diatom diversity was maintained, although the lowest irradiance (15 μmol m^{-2} s^{-1}) significantly limited phototroph growth in LC4 with very low substratum coverage and absorbance level constantly around 10%. Decrease in diatom diversity was particularly evident at the late developmental stages (reached at high light conditions only) when coccal cyanobacterial overgrowth was obvious in both runs. In a competition experiment with marine biofilm species, *Microcoleus chthonoplastes* resulted more successful than *Nitzschia* sp. and dominated communities at 25°C (97–98% of total biovolume), confirming observations in the field of cyanobacteria favoured by high temperature and coarser sediment while diatoms were dominant at low temperatures and on mud (Watermann et al., 1999).

Overall, the flow regime had no clear effect on biofilm development, although at initial stages diatoms were more represented in higher flow rate (Run1).

6. Experiments with Freshwater Biofilms

Freshwater biofilms were collected on four occasions, April, June, July and September 2004, from an overflow weir of the sedimentation tank (ST) of the wastewater treatment plant (WWTP) at Fiumicino Airport (Rome, Italy). Inocula were obtained according to Guzzon et al. (2005) to have safe-to-handle and homogeneous 100-ml biofilm suspensions to add to modified BG11 medium for inoculum preparation. Modifications included adding vitamins and silicates to support microalgal growth (40 mg l^{-1} and 57 mg l^{-1}, respectively) and using only nitrate as the sole nitrogen source and increasing the concentration of orthophosphate to obtain a final nitrogen:phosphorus (N:P) ratio of 15, thus ensuring balanced conditions for algal growth. Inoculation procedure followed as reported from marine communities. Four runs were conducted in the four separate LCs described in Sect. 4. In addition to light intensity, four parameter combinations featuring two temperatures (20°C and 30°C) and two flow rates (25 and 100 l h^{-1}) were tested. Each run lasted 30 days.

In this section, only compositional features of major community members in cultured biofilms have been reported, as the growth dynamics, biomass accumulation, nutrient retention and matrix EPS properties have been extensively described elsewhere (Guzzon et al., 2008; Di Pippo et al., 2009).

6.1. DIATOM COMPOSITIONAL PATTERNS

Microscopy observations, carried out as described above, showed that inocula had a low biodiversity and a stable composition throughout the sampling period with changes in the proportion of the major taxonomic groups, cyanobacteria, diatoms, green algae and euglenophytes, rather than actual taxonomic variations over time (Table 1 for a list of diatoms in the inocula). This confirmed previous findings on cyanobacteria and diatom assemblages within the Fiumicino WWTP phototrophic biofilms. 39 diatom taxa were found during a seasonal survey on biofilms scraped off the sedimentation tank (ST). Assemblages consisted mainly of readily recognised raphid taxa, mostly >20 μm long, with only one centric and four araphids. Relative abundance calculations indicated that the colonial biraphid *Diadesmis confervacea* dominated in winter and spring while *Nitzschia umbonata* and *Navicula gregaria* significantly contributed to summer assemblages and *Stephanocyclus meneghiniana* to the autumn diatom fraction (Congestri et al., 2005). In addition, long-term *in situ* observations of such communities highlighted almost constant successional patterns, possibly owing to the chemical characteristics of the water that are virtually constant during the years (Albertano et al., 1999; Congestri et al., 2003, 2005, 2006). Species composition was typical of eutrophic, organically polluted water bodies, and similar to those reported for other few WWTPs (Davis et al., 1990; Sládecková and Matulová, 1998).

Semi-quantitative assessment of phototroph relative occurrence in cultures during biofilm development showed a general decrease in phototroph diversity with only four chroococcalean and six oscillatorialean cyanobacteria, three raphid diatoms, five coccal green algae and one euglenophyte accounting for the majority of the biofilm biomass and phototrophic diversity in culture. Thus, an overall loss of diversity was also observed in freshwater cultures. Particularly, biofilms grown at the lowest irradiance (LC4) exhibited visible low biomass and low diversity with only few coccal green algae viable. *Diadesmis confervacea* was the only diatom that actively grows on slides in all experiments. *D. confervacea* was found especially at the two intermediate irradiance conditions (LC2 and LC3) and could be considered a sort of a proxy of LC3 assemblages (Fig. 3). It has been observed that, during growth in standing cultures, filaments of *D. confervacea* tended to attach firmly to the side of the glass vessels; a possible means of attachment was supposed to be the organic material that also allows maintenance of adhesion between adjacent cells (Rosowski, 1980). This could have increased the settling rate and adhesion of this species during the inoculation over other diatoms. The intertwined filaments (Fig. 3c) also formed a net-like structure that enhanced permanence and adhesion during the inoculation period. Thus, it appears that beside irradiance, that directed intermediate light (LC3) communities towards diatom dominance, the adhesion processes may have acted in selecting between diatom life forms present in the inocula. The higher relative abundance of *D. confervacea* found in low flow velocity experiments would confirm the hypothesis that shear forces conditioned, secondly, biofilm taxonomic structure.

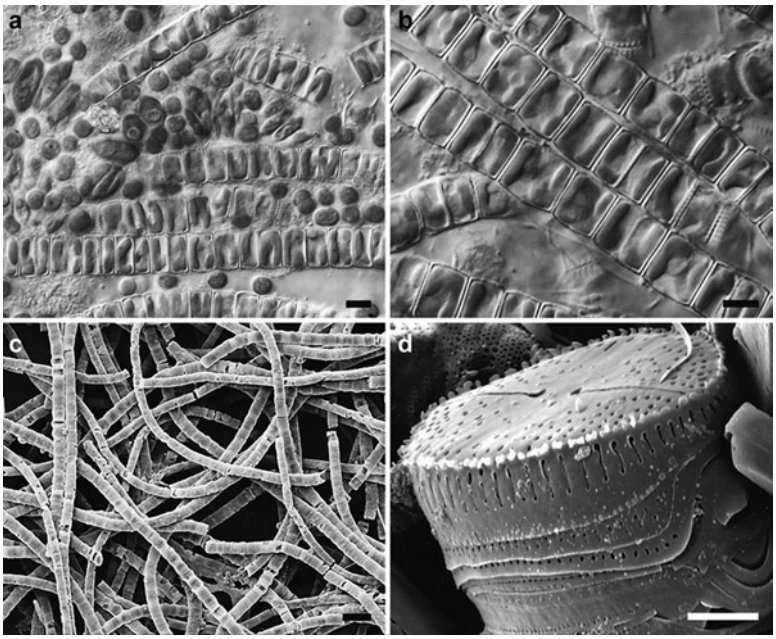

Figure 3. Light micrographs of *Diadesmis confervacea* filaments in culture (**a**, **b**). A dense network is also visible in critically point-dried material at SEM (**c**). SEM observation of acid-cleaned samples showed diacritical features of the species (**d**). Bars = 2 (**d**) and 10 μm (**a**–**c**).

Although less obvious than the case of diatoms, coccal chlorophytes tended to dominate biofilms grown at the highest irradiance (LC1), especially at the initial growth phases, then filamentous cyanobacteria as *Phormidium* spp. and *Oscillatoria* spp. increased over experimental time, while *Leptolyngbya* and *Pseudanabaena* spp. were more common in LC2 communities. During nutrient and light manipulation experiments on stream communities, it has been shown that chlorophytes prevailed over cyanobacteria in unshaded, nutrient-rich sites (Bourassa and Cattaneo, 2000), cyanobacteria outcompeted raphid diatoms in cultured multi-species biofilms at the late growth phase (Van der Grinten et al., 2004), while Barranguet et al. (2005) observed that filamentous cyanobacteria were successful late biofilm colonisers in biofilms grown on artificial substrata immersed in Dutch filtration dunes.

7. Conclusions

The incubator developed in PHOBIA provided for the first time the possibility of coculturing phototrophic biofilm components on artificial substrata in a closed system under controlled environmental conditions. Creating a maximum

surface-to-volume ratio, the precise control over irradiance was obtained and the flat topology was of additional advantage as the thin, running liquid film (supplying nutrients) prevented herbivore grazing and avoided the competition by phytoplankton.

The special design also provided non-invasive sampling of biofilm mass and, consequently, non-destructive estimates of biofilm growth over time that was indicated by the decrease of subsurface light. Inocula exhibited relatively low species richness and, consequently, represented good model systems to study microbial community development.

Light drove biofilm formation on the artificial substrata and mass accumulation was much slower in marine biofilms. Biofilms elaborated 3D architectures and microscopy revealed spatially and temporally defined phototrophic assemblages in marine and freshwater cultures. Mature biofilms produced filamentous streamers in most experiments that allowed access to regions with higher solute transport and fluxes, evidencing diffusion limitation, the existence of resource gradients and favouring species with filamentous growth habits. Light had also a stronger effect over the other environmental parameters in shaping composition of phototrophs in the experiments. Diversity was clearly influenced by light and decreased significantly at low light intensities. Thus, species competition and photosynthetic performance of microorganisms present were thought to have played a major role in biofilm development. Initial adhesion of organisms was related to the physiological features of biofilm components, the composition of the inocula and their prototroph lifestyles. Contact rate with substrata, cell morphology and adhesive properties in response to shear forces conditioned initial formation of biofilms on the substrata, then competition for available resources, growth and physiological characteristics of biofilm members influenced the light-driven maturation of the communities in culture.

8. Acknowledgements

We are grateful to Dr. Francesca Di Pippo, Dr. Antonella Guzzon and Dr. Silvia Bonavita for their help during the PHOBIA experiments conducted in the Laboratory of Biology of Algae.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 245–271
DOI 10.1007/978-94-007-1327-7_11, © Springer Science+Business Media B.V. 2011

POSSIBLE BUCKLING PHENOMENA IN DIATOM MORPHOGENESIS

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1. Introduction

We would like to present a new way of looking at diatoms: as buckled structures (Tiffany et al., 2009). Wrinkled, sometimes periodic structures can have alternative explanations, the best known being the reaction–diffusion equations of Alan Turing (Turing, 1952). While his paper was titled "The chemical basis for morphogenesis," he was well aware that in some cases "… in addition to the chemical instability, there is a mechanical instability causing the breakdown of … symmetry …. With the aid of a digital computer … [it] might even be possible to take the mechanical aspects of the problem into account as well as the chemical …." We now have available robust finite element methods (FEM) (Chandrupatla and Belegundu, 2000) which are contributing to the analysis of morphogenesis (Gordon and Jacobson, 1978; Gordon, 1985; Beloussov and Lakirev, 1991; Selker et al., 1992; Chen and Brodland, 2000, 2006; Conte et al., 2008; Holloway and Harrison, 2008; Ramasubramanian and Taber, 2008; Sen et al., 2009), including multiphysics FEM combining chemistry and mechanics (Muñoz, 2010). FEM could be used to see if the shapes of diatoms, that we allege may have been buckled mechanically, could be quantitatively emulated by computer simulation. Multiphysics FEM could bring in the chemistry (Gordon and Drum, 1994; Lenoci and Camp, 2008), and as we shall see, electrochemistry (Léger et al., 1999), of the pattern formation and precipitation of silica and the macromolecular dynamics of the cytoskeleton around the silicalemma (Parkinson et al., 1999; Gordon et al., 2009). Here we will simply catalog possible buckled structures in diatoms, leaving their computer simulation for later work. Beyond that, definitive answers will require watching morphogenesis unfold, perhaps via in vivo light microscopy of giant Antarctic diatoms (Gordon et al., 2010) or by scanning electron microscopy (SEM) of forming valves (Tiffany and Hernández-Becerril, 2005). As buckling is an instability phenomenon that can generally have two or more outcomes, small mechanical forces, applied via micromanipulation, could be used to alter the course of buckling in ways predictable by FEM (Hutson et al., 2009), as a further test of this hypothesis.

Figure 1. Buckling of linear structures (railroad tracks) into curves in a plane, due to thermal expansion, which generates compressive forces on the rails (Coxon, 1979; Reproduced with kind permission of the photographer, Dave Coxon). This is an example of buckling from one to two dimensions. The rails buckle the same way because they are coupled via the perpendicular ties.

Buckling involves a transformation of a structure to one of a higher dimension. A one-dimensional linear structure becomes wavy when pushed from both ends (Fig. 1). If the wave lies in a plane, the structure has become two dimensional, but bending into a nonplanar space curve can also be anticipated (Trahair, 1993), in which case it becomes three dimensional. An example would be a linear structure twisted into a helix (van der Heijden and Thompson, 2000): the supercoiling of a twisted rubber band or DNA molecule (Bates and Maxwell, 2005) is also an example of such buckling. A planar structure, like a sheet of paper, rubber or cloth, buckles into the third dimension when pushed from the sides or stretched along a line. We are most familiar with catastrophic buckling due to impacts at high speeds, such as in automobile crashes. But with few exceptions (Gordon, 1987), cellular level phenomena are mechanically more gentle. In general, buckling is thought of as mechanical failure of a structure:

> In engineering, buckling is a failure mode characterized by a sudden failure of a structural member subjected to high compressive stresses, where the actual compressive

> stress at the point of failure is less than the ultimate compressive stresses that the material is capable of withstanding. This mode of failure is also described as failure due to elastic instability (Wikipedia, 2010).

This attitude came about perhaps because most of the things we manufacture are rigid, and changes of shape are considered undesirable. But nature need not operate with our preconceptions. A tree sways and bends in a strong wind (buckles), usually without breaking, whereas the classical Greek column is expected to remain upright, and cracks or falls if pushed a fraction of the distance. But there are exceptions. We like our curtains and evening gowns nicely pleated, and we place galvanized roofing and cardboard into buckled sheets to begin with, for strength. In fact, corrugated materials become resistant to further buckling, at least in one direction, so that buckling in some cases is a source of increased mechanical strength. Buckling has been investigated in many biological contexts, few involving mechanical failure (Brodland and Gordon, 1990; Veitch and Naylor, 1992; Schlick et al., 1994; Coughlin and Stamenovic, 1997; Ramachandran and Schlick, 1997; Dias et al., 1998; Costa et al., 2002; Sharon et al., 2002; Hejnowicz and Borowska-Wykret, 2004; Needleman et al., 2004; Liu et al., 2006; Salicone et al., 2006; Volokh, 2006; Gentry et al., 2009).

2. Buckling of Long Narrow Structures: Chains of Diatoms

Diatoms frequently occur in chains (Pahlow et al., 1997; Karp-Boss and Jumars, 1998; Crawford and Sims, 2008; Musielak et al., 2009; Srajer et al., 2009). Any bending of the chains is a form of buckling (Fig. 2, sites and dates of collection of all diatoms are in Table 1). In general, in this case, the lateral bending and end on buckling forces are not generated by the diatoms themselves, but rather by the environment. When mechanical limits are exceeded as in a turbulent environment, chains are broken.

Quantitative analysis of the mechanics of chain diatoms has been started (Musielak et al., 2009). This work may account for the intricate adaptations that permit tremendous flexibility in turbulent environments (Tiffany et al., 2010), versus those adaptations that stiffen a chain against bending. In patchy environments, long, stiff chains have better access to nutrients (Musielak et al., 2009), but we can also anticipate that such stiffness leads to breakage and shorter chains in turbulent environments, so it will be interesting to see if there is an empirical trade-off between chain stiffness and turbulence.

3. Buckling of Long Narrow Structures: Pennate Diatoms

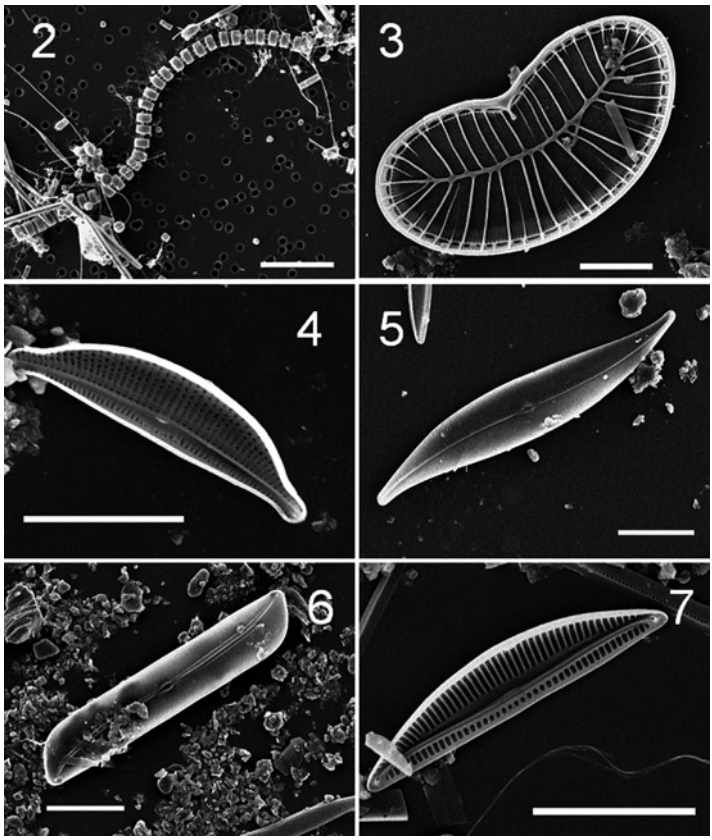
Less obvious is the buckling of single diatom cells. Diatoms do buckle and break when crushed (Hamm et al., 2003; Hamm, 2005; Hamm and Smetacek, 2007). But the valves and other structures of many diatoms look as if they were buckled during their formation. Most pennate diatoms are reasonably straight, presumably due to

the straight sternum or raphe-sternum made of unknown material inside the "pattern-centre" or "primary silicification site" (Mann, 2006). Why then are some pennate diatoms arched (arcuate, lunate) about their long axis (*Hannaea*, p. 366, *Semiorbis*, p. 456 in Round et al., 1990)? While it is true that the epitheca (older valve) constrains the shape of the hypotheca (younger valve), one would expect straightening after a few generations, by analogy to the restoration of "normal" morphology from auxospores (Jewson, 1992; Sato et al., 2008). There are two general ways that a straight raphe-sternum inside a developing valve could be buckled: (a) the structure could elongate, as by polymerization or sliding of filaments against one another, and on encountering the walls of the epitheca continue elongating by buckling, which allows a greater length in a confined space, before its growth makes a U turn at the distal ends of the cell (fig. 32 in Round et al., 1990) and (b) the microfilament ring around the silicalemma (Gordon et al., 2009), considered mechanically as two long arcs compressing the distal ends, causes the raphe-sternum to buckle. Of course, these two mechanisms could both be operating in some cases. What buckles could be the unknown material of the raphe-sternum on which silica precipitation nucleates and/or the silica itself. Similarly, when we speak of obstacles such as the central node, that obstacle may consist of nucleating material rather than silica, at the time of buckling.

Now, buckling of a linear structure results in one or more halfwaves whose wavelength depends on mechanical properties of the material being buckled, and on material attached laterally to that structure (Brodland and Gordon, 1990). For example, buckling of the raphe may be retarded by lateral support of the costae growing from it. We use the term halfwave because a full sine wave has two portions, bending one way, then the other. We show some pennate diatoms, then, that might be buckled structures with one halfwave (Figs. 3 and 4), two halfwaves (Figs. 5 and 6) or three (Fig. 7) or four (Fig. 8) such halfwaves. The bending may be marked only in the raphes or be reflected in the morphology of the whole cell. Cells of the same genus may be "crescent shaped or bilobate" (*Auricula*, p. 634 in Round et al., 1990), suggesting a partially nongenetic, i.e., mechanical variation in number of waves. This may be analogous to meristic variation in number of vertebrae and fin rays (Ali and Lindsey, 1974) or carpels (Charlton and Posluszny, 1991).

The raphe is an asymmetric structure, yet many pennate diatoms are bilaterally symmetric, except for the small Voigt discontinuity (Mann, 2006). It would therefore be interesting to learn whether the bending direction in bent pennates correlates with the primary/secondary sides of the raphe-sternum (fig. 32 in Round et al., 1990).

Buckling of pennates in three dimensions is possibly the basis for low-pitch helical structure of *Cylindrotheca* (p. 626 in Round et al., 1990). Ordinarily, the growing pair of raphes in adjacent daughter cells is perhaps constrained by the pair of cell membranes to which their silicalemmas are attached. However, those membranes may buckle, resulting in an out of plane warping of the raphes. This may be what leads to the interesting case where the buckling is perpendicular to the valve faces, so that the cells are "… arcuate in girdle view and thus with one concave and one convex valve" (*Gephyria*, p. 440 in Round et al., 1990) (see Figs. 9 and 10).



Figures 2–7. Figure 2 A buckled chain of *Cyclotella* sp. with flexible linkages between cells. This is an example of one dimensional buckling, in this case confined to a 2D plane, i.e., the Nucleopore® support. Figure 3 *Plagiodiscus* sp. with a bent raphe-sternum and valve, which we designate as one halfwave. Figure 4 *Amphora copulata* with one halfwave buckling. Figure 5 *Gyrosigma wormleyi*, with a two halfwave buckling pattern. Note that the valve margin reflects the raphe bending. Figure 6 *Gyrosigma* sp. with two halfwave buckling. Note that the valve margin reflects the raphe-sternum bending only distally. Figure 7 *Amphora* sp. with three halfwaves of raphe-sternum buckling. Scale bars 10 μm (Figs. 3, 4 and 7), 20 μm (Figs. 5 and 6), and 50 μm (Fig. 2).

4. Buckling Constrained by Stiff Internal Features

Some whole valves are buckled in a way that suggests that the silicalemma "outgrew" the constraints of the epitheca. Since the excess area has nowhere to go except into the third dimension, we can imagine this as a scenario for buckling. If the raphe has stiffened before the silicalemma growth is finished, then it will act as a constraint on that valve face buckling (Figs. 13–16).

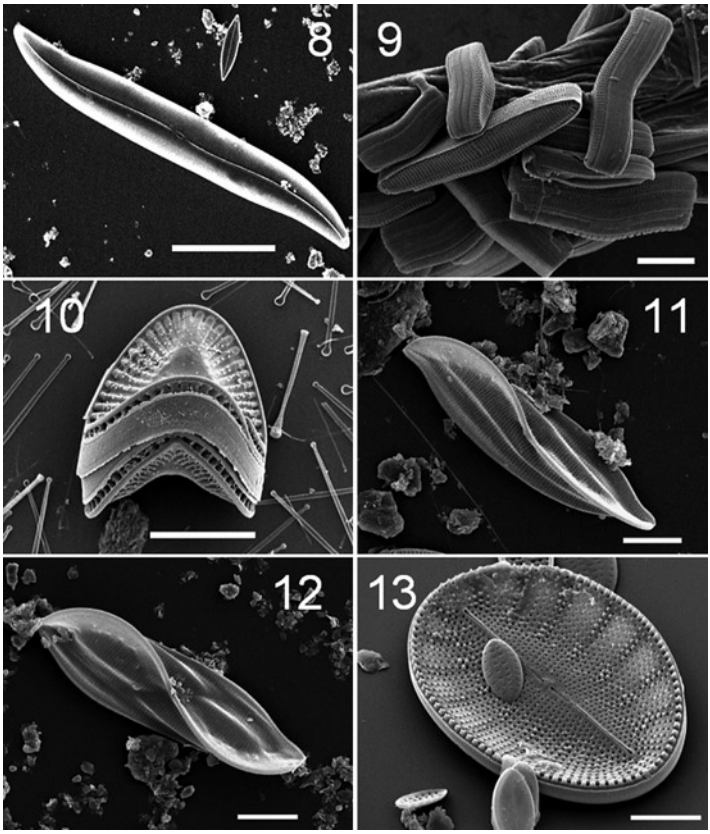
Table 1. Sources and collection dates (except for fossil specimens) for diatoms illustrated in this chapter. File names permit retrieval for further work.

Figure	Species	File name MAT database	Sampling site	Date sampled
2	*Cyclotella* sp. (new species)	antox500		1/15/99
3	*Plagiodiscus* sp.	SPYcx2500	San Diego Bay	10/22/00
4	*Amphora copulata*	ssdiatom24x5000	Salton Sea	9/21/98
5	*Gyrosigma wormleyi*	SCity_10_2_98jx1300	Salton Sea	10/2/98
6	*Gyrosigma* sp.	MontMfx1300	Montague Island, Sea of Cortez	5/30/99
7	*Amphora* sp.	SSBEACH6_12bx5000	Salton Sea Beach	6/12/99
8	*Gyrosigma balticum*	ssdiatom42x700	Salton Sea	9/21/98
9	*Achnanthes brevipes*	RKair1_12_99ax1000	Riviera Keys, Salton Sea	1/12/99
10	*Campylodiscus* sp.	San_Vin12x700	San Vicente Reservoir, San Diego	3/20/98
11	*Entomoneis* aff. *pulchra*	RedHillMhx2000	Red Hill, Salton Sea	2/22/99
12	*Entomoneis* aff. *pulchra*	SCUMs_4_2_28hx2000	Salton Sea	10/15/99
13	*Campyloneis* sp.	Tourmatiltx2500	Tourmaline Surfing Park, La Jolla	7/18/00
14	*Campyloneis* sp.	Tourmlx1800	Tourmaline Surfing Park, La Jolla	7/18/00
15	*Diploneis* sp.	bremerbay_25	Bremer Bay, Western Australia	10/5/05
16	*Surirella* sp.	bremerbay_42	Bremer Bay, Western Australia	10/5/05
17	*Pleurosigma ambrosianum*	S4_2_28kbx15000	Salton Sea	2/28/99
18	*Pleurosigma ambrosianum*	plk3_16cx20000	Salton Sea	3/16/99
19	*Gomphonema parvulum*	S1_5fx25000	Salton Sea	3/16/99
20	*Pleurosigma ambrosianum*	scumbax8000	Salton Sea	10/15/99
21	Unknown species	TJE7_21bx7000	Tijuana Estuary	7/21/99
22	*TerpsinoC musica*	WhfiCax250	Whitefield Creek, Salton Sea	12/8/99
23	*TerpsinoC musica*	WhfiCdx1100	Whitefield Creek, Salton Sea	12/8/99
24	*Biddulphia biddulphiana*	Be25	Bannister Island, Belize	3/31/10
25	*Biddulphia biddulphiana*	Be36	Bannister Island, Belize	3/31/10
27	*Stephanodiscus hantzschii*	San_Vinnx4500	San Vicente Reservoir, San Diego	3/20/98
28	*Craspedodiscus?*	electron_image_20	Dunkirk, Maryland	Fossil
29	*Glyphodiscus stellatus*	MBay6_11_02cctiltx1100	Mission Bay, San Diego	6/11/02
30	*Cyclotella litoralis*	MBy6_19atiltx2500	Mission Bay, San Diego	6/19/02
31	*Cyclotella litoralis*	M_B6_19etiltx3500	Mission Bay, San Diego	6/19/02
32	*Cyclotella chochtawhatcheeana*	almt12x8000	Alamo River, California	12/1/98
33	*Craspedodiscus elegans*	DUNKIRKbtiltx350	Dunkirk, Maryland	Fossil
34	*Auliscus sculptus*	SCPhytddx1300	Santa Clara, Sea of Cortez	5/10/97
35	*Auliscus* sp.	ob3_5bx500	Ocean Beach, San Diego	3/5/03

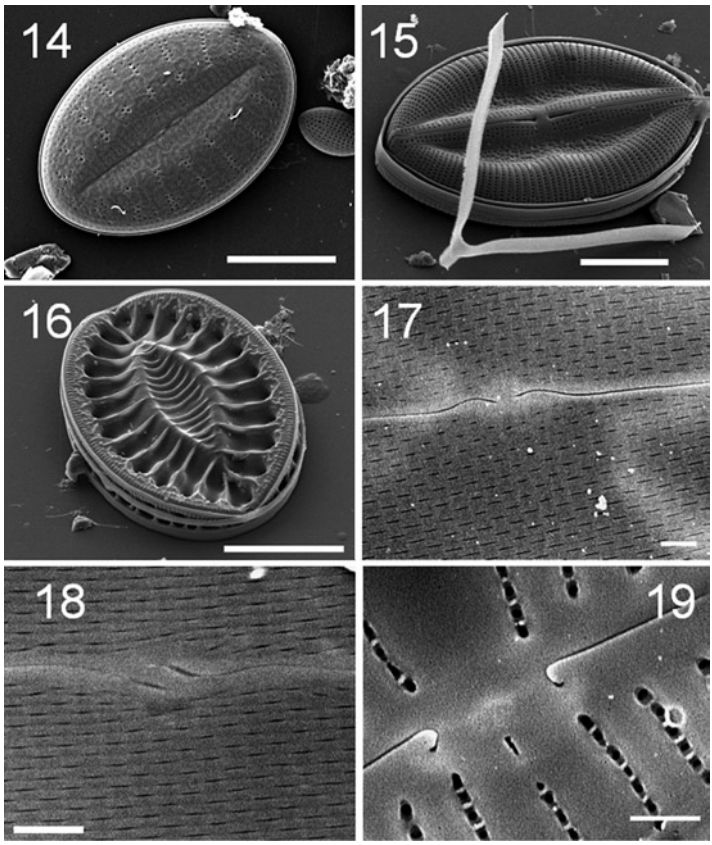
(continued)

Table 1. (continued)

Figure	Species	File name MAT database	Sampling site	Date sampled
36	*Actinoptychus senarius*	MisBy5_1cx2000	Mission Bay, San Diego	5/1/02
37	*Actinoptychus heliopelta*	DUNKIRK_ax600	Dunkirk, Maryland	Fossil
38	*Actinoptychus heliopelta*	DUNKIRK_ax600	Dunkirk, Maryland	Fossil
39	*Actinoptychus splendens*	MisBy5_1hx2000	Mission Bay, San Diego	5/1/02
40	*Actinoptychus splendens*	MiBayb800	Mission Bay, San Diego	2/22/01
41	*Cyclotella cryptica*	FreshWM12_27wx13000	Freshwater Marsh near Salton Sea	12/28/99
44	*Cymatopleura* sp.	MontMqx2000	Montague Island, Sea of Cortez	5/30/97
47	*Stephanodiscus, hantzschii*	San_Vinax1500	San Vicente Reservoir, San Diego	3/20/98



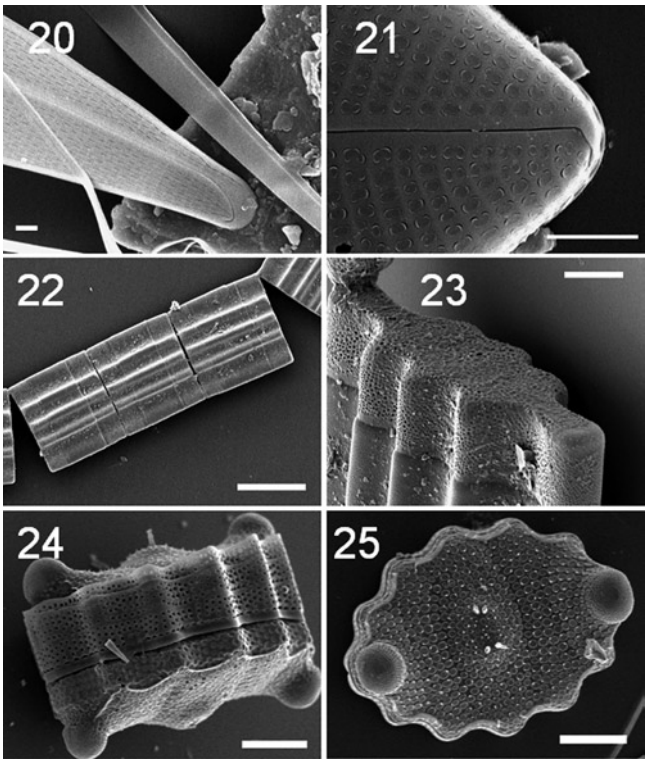
Figures 8–13. Figure 8 *Gyrosigma balticum*, with four halfwaves of raphe-sternum buckling, scale bar 50 μm. Figure 9 *Achnanthes brevipes*, in which buckling is perpendicular to the valve faces. Figure 10 *Campylodiscus* sp., severely buckled perpendicular to the valve face, producing convex and concave valves, and saddle shaped. Figure 11 *Entomoneis* aff. *pulchra*, with a three-dimensionally twisted raphe-sternum, with what look like long folds or pleats. Figure 12 *Entomoneis* aff. *pulchra*, with a three-dimensionally twisted raphe-sternum, with what look like split folds or pleats. Figure 13 *Campyloneis* sp., buckling of a pennate valve about an apparently stiff raphe-sternum viewed from the inside face of the valve. Scale bars 10 μm (Figs. 9 and 11–13) and 50 μm (Figs. 8 and 10).



Figures 14–19. Figure 14 *Campyloneis* sp., Buckling about an apparently stiff raphe viewed from the outside face of the valve. Figure 15 *Diploneis* sp., buckling of valve that in cross section would show two halfwaves, perhaps due to a stiff raphe. Figure 16 *Surirella* sp., buckling inside and outside presumably stiff sterna, as the raphes are on the perimeter of the valve. Figure 17 *Pleurosigma ambrosianum*, raphes buckling to the same side on encountering the central node, scale bar 1 μm. Figure 18 *Pleurosigma ambrosianum*, raphes buckling to opposite sides on encountering the central node. Figure 19 *Gomphonema parvulum*, sharp bending of raphes on encountering a perhaps stiff central node. Scale bars 1 μm (Figs. 17–19), 10 μm (Figs. 14), 20 μm (Figs. 15–16).

5. Buckling Constrained by Stiff Features

Raphes are often straight except at their distal and central pores (fig. 35 in Round et al., 1990). We can imagine that the growing raphe ends bend when they hit a stiff obstacle, such as the central node (Figs. 17–19) or epithecal wall (Figs. 20 and 21) and keep growing for a while, before the collision somehow feeds back to



Figures 20–25. Figure 20 *Pleurosigma ambrosianum*, severe bending of the distal end of a raphe on encountering the epitheca. Figure 21 *Unknown species*, bending twice of the distal end of a raphe, as it encountered the epitheca perhaps twice. Figure 22 *Terpsinoë musica*, corrugated buckling in girdle view. Figure 23 *Terpsinoë musica*, corrugated buckling. Figure 24 *Biddulphia biddulphiana*, girdle view, corrugated buckling. Figure 25 *Biddulphia biddulphiana*, valve view, corrugated buckling. Scale bars 5 μm (Fig. 20), 20 μm (Fig. 21, 23–25), 100 μm (Fig. 22).

halt the growth mechanism. The sharpness of the terminal bending may reflect the stiffness of the obstacle.

6. Buckling Around the Margins

About five undulations are found in *Undellata* (p. 604 in Round et al., 1990). Crenulation with 20 waves per cell is found in *Pseudorutilaria*, which overall is straight in form (p. 274 in Round et al., 1990), so that the correlation between cell shape and raphe shape is imperfect and needs investigation. Spectacular buckling of margins and girdle bands are reminiscent of corrugated "tin" roofs (Figs. 22–25).

7. Buckling Explains the Principle of Complementarity

The principle of complementarity asserts:

> After vegetative cytoplasmic division, the daughter protoplasts may be tightly appressed or may retract from each other. These two types of behaviour have been termed interactive and noninteractive division respectively (Mann, 1984). Since the new valves are formed in vesicles lying immediately beneath the plasmalemma on the faces of the recently completed cleavage furrow, valves formed during interactive division must have complementary shapes…. The valve shape is not controlled by shape and orientation of the cleavage furrow: any elevations, projections, etc. on the valve develop after cleavage, while the new valves are being deposited…. It is simplest to assume that each pair of complementary features … is produced by a single influence present during valve formation (pp. 34–35 in Round et al., 1990).

This is illustrated schematically in Fig. 26. The study of the buckling of laminates (Simitses, 1996; Zhang and Yang, 2009), as an explanation of biological structures, goes back to Wilhelm His (His, 1874, 1888; Gordon, 1985). The MTOC ⇔ silicalemma ⇔ plasmalemma ⇔ plasmalemma ⇔ silicalemma ⇔ MTOC (Gordon et al., 2009), when one counts the deposited silica inside each silicalemma, is a laminate of at least ten layers (MTOC = microtubule organizing center). In interactive division, there may be an eleventh extracellular layer temporarily binding the plasmalemmae together. It has been noted that:

> … the positions of the ribs in the SDV [silica deposition vesicle, i.e. silicalemma and contents] in one daughter cell appear to be largely or fully in register with the ribs in the corresponding SDV of the other daughter cell, implying linkage of morphogenesis across the cleavage furrow plasma membranes. However, the correlations could be coincidence – there are insufficient observations to be sure (Mann, 2006).

Mechanical coupling of this multilayer laminate might alter the local rates of silica deposition and cause them to be correlated between daughter cells, perhaps even more so when laminate buckling is occurring.

8. Bessel Functions for Valve Faces of Cylindrical Centric Diatoms

The vibrations of a circular musical drum are explained by Bessel functions. Any complex mode of a drum's vibration can be analyzed as a sum of Bessel functions much the way any function of one variable can be represented as the sum of sine waves. Just as perturbation analysis of the breakup of a cylinder of fluid into equally spaced drops begins with sine functions (Rayleigh, 1892; Gordon et al., 1972, 1975), deviations from planarity of a circle of material starts with Bessel functions (Nouri et al., 2008). Bessel functions, as functions of two variables, are characterized by two integers. We have tried to match up the first dozen Bessel functions

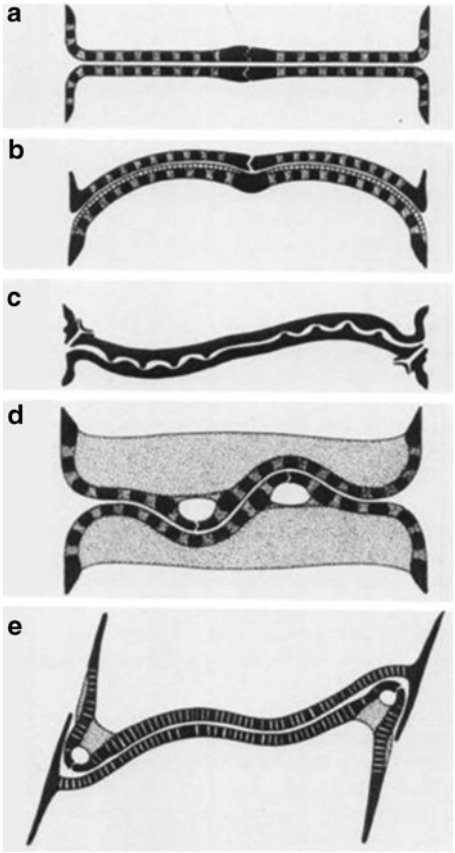
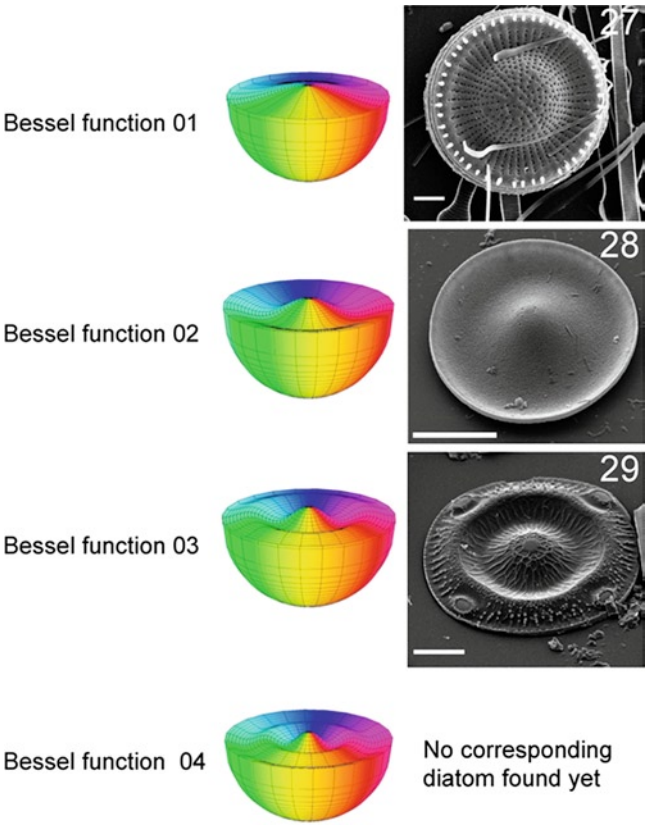


Figure 26. "Demonstration of the principle of complementarity; sections through sibling valves in diatoms showing interactive division: (**a**) *Navicula*: planar valve faces; (**b**) *Cocconeis*: heterovalvy, with one convex and one concave valve; (**c**) *Actinoptychus*: radial sectoring of the valves allows concave portions of one valve to complement convex portions of the other, without heterovalvy; (**d**), (**e**) *Denticula* and *Tryblionella*: bilateral asymmetry and opposite orientation of sibling valves allows the development of complex ridge-and-furrow systems" (fig. 27 in Round, et al., 1990), with permission of Cambridge University Press). The buckling modes 0, 1, 2, 4, 4 halfwaves may be assigned to these sketches, respectively, ignoring the fact that, of course, they would represent cross sections of three-dimensionally buckled, once planar laminates, and thus could also have Bessel function buckling modes out of the plane of the page.

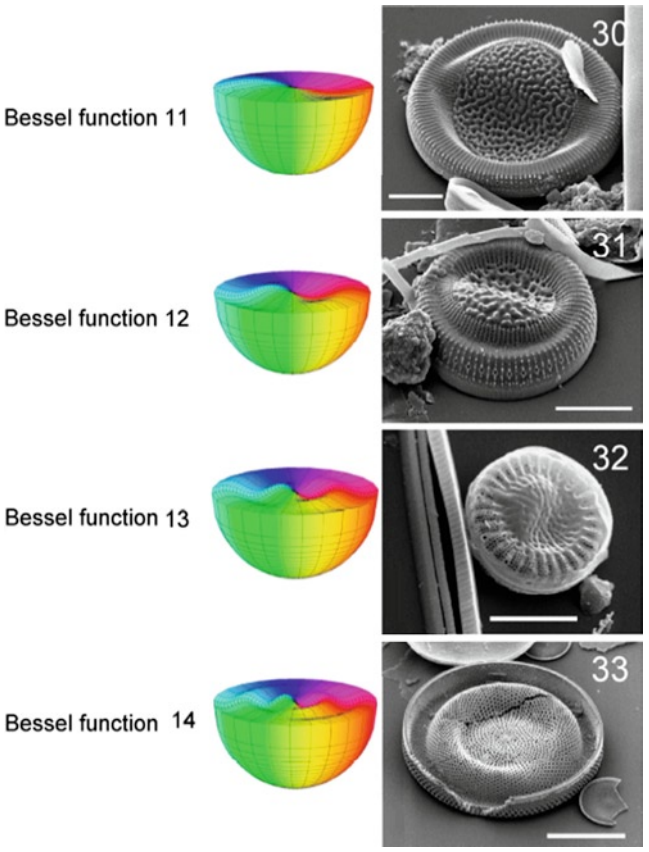
with centric diatoms whose valves resemble them (01: Fig. 27; 02: Fig. 28; 03: Fig. 29; 11: Fig. 30; 12: Fig. 31; 13: Fig. 32; 14: Fig. 33; 21: Fig. 34; 24: Fig. 35). The reader is challenged to find diatoms matching the Bessel functions for which we found no match: 04, 22, 23. Notice that cross sections of some of the Bessel functions would resemble the sketches in Fig. 26.



Figures 27–29. Bessel functions characterized by the two single-digit integers 01-04. Comparable diatoms are adjacent to Bessel functions. Figure 27 *Stephanodiscus hantzschii*. Figure 28 *Craspedodiscus*. Figure 29 *Glyphodiscus stellatus*.

9. Circular Buckling of Valve Faces of Cylindrical Centric Diatoms

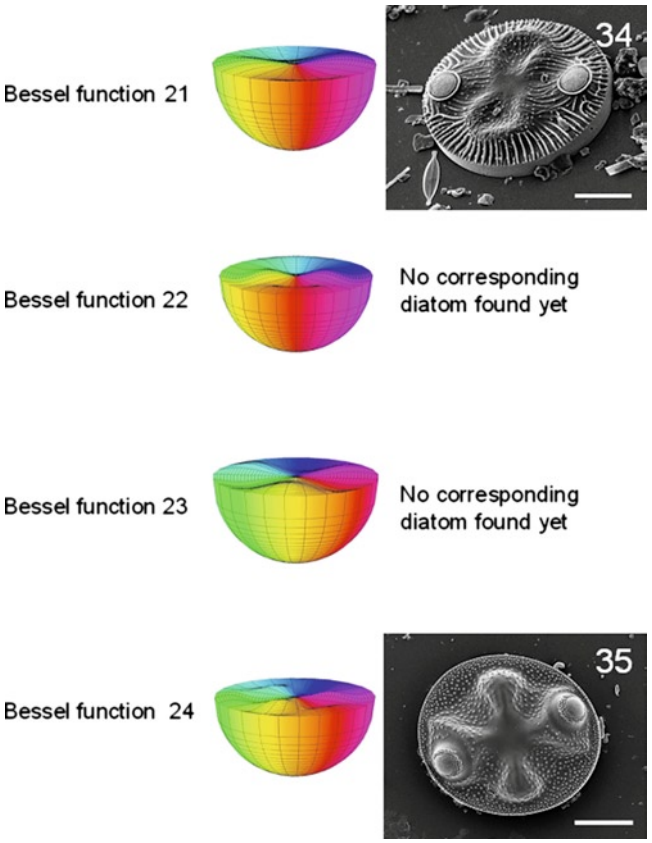
Stiffness in the annulus (fig. 26 in Round et al., 1990) or midring (Gordon and Drum, 1994) can lead to an entirely different class of buckling patterns: circular. Thus, we see a wave pattern that goes around the center. Where there is uniformity around the axis of a cylindrical centric diatom, we end up with an integer number of waves, i.e., the wave pattern is quantized. One wavelength includes a downswing and an upswing, so we will classify these buckling patterns by a single integer, the number of waves it takes to go around the circle once. Note that the Bessel functions 01 (Fig. 27) and 11 (Fig. 30) could also be looked at as circular buckling patterns of 0 and 1 wave, respectively. We give some examples with 3 waves (Fig. 36), 4 waves (Figs. 37 and 38), and 14 waves (Fig. 39). Some genera, like



Figures 30–33. Bessel functions characterized the two single-digit integers 11–14. Comparable diatoms are adjacent to Bessel functions. Figure 30 *Cyclotella litoralis*. Figure 31 *Cyclotella litoralis*. Figure 32 *Cyclotella chochtawhatcheeana*. Figure 33 *Craspedodiscus elegans*.

Actinoptychus, have been illustrated with 3, 5, and 8 waves, with reports of up to 20 "sectors" or 10 waves (pp. 200–201 in Round et al., 1990). A diatom with 11 waves is shown in Fig. 40.

The perfection of some diatom circular buckling patterns has been tested by rotating a digital image by 360/*n* degrees, where *n* is the number of waves, and subtracting the pictures from one another. For a perfect pattern, the resulting picture should be solid black, and some diatoms come uncannily close to perfection (Sterrenburg et al., 2007). On the other hand, others are far from perfect (Fig. 41), suggesting a dynamic process that can be "frozen" by firming up of the silica before near perfection has been achieved. This is consistent with the notion that many perturbation modes of buckling compete with one another, with the faster growing modes generally dominating if the process has

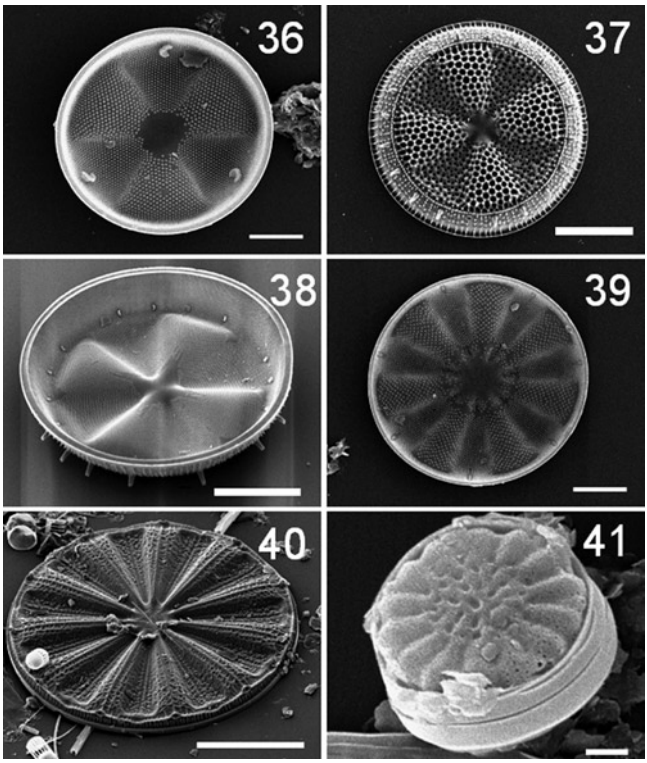


Figures 34–35. Bessel functions characterized by the two single-digit integers 21–24. Comparable diatoms are adjacent to Bessel functions. Figure 34 *Cyclotella litoralis*. Figure 35 *Cyclotella litoralis*.

enough time to reach a stationary state (Nouri et al., 2008). As perfection may be sought for diatom nanotechnology, these mechanisms need to be understood (Gordon, 2010).

Cracking is an extreme result of buckling of a brittle material. Patterns similar to circular buckling have been generated in silica gel (Fig. 42) and concrete (Fig. 43). These are reminiscent of *Glorioptychus* with an inner portion with 3 waves and an outer ring of 9 waves (p. 202 in Round et al., 1990).

Pennate diatoms would have to have rectangular valves to allow for regular waves perpendicular to their axes, which would be analogous to circular waves around a cylindrical centric diatom. With their curved margins, such longitudinal waves (*Cymatopleura*, pp. 648–649 in Round et al., 1990) would be expected to not be spaced equally (Fig. 44).

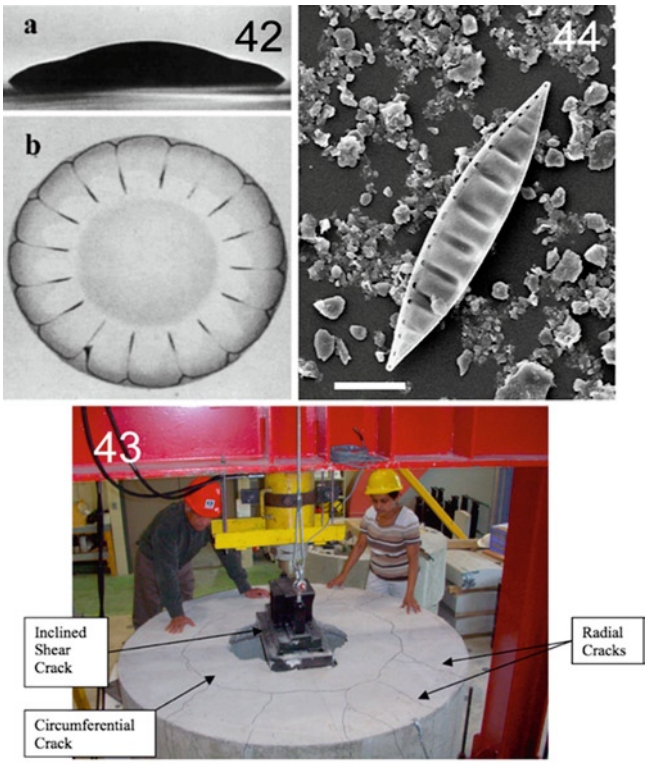


Figures 36–41. Figure 36 *Actinoptychus senarius*, a circular buckling with three waves around in a centric diatom valve. Figure 37 *Actinoptychus heliopelta*, circular buckling with four waves around. Figure 38 *Actinoptychus heliopelta*, inner aspect of a valve with circular buckling of four waves around. Figure 39 *Actinoptychus splendens*, circular buckling with 14 waves. Figure 40 *Actinoptychus splendens*, circular buckling with 11 waves around. Figure 41 *Cyclotella cryptica*, circular buckling with some irregularities, perhaps due to "freezing" of the dynamic pattern of buckling in silica before the pattern had a chance to settle down. Scale bars 1 μm (Fig. 41), 10 μm (Figs. 36, 39), 50 μm (Figs. 37–38, 40).

10. Toy Models for Buckling

Analog simulations using sheets of material are probably the best way to qualitatively explore the possibilities for explaining diatom buckling patterns. Samir S. Badour (1997, personal communication) noted the flip-flop of the valves in the centric chain diatom *Cyclotella cryptica.* This may be explained by overgrowth of the nascent valves: a circle whose diameter exceeds the inner diameter of the cylinder constraining it will buckle (Figs. 45 and 46).

With a little imagination, scissors, needlepoint frames, and dental dam, various toy models could be built before we get into quantitative computer simulations,

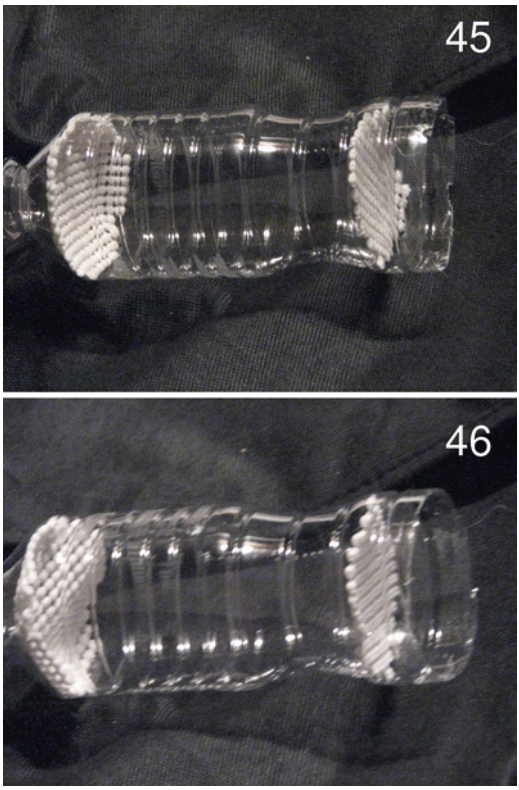


Figures 42–44. Figure 42 A 4 mm diameter silica gel disk cracks radially on drying out, creating an analogy to buckling patterns of some centric diatoms, including a hyaline region in the middle (Pauchard, et al., 1999, with permission. Copyright (1999) by the American Physical Society). Cracking is an extreme consequence of buckling. Figure 43 Punching failure cracks in concrete roughly imitate some centric diatom patterns (Mediwake, 2006), with kind permission of thesis advisor Nipon Rattanawangcharoen. Figure 44 *Cymatopleura* sp., a pennate diatom with buckling of the valve perpendicular to its axis. This is analogous to circular buckling in cylindrical centric diatoms, but the wave spacings are not uniform. Scale bar 10 μm.

which while more flexible in many ways, such as allowing us to specify the constitutive properties of each component, do not [at least at present (Dobashi et al., 2007)] provide the tactile, haptic feedback of a physical model.

11. Discussion: How Buckling Warps Our View of Diatom Morphogenesis

We have shown that a wide range of phenomena in diatoms resemble buckling patterns, and may indeed be caused by mechanical buckling of raphes, nascent valves, etc. Proof or disproof of this concept will come via computer simulation,



Figures 45–46. Figure 45 Toy models. A toy model for Bessel function 01 buckling of a nascent centric valve that has slightly overgrown its epitheca. Figure 46 Same for Bessel function 11.

time sequence microscopy, micromanipulation, and direct measurement of the constitutive properties (like stiffness) of the materials involved (cf. Dugdale et al., 2005; Francius et al., 2008). However, the study of buckling in diatoms may have many other ramifications.

For example, we have seen how the pair of daughter cells may contain a laminated structure with at least 11 layers, raising the interesting questions of how these layers are all kept together during valve morphogenesis, and then released from one another. Could there be symmetry breaking instabilities in this process that somehow lead not just to complementary valves (Fig. 26) but to more profound heterovalvy (Round et al., 1990), the deeply mysterious phenomenon whereby two valves with presumably identical genomes involved in their morphogenesis become so vastly different that we would assign them to different orders if we did not see them attached (Gordon, 2010)? Contrary to the suggestion "… that

heterovalvy illustrates the ability of at least some diatom genotypes to respond to their environment by coding for more than one phenotype" (Cox, 2006), it may be a mechanical phenomenon in which the role of the genome is to set up the initial or "boundary" conditions (Gordon, 1999). Here is another example:

> No genetic coding is needed to instruct pieces of a leaf to curl up and curl down. All that is required is a growth process to elongate the sheet along its edge — elasticity takes care of the rest (Sharon et al., 2002).

There are some caveats we have to place on these observations. The matches are obviously not quantitative, for instance to the Bessel functions, but the discrepancies could be attributed to changes in the nascent valve as the silicalemma grows (Gordon and Drum, 1994) or nonlinear phenomena (Huang et al., 2005) that are not simple amplifications of small perturbations. For example, the domain pattern of costae in *Thalassiosira eccentrica* (shown in fig. 1g of (Gordon and Drum, 1994)), which evaded understanding via a DLA model (Gordon, 2003), may find its explanation in herringbone wrinkling patterns (Huang et al., 2005).

While diatom silica seems to be amorphous (Gordon and Drum, 1994) and thus isotropic in its mechanical properties, microtubules and other macromolecular structures attached to the silicalemma are mechanically anisotropic, leading to the possibility of more complex buckling modes (Im and Huang, 2008; Yin et al., 2009). Stiffness at the margin can alter the buckling patterns, which would constitute a hinge effect (Veitch and Naylor, 1992; Gebeshuber, 2007). We do not know when buckling occurs relative to the steps of valve morphogenesis (Gordon et al., 2009), but can only presume that it is while structures are thin. The mechanical roles of ocelli may be to bias which way the initial buckling perturbation goes (Figs. 34 and 35), if their development starts prior to buckling, or they may merely distort an already buckled structure (Fig. 29).

In general, diatom valve formation occurs in two steps ("… an almost two-dimensional array of ribs and striae first, then any additional layers and structures external or internal to the first-formed layer" Mann, 2006; cf. Pickett-Heaps et al., 1990): (a) a thin layer of silica precipitates within the silicalemma as it grows, typically in 10 min and (b) the valve thickens, over about 6 h (Gordon and Drum, 1994). This has two mechanical consequences. First, a thin layer of silica is likely to be easy to bend and buckle, just the way a glass capillary bends more readily the thinner it is. Second, diatoms can do something we rarely indulge in to increase mechanical strength of a manufactured structure: the valve thickens after it is shaped. Corrugation also increases strength; thickening after corrugation would do so that much more.

While we do add coatings to things by electrodeposition, this is usually to alter surface properties or appearance, not to increase strength by thickening. Nevertheless, the similarities between diatom morphogenesis and electrodeposition may lead to new avenues in diatom nanotechnology: both may occur via DLA (diffusion limited aggregation) (Gordon et al., 1980; Witten and Sander, 1981; Léger et al., 1999; Gordon, 2010) and both lead to nanostructured three-dimensional

growth, not necessarily simple thickening (Bicelli et al., 2008; Gurrappa and Binder, 2008). But in electrodeposition, one physical phenomenon occurs that has not been delved into in diatom morphogenesis: increasing the electric potential alters the morphology of the precipitate from branched to straight (Fig. 46). This may solve a major outstanding inadequacy of DLA computer simulations of diatom morphogenesis: why are costae mostly long and straight, branching only when there is more space to fill in (Gordon and Drum, 1994; Mann, 2006)? While sintering is an alternative, not mutually exclusive, explanation (Gordon and Drum, 1994), electric potentials could provide long-range order to the precipitation process (Fig. 47). The mechanisms for the formation of sculptured thin films (Lakhtakia and Messier, 2005) may also find application here. Note that despite straight growth, costae may buckle when they reach the margin of the epitheca (Fig. 48).

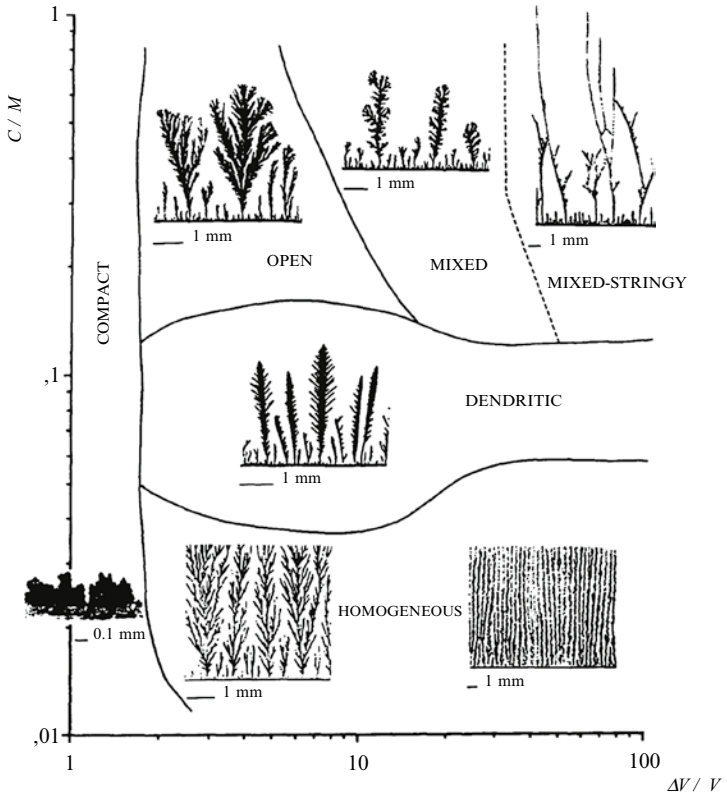


Figure 47. "Diagram of morphologies for zinc electrodeposits obtained from a non deaerated aqueous zinc sulphate solution in a parallel film cell. Electrode length: 3.5 cm.; electrode separation: 3 cm; cell thickness: 70 μm" (Lopez-Tomas, et al., 1995, with permission of Elsevier). Axes are concentration of electrolyte versus voltage.

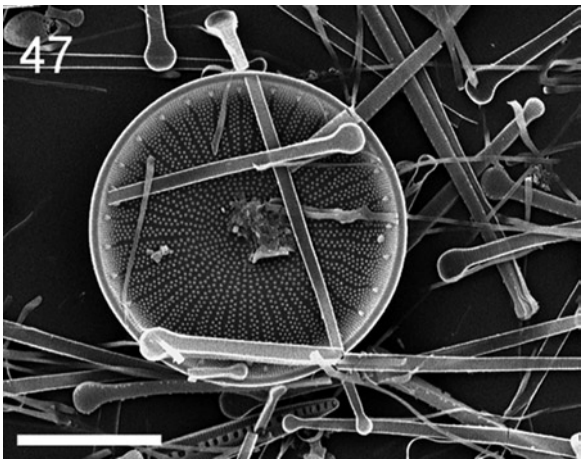


Figure 48. *Stephanodiscus hantzschii*, buckling of costae as they reach the inner surface of the epitheca.

Deliberate buckling of materials is now being used in nanotechnology (Schweikart et al., 2010; Yin and Chen, 2010), an approach which might be enhanced by taking lessons from diatoms, or using them directly in diatom nanotechnology (Drum and Gordon, 2003; Gordon et al., 2005, 2009, 2010; Gebeshuber, 2007; Gordon, 2010). Diatomists can also learn new approaches to explaining diatom morphogenesis, such as "controlled buckling instability" and "wrinkling as strategy for building lithography-free hierarchical structures" (Schweikart et al., 2010). Hierarchical buckling may be involved in the formation of wavy leaves (Sharon et al., 2007). The hierarchical or multiscalar (Pouget et al., 2007; Gordon, 2008) aspects of diatom morphogenesis still await investigation.

Thus, buckling has led us to consider the possibility that the silicalemma has electrical properties, which may alter the morphology of the precipitating silica within. While the silicalemma is not the same shape as nerve dendrites, the theory of electrical fields of complex-shaped cell membranes has been developed there (Segev and Rall, 1998; Segev, 2006). Further analogous effects applicable to diatom morphogenesis might be found in the effect of these electric fields on microtubules (Alvarez and Ramirez, 1979; Kaimanovich et al., 1989; Tuszynski et al., 1997). Direct mechanical effects are also known (Heidemann and Buxbaum, 1994), so that buckling could alter the pattern of microtubule polymerization, and microtubules are also capable of some patterned self-organization (Tuszynski et al., 1997). A buckled surface is a patterned surface (Chen et al., 1998; Lin et al., 2000), to which adhered microtubules might take orientations following the contours. Thus, the morphology of the growing, nascent valve, reflected in its mechanical buckling and changing electric fields, may alter the positioning of the microtubules attached to the silicalemma along which silica transport vesicles may move (Parkinson et al., 1999; Mann, 2006). This brings us back to the need

to observe valves in the process of buckling, including changing electric potentials (Tominaga et al., 1999) and the dynamics of their associated microtubules (Altinok et al., 2007), to test all these ideas.

12. Acknowledgments

We would like to thank Nipon Rattanawangcharoen for discussions on the mechanics of buckling and William R. Buckley for a critical reading. We also thank Steve Barlow for use of the Electron Microscope Facility. Dedicated to diatomist Samir S. Badour, to Bonnie and Simone Hurwitz, to Lana G. Hunstad on her 24th birthday, and to Leland T. Gordon on a big step forward.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 273–289
DOI 10.1007/978-94-007-1327-7_12, © Springer Science+Business Media B.V. 2011

DIATOM FRUSTULES: PHYSICAL, OPTICAL, AND BIOTECHNOLOGICAL APPLICATIONS

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1. Diatom Structure

Diatoms are unicellular photosynthetic eurkaryotes within the class Bacillariophyceae whose peculiarity amongst other microalgae is a siliceous cell wall (Round et al., 1990). Traditionally, diatoms have been subdivided into two major groups based upon their symmetry: the *centrales* have radial symmetry, whilst the *pennales* have bilateral symmetry. Diatoms are found in both freshwater and marine environments, as well as in soil, and on moist surfaces. Individual diatoms vary in size from 2 nm up to several mm in size, although only a few species are larger than 200 μm.

It is estimated that there are at least 100,000 species, separated into 250 genera (Norton et al., 1996; Van den Hock et al., 1997); the complex morphological features of the silica cell wall (frustules) are being used to delineate the genera and species. Indeed, it is the myriad intricate patterns of the frustule which have caught the imagination of nanotechnologists.

The basic architecture of the diatom is that of a hat-box-type structure composed of amorphous silica in which the cell protoplasm is completely enclosed. The nanopores and slits in the frustule allow the living cell to interact with the surrounding environment. It has also been postulated that the frustule may further function to physically protect the cell and to assist in light collection.

2. Exploitation

The development of synthetic nanotechnology has been driven by the global demand for ever smaller structures in electronic, optical, chemical, and biomedical devices. The establishment of an industry that produces nanotechnology-based devices critically depends on methods for inexpensive manufacturing of nanoscale materials on a massive scale. It is highly desirable to be able to produce hard ceramic materials at low temperatures, without aggressive chemicals, and with tight control over nanometer scale structures. Nature has already optimized such nanostructured materials during its evolution in the form of diatoms which

achieve a high degree of complexity and hierarchical structure under mild physiological conditions, requiring no more than basic nutrients and sunlight.

The economic feasibility of the industrial production of nanomaterials by engineered organisms is dependent on cheap culturing. Like other photosynthetic organisms, diatoms require CO_2, water, inorganic salts, and light, thereby enabling culture in readily available fresh or seawater with no need for expensive supplements (Lopez et al., 2005a). Furthermore, diatom culturing would not compete for agriculture cropland, and growth in fermentors could be located on unproductive land such as deserts (Gordon and Polle, 2007). Current estimates show that the diatom biomass production rate of a single pilot-scale facility with 45 vertical bubble column photobioreactors can be in excess of 700 kg (dry weight) per annum (Miron et al., 1999), which corresponds to an annual silica frustule yield in excess of 70 kg (assuming a 10 wt% silica content in dry cells).

These structures have evolved over millions of years to generate intricate nanostructures more complex than anything that could be produced using artificial techniques and may provide materials ideal for biotechnological exploitation. These quasi-regular structures have been proposed for applications as diverse as optics, biophotonics, biosensing, filtration, microfluidics, and drug delivery (Drum and Gordon, 2003; Hamm, 2005; Fuhrmann et al., 2004; Rosi et al., 2004).

3. Material Properties

Nature has provided us with a chocolate box assortment of diatom structures from which to choose. Given specimens vary in shape, size, nanopatterning, internal volume, mechanical and optical properties, and diffusion potential through pores.

As such, the frustule may be exploited for technical applications in a number of ways: direct use of the silica structure, biomimicry of silicification routes, the use of surface chemistry for functionalization, and a template for structures made from other materials or nanocomposites.

3.1. SILICIFICATION

Biomineralization is the formation of inorganic materials under the control of a living cell. There are several examples of single-celled eukaryotes which use biomineralization to produce intricately structured cell walls. Many species produce structures made of $CaCO_3$ (e.g., coccolithophores) whereas silica (SiO_2) is much less abundant in biomineralizing organisms (e.g., diatoms, radiolaria). It is silica, however, which may be used in a large panel of applications, e.g., catalysis, separation science, or optics (Gomez-Romero and Sanchez, 2003). Thus, while it may seem that elucidation of the mechanisms of diatom silicification is a fundamental biological problem, insight into the mechanisms of biomineralization may inspire

and enable the synthesis of novel patterned inorganic materials with complex morphologies and advantageous properties. To achieve this, it is essential to understand the control of silica formation at the molecular level and the relationship between a one-dimensional linear genotype and the chemical and physical properties of the multidimensional phenotype (Losic et al., 2009).

Diatoms fabricate the silica shell in a bottom-up self-assembly process using soluble silicon to generate silica nanoparticle building blocks (Sumper and Brunner, 2006). The silicon enters the diatom from the aqueous environment in the form of silicic acid ($Si(OH)_4$; Del Amo and Brzezinski, 1999) *via* specific membrane transmembrane proteins (SITs: Silicic acid transporters; Hildebrand et al., 1997, 1998). The silica polycondensation and assembly then occurs in specialized vesicles known as silica deposition vesicles (SDV; Vrieling et al., 1999b) where silica can accumulate at concentrations up to 1,000× higher than in the surrounding aqueous environment (Lopez et al., 2005b). The SDV appears to be a general organelle for silica biogenesis in protists and has been identified in sponges, radiolarians, synurophytes, and choanoflagellates (Simpson and Volcani, 1981). The exact chemical form of Si incorporated and condensed within the SDV is unknown, but importantly, the SDV is known to be acidic (Vrieling et al., 1999b). Each diatom species appears to contain silaffins and long chain polyamines (LCPA) which accelerate and control silica morphogenesis from silicic acid and are believed to be involved in the morphogenesis of the species-specific nanopatterns in the SDV. Silaffins (proteins with silica affinity) are polycationic peptides which have signal peptides for co-translational import into the endoplasmic reticulum (ER), where posttranslational modifications occur. Silaffins were first isolated from the cell wall of *Cylindrotheca fusiformis* and it was demonstrated that in the presence of silaffins, nanospheres can be synthesized *in vitro* from silanes at nearly neutral pH and ambient temperature and pressures (Kroger et al., 1999; Cha et al., 2000). Such properties would be highly desirable for *ex vivo* synthesis of silica spheres which currently requires either strongly alkaline conditions (Stober et al., 1968) or high temps and long incubation time (Iler, 1979; Brinker and Schere, 1990). Indeed, a synthetic 19-amino acid peptide unit of the silaffin-1 precursor polypeptide from *C.fusiformis* is able to catalyze the formation of silica nanospheres within minutes.

In future biomimetics, it may also be wise to take account of the organic component of the silica cell wall. Polarized infrared (IR) absorbance spectra showed structural anisotropy of Si-O bond of SiO_4 in frustules of pennate diatoms (Asada et al., 2002).This structural distortion is thought to be a result of the organic matter in the network structure. Additional variation in silica synthesis may also be attained through the selection of peptides from differing species and which are involved in the synthesis of different structures. AFM (atomic force microscopy) analysis of *Pinnularia viridis* frustules cleaved in cross section revealed the nanostructure of the valve silica to be composed of silica spheres that were 44.8 ± 0.7 nm in diameter whereas those in the girdle band were 40.3 ± 0.8 nm. In contrast, the heavily silicified *Hantzschia amphioxys* (Ehrenberg) Grunow showed valve silica spheres to be 37.1 ± 0.5 nm and girdle band sphere to be 38.1 ± 0.5 nm (Crawford et al., 2001).

3.2. SURFACE CHEMISTRY FOR FUNCTIONALIZATION

The combination of the silica chemistry of the frustule coupled with a high surface area makes it an ideal template for the attachment of active biomolecules. The intricate structure of the frustule provides a larger surface area than either a planar glass slide or silica microbeads. Hence, in addition to the provision of a low-cost material, a frustule could perform better with regards to biotechnological applications (De Stefano et al., 2008).

The frustule essentially comprises hydrated glass ($SiO_2.nH_20$) which therefore has free hydroxyl groups on the surface. These reactive groups allow the chemical modification of the surface and subsequent functionalization (Townley et al., 2008). Silanes are one of the most commonly used chelating agents due to their reactivity with hydroxyls: In fact, they can couple organic groups to almost any oxide surface (Nashat et al., 1998). Using silane chemistry, De Stefano et al. (2008) linked a fluorescently labeled monoclonal antibody to the surface using Protein A as a biospecific spacer arm and showed that a dose response curve could be made for fluorescence intensity as a function of antibody concentration. Coupling *via* proteins A or G ensures free access to the antigen-binding domains of the antibody. However, this method is not always suitable for all applications, and the affinity of protein A and G differs for antibodies from different species and for different isotypes within a species. An alternative chemistry entails binding to the silica surface *via* the amine or carbohydrate moieties of the antibody. Coupling *via* amine groups is highly effective but may result in multiple attachment points and consequent distortion, or impairment of the antigen-binding site. Conversely, conjugation through the carbohydrate group employs correct orientation, and therefore optimal binding capacity, but is not possible for non-glycosylated proteins (Townley et al., 2008).

The frustule easily lends itself to the concept of microscale total analysis system (μTAS) or lab-on-a-chip. Such a system would require only very small sample sizes and be cheap and disposable. Such a system could detect multiple antibodies or antigens in a complex mixture such as sera. As we demonstrated (Townley et al., 2008), it is possible to tether total antibody complement from a serum sample and to probe with multiple secondary antibodies conjugated to different fluorescent dyes as a proof of principle for use in a TAS. Tethered antibodies may also be used for immunoprecipitation which involves the selective precipitation of an antigen from a complex mixture using an antibody specific to that antigen. The frustule–antibody complex provides a solid phase matrix which brings the antigen out of solution.

In addition to antibodies, the diatom wall has been shown to be amenable to functionalization with DNA (Rosi et al., 2004). The cell wall was coated with DNA and then used as a template for the sequence-specific assembly of DNA functionalized nanoparticles. The DNA can further be used to programme the assembly of multiple layers of nanoparticles onto the template. This can be visualized as a dramatic decrease in the strong absorption at 520 nm from the

DNA-functionalized gold colloid upon the hybridization of complementary DNA which directs the assembly of the nanoparticles onto the diatom template.

Functionalization of the silica surface can also incorporate the intrinsic photoluminescent (PL) properties (see Section 4.2) of the biosilica. If nucleophilic moieties or biomolecules are attached to nanoscale semiconductors or other photoluminescent surfaces with nanoscale topology, it is possible to increase the PL emission. This is proposed to occur due to nucleophilic groups donating electrons to non-radiative defect sites on the photoluminescent surface. Functionalization of the diatom biosilica with IgG was shown to increase the PL intensity over six-fold consistent with this mechanism due to the nucleophilic nature of rabbit IgG antibody (Gale et al., 2009). Binding to the complementary antigen (also nucleophilic) further increased the PL intensity by a factor of three, whereas noncomplementary antigens showed no increase in PL. This could therefore form the basis of a label-free method for biomolecule detection. Since the increase in PL intensity is dependent upon antigen concentration, biosensing is also quantitative.

3.3. MECHANICAL PROPERTIES OF SILICA

The growing demand for mesoporous silica for uses such as filter agents, ion exchange materials, and abrasives has ignited an interest in the structural and micromechanical characterization of diatoms. In all probability, the siliceous coverings of diatoms have been evolutionarily optimized to support the greatest possible stresses with the minimal amount of material. Indeed, they would be expected to have a high compressive strength due to the shell architecture, specifically, the presence of ribs or pores that can dissipate stresses. Seminal experiments performed by Hamm et al. (2003) measured the force necessary to break single cells. Using calibrated glass needles to load and break the frustules with defined forces, it was determined that mechanical strength and size were inversely related. Mechanical strength was also found to vary between species, which would correlate with differences in architecture.

Species	Diameter (m)	Force (N)
Thalassiosira punctigera (centric)	50	260
T. punctigera (centric)	100	180
Coscinodiscus granii (centric)	130	90
Fragilariopsis kerguelensis (pennate)	30 (longest axis)	730

Furthermore, it is likely that different regions of the frustule would have different structures of silica and degrees of silicification (Almqvist et al., 2001).

The enormous surface area of the shells in conjunction with the ability to functionalize the surface could, for example, be used in the controlled release of a drug either through the pores or by diffusion from substances embedded in or on the silica (Gordon et al., 2008). Applications which make use of the surface

area of the frustule will require judicious selection of the species of the diatom. As an illustration of this point, *Thalassiosira descipiens* has a surface area of 258 m^2g^{-1} (Kamantani and Riley, 1979) whereas the closely related *Thalassiosira eccentrica* has a surface area of only 9 m^2g^{-1} (Vrieling et al., 1999a).

3.3.1. Bioadhesives

In vivo, the silica frustule of the diatom has an organic coating, primarily composed of polysaccharides, proteins, and some lipids (Hecky et al., 1973). Analysis of the mucilage by force mode AFM demonstrated it to be a nonadhesive, soft, and compressible material (Crawford et al., 2001). The organic layer is thought to play a role in the prevention of the dissolution of the silica in its aqueous environment. Modern technology also suffers from similar problems, for example, man-made glass-fiber-reinforced polymers show rapid quality deterioration when used in water (Connor et al., 1997). Further characterization of the natural coatings could therefore be applied to engineered systems.

In addition to the mucilage layer, specialized organic secretions from the diatom function as natural adhesives, either in the attachment of cells to each other in colonial forms, or to fix themselves to a substrate in the case of benthic species. These adhesives are extremely strong and robust in both fresh and seawater environments (Gebeshuber et al., 2003). This is in stark contrast to man-made adhesives which fail in wet conditions owing to chemical modification of the adhesive or its substrate. As such, engineering stable underwater adhesives is a major technical challenge and characteristics of natural adhesives may be of use in designing man-made analogues.

One example of a diatom which has superb natural adhesion (as assessed by the ability to withstand coarse snail grazing) is *Eunotia sudetica*. *Eunotia* forms chains by the adhesion of the valve faces and also adheres at the valve face to external surfaces. AFM showed the adhesive to form bead-like structures with a height of 10–20 nm and lateral dimensions of 1 m, with a spacing of 1 m. Force-pulling experiments revealed a strong and tough adhesive which was stable and robust in a wet environment (Gebeshuber et al., 2003).

3.4. DIATOMS AS TEMPLATES

Nanostructures are currently expensive and difficult to manufacture. Generally, 3D structures are fabricated using layer-by-layer lithographic technology. Therefore, the inherent hierarchical structure and the vast range of species-specific morphology are ripe for exploitation. However, applications for the direct use of diatom frustules within nanotechnology are limited by the chemical and physical properties of silica which do not provide the optimum chemistry/refractive index for many applications. It would therefore be desirable to retain the morphology while extending the range of applications by converting the silica into technologically more suitable functional materials.

Pioneering work by Sandhage et al. (2002) showed an inorganic molecular conversion reaction which retained the size, shape, and morphology of the diatom whilst changing its composition. In the displacement reaction, the SiO_2 of the diatom was converted into MgO by a vapor phase reaction at 900°C. Similar experiments have also shown that titanium fluoride gas can be used to displace the silicon, resulting in a structure entirely comprising titanium dioxide, a material useful for commercial solar cells. Furthermore, nanocrystalline F-doped anatase TiO_2 derived from diatom frustules was found to induce rapid hydrolysis of organophosphorus esters found in pesticides and nerve agent mimics (Lee et al., 2007b). Hydrolysis rates are strongly dependent on the level of fluorine doping, with a reduced effect at lower fluorine concentrations, consistent with an increase in the surface Lewis acidity of the titania with increased fluorine doping. Such materials have promise for environmental remediation applications. Other applications suggested for Silica replicas include an ultrarapid, low-voltage NO sensor. A low temperature magnesiothermic reduction process was derived for the conversion of the frustule into microporous nanocrystalline silicon replicas. The silicon replicas exhibited rapid changes in impedance upon exposure to gaseous nitric oxide, suggesting a possible application in microscale gas sensing (Bao et al., 2007).

Similarly, the silica shells of *Aulacoseira* diatoms have been converted into Eu^{3+} doped $BaTiO_3$-bearing microparticles that exhibit bright red fluorescence (Weathersoon et al., 2006), and a ZnO precipitation process has been shown to generate Zn_2SiO_4-coated frustules (Cai and Sandhage, 2005) .

3.5. IN VIVO GENERATION OF NANOCOMPOSITES

An alternative approach to modifying the diatom frustule is the generation of a silica composite material using the inherent mechanism of silica deposition. This in vivo approach has been used to incorporate germanium directly into the frustule of *Nitzschia frustulum* to produce a Si-Ge oxide nanostructured composite material (Rorrer, 2004). Since germanium is a semiconductor, these structures could have applications in electronics, optoelectronics, photonics, thin-film displays, and solar cells.

In addition to metals, the incorporation of organic dye molecules into porous glasses have potential for incorporation into tuneable lasers and nonlinear optical devices, luminescent solar concentrators, gas sensors, and active waveguides. The in vivo incorporation of a number of laser dyes into diatom cell walls has been demonstrated (Kucki et al., 2006). The dyes were added to the culture medium and confocal laser scanning microscopy used to examine the dye doped frustules. While Rhodamine 6G was lethal to the diatoms, Rhodamine B, -19, -101, -110, -123, and Lysosensor DND-160 were all incorporated into the silica. This enabled a broad spectral range to be covered, with emissions at 433, 539, 545, 561, 592, and 606 nm. Interestingly, the authors showed an example of a shell where only one half is

fluorescent because the older half was already formed before the addition of the dye. During cell division, a new second half was then built with incorporated dye. By this technique, bicolored cells are also possible using cultivation media with different dyes successively. The simultaneous presence of different dyes, on the other hand, allows dye combinations leading, for example, to Forster-transfer system, in order to enhance the quantum efficiency of emission.

4. Optical Properties

4.1. DIFFRACTION AND INTERFERENCE

The fascination with the optical properties of diatoms was apparent even in Victorian times when early microscopists created elaborate mosaics using individual diatoms on slides. When observed under a light microscope, the interactions of the diatom silica with light results in vivid structural colors with intense diffraction and interference effects. This is due to multiple reflections arising from the multilayered, semitransparent surfaces such that the phase shift and interference of the reflections modulates the incident light resulting in the amplification or attenuation of some frequencies more than others (Gordon et al., 2008). The angle at which the light strikes the surface will determine the precise interference effect such that the diatom will appear to change color dependent upon the position of observation. The optical properties exhibited by the frustule are therefore a combination of the properties of the silica and the hierarchical structure. By studying, mimicking, or optimizing these characteristics, the frustule may be exploited for nanotechnological applications.

4.2. PHOTOLUMINESCENT PROPERTIES OF SILICA

New functional luminescent materials are in great demand for products such as flat panel displays, cathode ray tubes, and electroluminescent displays (Rack et al., 1996; Ziemelis, 1999; Weder et al. 1998). Silicate-based phosphors are promising luminescent materials because of their chemical stability, moisture resistance, and low cost (Kong et al., 2005; He et al., 2003). The interest in porous semiconductor and insulating materials stemmed from the realization that porous silicon luminesces efficiently in the visible region when irradiated with ultraviolet light (Cullis et al., 1997).

The same UV-induced visible luminescence seen with fabricated porous silicon has been shown in the amorphous biosilica of the frustule (Butcher et al., 2003). Interestingly, this photoluminescence is species dependent and based on the frustule structure and the surrounding environment. The ability to alter the frustule by changing growth conditions or by forming nanocomposites may be useful for biotechnological applications.

4.2.1. Manipulation of the Photoluminescence

Jeffryes et al. (2008) describe the biological fabrication of Ge-doped biosilica frustules by two-stage cell culture of the diatom *Pinnularia.* After the metabolic insertion of 1.6 wt% Ge, the overall frustule architecture was unchanged; however, the frustules no longer possessed the nanopore array lining the base of the pores. The doped biosilica showed blue photoluminescence (PL) with peak emission at 460 nm after excitation with 337-nm UV light. This is analogous to the emission from mesoporous silica (Glinka et al., 2002) and most likely originates from surface defects, including silanol groups and oxygen defect centers. The frustule containing Ge, however, had a much higher PL intensity, possibly due to the superimposition of Ge-O defects on the PL emissions of the biosilica. Furthermore, when incorporated into a device, it was found that in contrast to undoped frustules which did not emit any discernible electroluminescence (EL), frustules containing 1.6 wt% Ge showed EL spectra at an applied voltage of 150 V. Two bands of EL emission were observed, one with a series of bands between 300 and 500 nm, and the other with a series of broader emissions between 640 and 780 nm. Whilst it is common for Ge-implanted SiO_2 thin films to possess similar EL and PL spectra (Butcher et al., 2005), the EL and PL spectra seen in the diatoms have different spectra and therefore probably arise from different mechanisms. Indeed, it is suggested that it is the unique interaction of UV-visible emissions arising from the periodic holes array of the frustule combined with electronic excitation of the defect centers within the interior of the Ge-doped biosilica which gives rise to these unique properties.

Another study demonstrated that sublethal concentrations of nickel were able to alter the optical, physical, and cytological properties of the marine diatom *Coscinodiscus wailesii* (Townley et al., 2007). Photoluminescence was shown to be quenched as a result of growth in nickel in accordance with other studies which show that metals can quench the inherent PL of silica (Andsager et al., 1993).

An alternative approach involves coating the surface of the diatom with inorganics to modify the PL characteristics. The intrinsic PL from the frustule spans a broad peak from approx. 370 to 700 nm. This makes the material unsuitable for applications that require narrow emissions and high sensitivity, such as photodetectors. When coated with CdS, the intrinsic PL of the frustules is totally quenched by the disappearance of the broad PL signal centered at 457 nm and is replaced by a much sharper emission peak from 560 to 620 nm, centered at 588 nm (Gutu et al., 2009). Similarly, deposition of nanocrystalline Zn_2SiO_4:Mn on the surface of frustules from the marine diatom *Pinnularia sp.* resulted in bright green fluorescence with PL peaking at 528 nm (Lee et al. 2007a).

4.2.2. Environmental Effects on Photoluminescence

De Stefano et al. (2005) put forward the suggestion that diatoms may be used as optical chemical sensors after demonstrating that the PL spectrum is affected by the surrounding atmosphere. In particular, they showed the response of the centric diatom *Thalassiosira rotula* Meunier, to several gasses and volatile substances. The electrophiles NO_2, acetone, and ethanol were shown to quench the PL by

attracting the electrons from the silica skeleton. Also, due to absorption by the nanopores, which increases the average refractive index of the structure, the principal PL band is redshifted. The converse situation was observed when the diatom silica was exposed to nucleophilic substances: Xylene and pyridine were shown to increase the PL tenfold and redshift the main peak. For all the substances tested, both quenching and enhancement of the PL were fully reversible, once the environment was returned to normal. Building on such studies, Setaro et al. (2007) carried out experiments to show that such changes in PL may be used for quantifiable optochemical gas detection. The diatom species *Thalassiosira rotula*, *Coscinodiscus wailesii*, and *Cocconeis scutellum* were exposed to NO_2, CO, or CH_4. In all cases, the addition of the gas resulted in no significant modification to the spectral shape, peak position, or spectral width. Exposure to NO_2 or CO showed a significant quenching of the PL signal, with a response time of 2–3 min. This response was almost completely reversible and showed significant decreases even at very low gas concentrations. The situation with CH_4 was shown to be slightly more complex. Presentation of *T.rotula* to the gas showed an enhanced PL signal; however, *C.wailesii* and *C.scutellum* showed an initial decrease in PL at low concentrations of gas, while higher gas concentrations always resulted in an increase in PL. The rationale for this behavior is attributed to the reaction of methane molecules with the few hydroxyl groups present on the silica surface producing methane hydrate. Since the hydroxyl ion is no longer available for the PL transition, the result is a decrease in the signal. Once the hydroxyl groups are saturated, the other methane molecules covering the frustule surface have available electrons for PL.

4.3. PERIODIC NANOSTRUCTURES

Traditionally, the manipulation of optical photons has relied, in general, on the mechanism of total internal reflection. Light propagating in a high-dielectric material is reflected at the interface with a low-dielectric material. This severely limits the degree of miniaturization of optical components because the interface must be smooth with respect to the wavelength of light. Photonic crystals offer a completely different mechanism for the control of light. The difference lies in the concept of the photonic bandgap – the optical analogue of the electronic bandgap in semiconductors. Light has several advantages over the electron. It can travel in a dielectric material at much greater speeds than an electron in a metallic wire and can carry a larger amount of information per second (Joannopoulos et al., 1997). Applications for photonic crystals include waveguides, diffraction elements for solar cells (Gombert et al., 1998), and active devices such as photonic crystal lasers (Mekis et al., 1999; Riechel et al., 2000; Notomi et al., 2001). Diatoms may have potential in photonic devices because of their regular structure in the range of visible light wavelengths.

The role of the frustule in light collection by diatoms was recently brought to the fore by the study of Fuhrmann et al. (2004) who indicated the possible role of

waveguiding in the visible range for valves of *C.granii*. From an optical point of view, the diatom cell wall can be regarded as a slab waveguide with a two-dimensional photonic crystal pattern (Fuhrmann et al., 2004). Such periodic structures confine light vertically within the slab *via* index guiding with the optimum height equal to approximately half a wavelength. This is crucial since if the slab height is too small, then the modes will be weakly confined and if it is too large then higher order modes will fill the gap. Other criteria which are important for the spectral range in which the cell wall may act as a photonic crystal are first the refractive index contrast and second the physical dimensions of the periodic structure. From comparison with index matching liquids, the refractive index of the silica shell has been determined to be 1.43 (Fuhrmann et al., 2004). The valves of *C.granii* have been determined to have a lattice constant of 900–950 nm and the thickness of the silica slab to be approximately 700 nm. The girdle bands exhibit a square lattice of holes with a lattice constant of 250 nm and a thickness between 200 and 600 nm (Fuhrmann et al., 2004). It should be noted, however, that the thickness of the cell wall decreases from generation to generation (Fritsch, 1975).

The interpretation of the frustule acting as a photonic crystal is, however, based on the assumption of perfectly periodic valve ultrastructures, with predicted guided modes at highly specific wavelengths. Studies with red light have shown that the inherent deviations from perfection in real valve ultrastructures leads to weak coupling conditions outside the theoretically predicted guided-mode wavelength range (Noyes et al., 2008). Further studies were carried out using the diatom *C.wailesii* which has near perfect radial symmetry and an easily identifiable triangular elementary cell (De Stefano et al., 2009). The optical properties of the valve structure of *C. wailesii* were explored using data obtained from scanning electron microscopy (SEM), denoting a lattice constant of approximately 660 nm, and a value of 1.45 for the refractive index of amorphous silica (De Stefano et al., 2009). Theoretical calculations showed that there is not an optical gap in the range of frequencies explored, for neither TE nor TM modes. However, calculations using a diatom structure in which the silica had been replaced with titanium oxide which has a refractive index of 2.4, showed a complete optical gap at approximately 2.2 m. In addition, De Stefano et al. (2009) investigated the propagation of the electromagnetic field in the direction perpendicular to the diatom plane. From this aspect, the radial symmetry resembles that of a photonic crystal fiber. Finite element modeling propagation simulations show that the field is concentrated into the defect center, that is, the compact zone in the center of the porous structure. Such a focussing effect could have an application for the diatom as a fibre integrated beam collimator.

4.3.1. Photosynthetic Effect

The silica structure of the cell wall is not the only peculiarity of diatoms; their photosynthetic efficiency is also remarkable. They can survive under relatively weak ambient light intensities due to a high light conversion quantum efficiency throughout the visible range. This can, in part, be attributed to the dye composition

of the photosynthetic antennae which contain specialized carotenoids such as fucoxanthin which cover wavelengths not accessible by chlorophyll (Dutton et al., 1943). However, it has been postulated that the properties of the silica structure could help the transmission and collection of light to the photoreceptors to improve their photosynthetic efficiency (Parker and Townley, 2007). Photosynthetic receptors are located in chloroplasts which are situated close to the silica cell wall. Interestingly, there are reports that high light intensities cause a redistribution and migration of chloroplasts away from the shell to the center of the cell (Furukawa et al., 1998).

If the frustule does indeed act as a photonic crystal slab waveguide, then any guided modes which are present in the slab may also penetrate into the outer medium as an evanescent wave. It is anticipated that the evanescent field will still have substantial amplitude within a distance of 250 nm from the slab (Yamanaka et al., 2008). This is the region where the chloroplasts are located, indicating that coupling of excited states in the light-harvesting systems to optical modes of the silica slab is possible (although coupling is not expected to be very pronounced due to the low RI contrast). Further evidence for an active role of the frustule in photosynthesis has been seen in the freshwater diatom *Melosira varians.* Analysis of the optical properties showed absorption mainly in the blue light wavelength region (Yamanaka et al., 2008). This is proposed to be caused by an interaction between the light and the inner nanostructure. An excess of blue light could potentially give rise to harmful active oxygen and therefore the nanostructures may serve to reduce the amount of blue light under high intensities. Conversely, when the incident blue light is weak, chloroplasts may approach the inner nanostructures to enhance the interaction with light to increase photosynthesis.

5. Conclusion

The diatom cell wall appears unnecessarily ornate simply to separate the cell from its external environment. Nature is seldom wasteful, and it is almost certain that evolution has selected designs to maximize characteristics such as mechanical strength or the propagation of light. It is our role as scientists to understand the unique characteristics of the frustule and to determine how we can apply this knowledge for technological advancement.

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PART 3:
FRESHWATER ECOLOGY

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Witkowski
Radziejewska
Wawrzyniak-Wydrowska
Lange-Bertalot
Bak
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Rex L. Lowe received his Bachelor of Science and Doctor of Philosophy degrees in botany at Iowa State University. He is currently Professor Emeritus at Bowling Green State University (BGSU) where he has won awards for outstanding teaching and research. Dr. Lowe teaches courses on freshwater algal ecology, limnology, and Great Lakes Ecosystems. In addition to BGSU, Dr. Lowe also teaches in the summers at the University of Michigan Biological Station. In 2008, Lowe was awarded the Wilder Chair for a distinguished botanist at the University of Hawaii. Professor Lowe has trained and graduated over 70 graduate students including 16 Ph.Ds. Lowe's scholarly books and manuscripts (>150) cover topics concerning algal ecology and diatom systematics.

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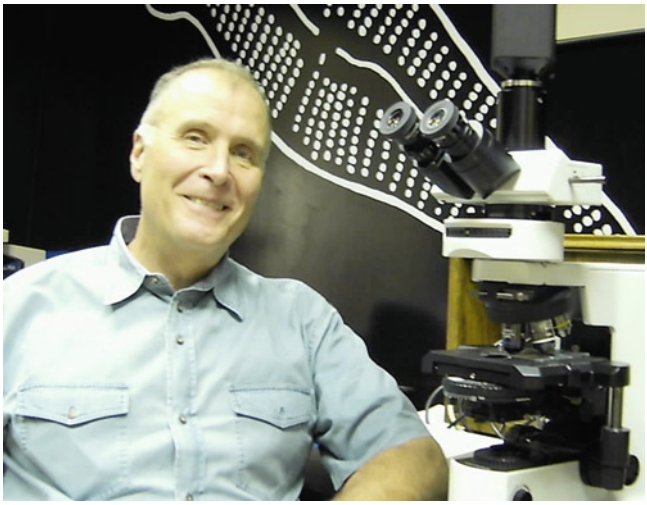

J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 293–311
DOI 10.1007/978-94-007-1327-7_13, © Springer Science+Business Media B.V. 2011

THE IMPORTANCE OF SCALE IN UNDERSTANDING THE NATURAL HISTORY OF DIATOM COMMUNITIES

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1. Introduction

Regardless of our attempts to study organisms in a bias-free manner, scientists often neglect to design hypotheses or experiments at spatial and/or temporal scales appropriate for the organisms to be studied. This problem is particularly acute in the study of periphyton including diatoms where powerful light and scanning electron microscopes greatly magnify organisms and allow us to forget that they are truly microbial. Periphyton communities are relatively small intricate communities and are often quite structurally complex (Fig. 1) but are regulated by many of the same factors that regulate macrobial communities.

Diatoms range in size from <10 μm in length (e.g., *Diadesmis perpusila* (Kützing) D.G. Mann) to several hundred μm (e.g., *Pinnularia latevittata* v. *domingensis* Cl.) and range in biovolume from <50 to >160,000 μm^3. This range in size is comparable to the difference between an average-sized human (1.8 m) and a mayfly and has many ecological implications for diatoms such as vulnerability to grazers, resource acquisition, reproduction, vulnerability to disturbance, etc. Periphyton biologists often study algal communities at scales too large to accommodate microscale differences in environmental gradients. The algal community of a stream reach is routinely employed to characterize the "health" or biological integrity of the stream (Stevenson et al., 2006).

Periphyton is scraped from submerged substrata, put into containers and transported back to the laboratory for analysis. Although this protocol has been useful for employing periphyton and, specifically, diatoms as indicators of stream condition, the technique sacrifices an appreciation of the physical relationship among individual diatoms and their microhabitat. One can analyze numerical community structure in a strewn wet mount but the spatial relationships of individuals and the structural physiognomy of the community is lost. If we studied macrobial plant communities by chopping down a forest or mowing down a prairie and spreading the plants on a large surface for analysis, our knowledge of plant community ecology would be poor indeed. Scanning electron microscopy (Greenwood et al., 1999) and specialized techniques for light microscopy (Francoeur et al., 2001) have fostered a greater appreciation of periphyton com-

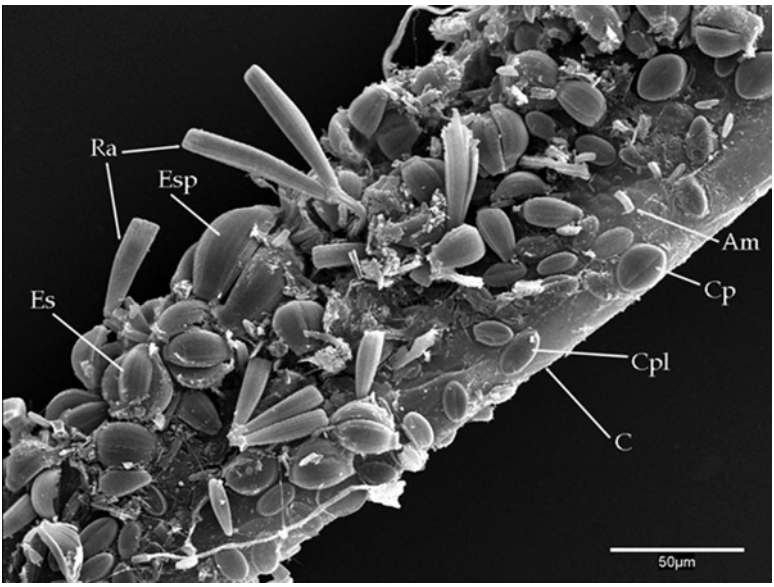

Figure 1. An epiphytic diatom community attached to the green alga *Cladophora* (C). Diatoms in the community include *Cocconeis pediculus* Ehr. (Cp), *Cocconeis placentula* Ehr. (Cpl), *Achnanthidium minutissimum* (Kütz.) Czarn. (Am), *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot (Ra), *Epithemia sorex* (Kütz.) (Es), and *Epithemia* sp. (Esp).

munity physiognomy. Aquatic ecosystems harbor dozens of different microhabitats, each with a distinct algal community regulated by local parameters.

In contrast, macrobiologists who are focused on larger organisms such as aquatic insects or fish may often treat the benthic algal community as an organism that responds to environmental parameters in a predictable way (Rosemond et al., 1993). In fact, several recent papers have been published that refer to algae in the singular, with statements such as "the algae is limited by …,", failing to recognize or at least acknowledge that benthic algae form a complex community of often hundreds of populations from several different kingdoms each regulated by a unique set of abiotic and biotic parameters. Ecosystem variables that influence a diatom cell operate on very different spatial and temporal scales relative to larger members of aquatic ecosystems.

I will use diatoms to illustrate my thesis of the importance of scale. And, where appropriate, compare diatoms to macroscopic aquatic community members to provide a perspective. The objective of this manuscript is to encourage benthic biologists and particularly diatomists to "step back" occasionally and appreciate the spatial and temporal scale of communities that we examine and to "think like a diatom."

1.1. TEMPORAL AND SPATIAL SCALES

To understand the importance of scale in the aquatic community it is instructive to compare size and speed of motility of community members Hay and Maitland (1993) (Table 1).

It is clear that diatoms cannot avoid predation by invertebrates such as snails or mayflies through speed of retreat and must resort to other behaviors to avoid predation. Diatoms also differ from other members of the aquatic community in their response time to changes in the environment (Table 2). These spatial and temporal differences among aquatic community members are important factors when posing research hypotheses regarding aquatic ecosystems or when selecting suites of aquatic organisms as environmental monitors. Diatoms with their relatively rapid rate of division and their ability to increase their population clonally respond quickly to changes in environmental variables. In the proceeding chapter, the importance of scale will be discussed in the context of environmental variables and microhabitats.

Table 1. Comparative traits of diatoms and aquatic cohorts.

	Bacterium	**Diatom**	**Gastropod**	**Mayfly**
Size	1 μm	50 μm	1 cm	1 cm
Diatom equivalent size	0.02	1	200	200
Speed	10 μm/s	10 μm/s[a]	200 μm/s	20.8 cm/s[b]
Diatom equivalent speed	1	1	20	20,000
Body lengths/s	10	0.2	0.02	21

There is considerable variability in these values among species but these examples allow comparison across organism groups

[a] Mean; Harper and Harper (1967), Harper (1977)

[b] Brackenbury (2004)

Table 2. Comparative population response rates based on reproduction rates.

Taxon		
	Time to reproductive age	**Population response**
Clonal organisms		
Bacteria	<0.1 day	Days
Diatoms	0.5 days	Weeks
Protozoan	0.5 days	Weeks
Rotifer	8 days	Weeks
Non-clonal organisms (time to sexual maturity and reproduction)		
Stenonema (mayfly)	1 year	Year
Sculpin	2–3 years	Years
Humans	20 years	Years

1.2. PREDATOR–PREY RELATIONSHIPS

There is a rich literature on predator–prey relationships for macroscopic organisms, especially vertebrates (Shoener, 1974; Scharf et al., 2000; Vézina, 1985). This research has led to some important ecological theories such as the optimal foraging theory (Krebs, 1978). However, investigation of predator prey relationships at the scale of diatoms is limited (Tall et al. 2006a; Gresens and Lowe 1994). In fact diatom-containing periphyton is often treated as a "super organism" when research is focused on benthic grazers (Gettel et al., 2007; Kawata et al., 2001; Marks and Lowe, 1989). Grazers with a relatively large mouth gape size may not be selective ingestors of periphyton but may post-select grazed periphyton, including diatoms, in the gut (Petersen, 1987). Petersen et al. (1998), in a study of diatom survival during gut passage, found that diatoms survived ingestion and gut passage better in caddis larvae than in mayfly nymphs. Smaller grazers, however, may more selectively ingest algae. Tall et al. (2006b) found a significant relationship between invertebrate head width and maximum size of ingested diatoms and also found that grazers fed preferentially on different diatom size classes in accordance with their head width. This relationship held among a broad spectrum of grazer taxa including insects, crustaceans, and worms. In addition, diatom species can vary widely in relative quantities of protein, lipid, carbohydrate, and ash (summarized in Lamberti, 1996). Based on optimal foraging, one might expect grazers that are capable of selecting diatom species to select abundant species that are easy to handle and offer high nutritional value. Predators are also constrained in their choice of diatom prey by the microhabitat that they occupy. Nematodes and chironomids that are meiofaunal in fine sediments have access to a limited assemblage of diatoms that are epipelic and endopelic (Greenwood et al., 1999). Epipelic diatom assemblages are often rich in keeled and sigmoid diatom taxa (*Gyrosigma*, Pleurosigma, *Nitzschia,* etc.) These taxa are of suitable size to be prey for meiofauna (Fig. 2).

At a smaller scale, there is strong evidence that ciliated protozoa can differentiate between diatom species and consume them selectively; Epstein et al. (1992), who conducted a feeding study of 18 benthic ciliate species found that they selectively grazed only 4 of 42 species of diatoms. McCormick (1991) documented selectivity of diatom prey by protozoa that were not based entirely on diatom size, suggesting that other factors such as abundance or accessibility might be of importance. Hamels et al. (2004) offered a choice of diatom prey species to four different species of ciliate predators. The predators exhibited a preference for diatom prey species that varied among the ciliate predators. They further showed that preferred diatom species did not have to be physically contacted in order for the predator to identify them but were selected on the basis of soluble chemical cues. Such highly specific predator–prey interactions may play an important role in structuring diatom communities, especially considering that ciliates can consume over 30 diatoms per hour (Balczon and Pratt, 1996). These microbial predator prey studies illustrate the importance of recognizing scale in understanding invertebrate–periphyton trophic links.

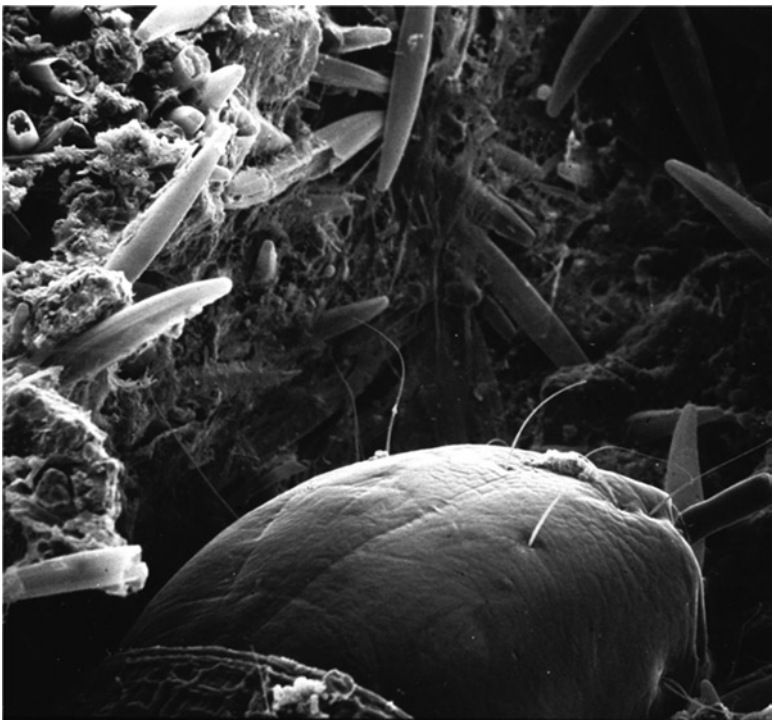

Figure 2. A larval dipteran (Chironomidae) grazing through an epipelic diatom community. The dominant diatom is *Gyrosigma* spp.

1.3. SPATIAL SCALES AND RESOURCES

When researchers measure environmental variables such as inorganic nutrients (N, P, Si, etc.) or light, data are normally collected from the water column at some distance from the diatom assemblage that requires these resources. These methods are probably adequate for sampling the variables that potentially regulate planktonic diatoms since they are not in contact with surfaces that may alter resource concentrations, although, even in the water column, diatoms can be exposed to patchy resources (Turpin et al., 1981; Lehman and Scavia, 1982).

Benthic or periphytic diatoms occupy a variety of substrata, both organic and inorganic, that may present resource conditions and opportunities very different than those measured in the water column. It is difficult to measure the concentration of resources at a scale relevant to benthic diatoms; however, some tools such as microprobes have illustrated the nature of fine-scale resource gradients (Losee and Wetzel, 1983; Dodds, 1992; Wetzel, 1993; Kemp and Dodds, 2001). Using an oxygen microprobe, Carlton and Wetzel (1988) demonstrated significant

light-mediated temporal shifts in sediment oxygen concentration through 4 mm of sediment. Temporal oxygen concentration shifts mediated the release of soluble reactive phosphorous (SRP) flux from the sediment during darkness. These resource shifts at milimeter scales would not be detected through traditional water column sampling but are generated by, and extremely relevant to, epipelic diatoms.

Substrate chemistry has been shown to strongly influence benthic diatoms. Carrick and Lowe (2007) demonstrated that benthic diatoms in Lake Michigan living on sand (epipsammic) were able to use the quartz-rich (SiO_2) sand as a silicon source when Si concentrations appeared to be limiting in the water column (Si < 0.5 ppm). Diatoms living on $CaCO_3$ sand remained Si limited.

Epiphytic diatoms display several different physiognomies that impact their access to resources at a scale of μm. Moeller et al. (1988) and Burkholder et al. (1990) used track autoradiography with both light and scanning electron microscopy to examine phosphorus uptake for single epiphytic cells of various contrasting attachments to the host plant. This powerful technique allowed them to document the relative importance of the host versus the water column as a phosphorus source. *Achnanthidium minutissimum* (Kütz.) Czarn. an adnate diatom in close contact with the host plant (*Najas flexilis*) received as much as 60% of its phosphorus from the host. In contrast, *Gomphonema parvulum*, (Kütz.) Kütz, an attached but stalked diatom several micrometer from *Najas,* received only 20% of its phosphorus from the host. Traditional measurement of phosphorus resources in the water column would miss the importance of these micrometer scale differences in nutrient resources. Phosphorous loss from host plants to epiphytes, whether it is passive or epiphyte mediated can have strong effects on diatom community structure. In the Eel River, (Angelo Coastal Reserve, California) nitrogen-fixing species of *Epithemia* strongly dominate the epiphyte community on *Cladophora* and significantly increase water column nitrate levels (Power et al., 2009). The relationship between diatoms and their host probably varies based on context (plant species, water body, and nutrient availability. For example, Dodds (1991) found there was little nutrient competition between the epiphytes and *C. glomerata* in Montana streams that he was studying. The presence of relatively small diatom substrata in aquatic ecosystems provides habitat for species that might otherwise be excluded, thus increasing overall biological diversity. For example, the presence of small particles of natural organic matter in an oligotrophic lake can host eutrophic diatom species requiring nutrients not available in the water column organics.

1.4. SPATIAL SCALE DISTURBANCE

Physical disturbance can play a strong role in regulating periphyton communities and its impact is a function of frequency, intensity, and season. Peterson (1996) presented a conceptual model predicting that diatom assemblages growing in

relatively thick mats are more likely to be removed by physical disturbance than assemblages with a lower biomass. Within the algal mat assemblage, several diatom species possess adaptations that should render them more resistant to disturbance such as small size, enabling cells to occupy surface-associated reduced current speeds; prostrate positioning of cells; synthesis of strong mucilage pads or films; and rapid reproduction rates, making surviving species more resilient. Francoeur et al. (1998) examined microform bed clusters and boulders as refugia for algae, including diatoms, during bed-scouring disturbance events in a New Zealand stream. They found that while disturbance greatly reduced diatoms on stream cobble, microform bed clusters and boulders conferred increased resistance to flood disturbance and served as refugia for diatoms and other algae able to occupy these stable microhabitats. Diatoms more resistant to disturbance were low-profile taxa. They found that high-profile taxa (long-stalked *Gomphoneis minuta* (Stone) Kociolek and Stoermer, filamentous *Diatoma hiemale* (Lyngbye) Heiberg) were more susceptible to disturbance than *Gomphonema minutum* f. *syriacum* Lange-Bertalot *et* Reichardt which was of lower profile.

1.5. SPATIAL SCALE MICROHABITATS AND MOVEMENT

As microbial producers in spatially variable complex habitats, diatoms have evolved remarkable morphologies and behaviors to cope with microhabitat variability. Various diatom cell morphologies appear to confer an advantage in specific microhabitats.

2. Epipsammon

Living on sand grains presents both a challenge and an opportunity for diatoms. Several diatom species are typical of epipsammic habitats from studies of both lakes, rivers, and marine ecosystems (Moore, 1977; Kingston et al., 1983; Krejci and Lowe, 1986, 1987; Miller et al., 1987; Passy-Tolar et al., 1999; Vilbaste et al., 2000; Muylaert et al., 2002). Epipsammic diatoms can be categorized into two guilds of attachment strategies, either prostrate on the surface of the sand grain or raised on short flexible stalks (Round, 1981). Some common prostrate taxa include *Achnanthes delicatula* (Kütz.) Grunow, *Amphora ovalis* Kütz., *Planothidium dubium* (Grun.) Round and Bukht, *Karayevia clevei* (Grun. in Cl. and Grun.) Round and Bukht. Some common stalked forms include *Martyana atomus* (Hustedt) Snoeijs, *Opephora olsenii* Mùller, *Staurosirella leptostauron* (Ehrenberg) Williams and Round, *Staurosira construens* var. *venter* (Ehrenberg) P.B. Hamilton, *Achnanthes exigua* Grun., and *Staurosirella pinnata* (Ehrenb.) D. M. Williams and Round. The two attachment strategies are employed differently on sand grains. Prostrate species are

more abundant in depressions or hollows on the grains while stalked forms occur primarily on elevated areas of the grains (Krejci and Lowe, 1986). While studying colonized sand grains microscopically, we observed that stalks of the diatoms on elevated portions of the sand grains were relatively short and quite "springy." As grains came in contact with each other, the stalked diatoms would bend and then spring back into an erect position when the grains separated from each other. The relatively immobile prostrate species were protected from crushing between the grains due to their occupation of depressions on the grains. Furthermore, Krejci and Lowe (1986) examined the mineralogy of the sand grains and found that stalked diatoms occurred significantly more often and in greater abundance on quartz grains, while prostrate diatoms occurred equally in the depressions in quartz and feldspar grains.

3. Epipelon

Epipelic diatoms inhabit the surface of sediment with particles normally smaller than algal cells. This microhabitat has been studied extensively in both marine and freshwater ecosystems where much of the focus has been on rhythmic movement (Happey-Wood and Jones, 1988; Hopkins, 1983; Palmer and Round, 1967; Paterson, 1986; Round, 1978, 1979, 1981). Epipelic habitats present some unique challenges and opportunities for diatoms (Burkholder and Cuker, 1991). Perhaps, the greatest challenge is becoming buried under the sediment of these depositional habitats. Epipelic algae have evolved several adaptations for successfully occupying this microhabitat (Moss, 1977). Common diatom morphologies in the epipelon such as elevation of the raphe on a keel or wing (*Nitzschia*, *Surirella*, *Cymatopleura*, *Stenopterobia*, *Campylodiscus*, *Entomoneis*, and *Plagiotropis*) allow cells to move through the sediment and avoid burial. Diatoms with a sigmoid shape (*Gyrosigma*, *Pleurosigma*, and some species of *Nitzschia and Stenopterobia*) are also abundant in the epipelon. The sigmoid shape seems to confer increased mobility through unconsolidated sediments.

Scanning electron microscopy and freeze–fracture techniques have shown epipelic and endopelic communities to be highly structured and spatially variable (Greenwood et al., 1999) (Fig. 4). These techniques in combination with chemical microprobes (Carlton and Wetzel, 1988) demonstrate that distances of a few micrometers can be significant to algal cells. Nonmotile cells buried beneath a few micrometers of sediment may be out of their primary habitat. For example, *Melosira varians* C. Agardh is normally benthic in slow-flowing water. When buried by a few micrometers of sediment (Fig. 3), it can be left in the dark with no means of propelling itself to the top of the sediment. Other endopelic algae such as *Gyrosigma* (Fig. 4) can travel through sediment when buried to find more suitable habitat (Jönsson et al., 1994; Greenwood et al., 1999).

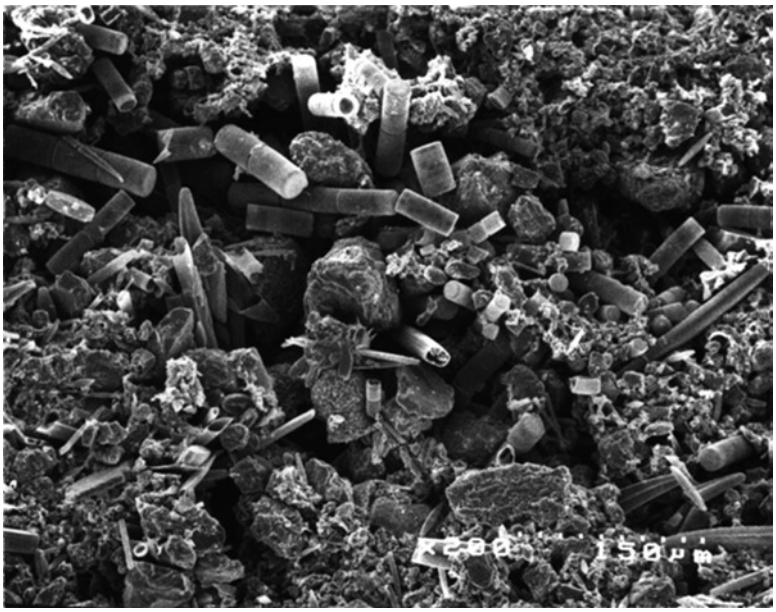

Figure 3. An endopelic diatom community from stream depositional zone. Nonmotile *Melosira varians* is buried.

4. Subaerial

Subaerial diatoms occur in habitats that are not submerged but permanently or, more often, temporarily moist. Moisture may be from the atmosphere (rain and fog), from groundwater seeps, or from splash zones near lakes or waterfalls. Vertical wetwalls have received the most research attention and much of the research on diatoms has focused on taxonomy and floristics (Skvortzov, 1937; Dodd and Stoermer, 1962; VanLandingham, 1965; Carter, 1971; Lowe and Collins, 1973; Dayner and Johansen, 1991; Camburn 1983; Johansen et al., 1983a, b; St. Clair and Rushforth 1976; Rushforth et al., 1984; Johansen, 1999; Poulíčková and Hašler, 2007). A challenge for subaerial diatoms is balancing the need for light for photosynthesis with the need to prevent desiccation of the cell. Many diatom species that are typical of subaerial microhabitats have reduced aereolae or additional siliceous lamina over the cell wall occluding many of the aereolae, ex. *Diadesmis* spp., *Nupela* spp. *Melosira dickiei* (Thwaites) (Kütz) (Lowe et al., 2007). In an extensive survey of wetwalls in the Great Smoky Mountains National Park, USA.

Lowe et al. collected diatoms from 49 subaerial habitats representing 11 different bedrock types. They found differences in distribution of diatom species at

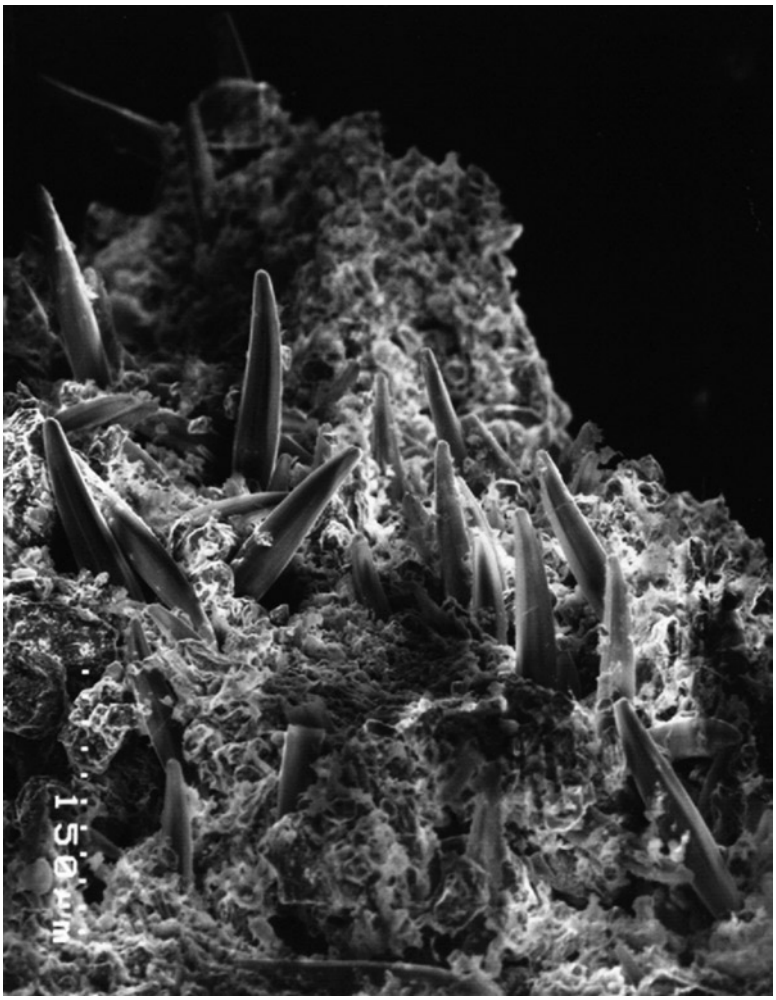

Figure 4. Epipelon from the same sediment as Fig. 3. Motile *Gyrosigma* has moved to the surface following burial.

very small scales on wetwalls. Diatom collections from the same acidic wetwall separated by a few centimeters were often very dissimilar. They attributed small-scale species patchiness to subtle differences in shading and moisture on the wetwalls. Diatom communities that are only a few centimeter apart can occupy very different microhabitats as rock faces possess subtle differences in surface morphology that influence exposure of the community to sunlight and drying.

5. Epiphyton

Of all the potential benthic diatom substrata, plants have received the most research attention (Burkholder, 1996). Much of this research has been focused on resource acquisition of periphyton or competition between the host plant and periphyton (Power et al., 2009; Cattaneo, 1978; Cattaneo and Kalff, 1978; Burkholder and Wetzel, 1989, 1990).

However, in consideration of the scale of the physical relationship between epiphytic diatoms and their hosts many interesting patterns have been documented. An epiphytic diatom can maintain close physical contact with the host (e.g., *Cocconeis pediculus* Ehr., Fig. 5) or attach to the host by one end or by a mucilage stalk or tube (e.g., *Ulnaria ulna* (Nitzsch) Compère, *Rhoicosphenia abbreviata*((C. Agardh) Lange-Bertalot, *Gomphonema* spp.) (Fig. 6). These two attachment strategies impact the diatom's access to host-source versus water-column-source nutrients, light resources and vulnerability to grazers. Prostrate diatoms are thought to be less vulnerable to grazers; however, there is evidence that these forms are also consumed (Tall et al., 2006b). One might expect diatom attachment strategies to be predictable based on grazer (top-down) and nutrient (bottom-up variables although in grazer-nutrient studies little attention has been focused on epiphyte physiognomies (Neckles et al., 2003). Low water column nutrients should be advantageous for prostrate epiphytes and their dominance would be further enhanced with increased pressure from benthic grazers. Under conditions of high water column nutrients stalked and erect diatoms should have increased dominance as they would be more competitive for light resources. However, the intensity of grazing pressure might constrain their dominance. The importance of these two variables in combination needs further research attention.

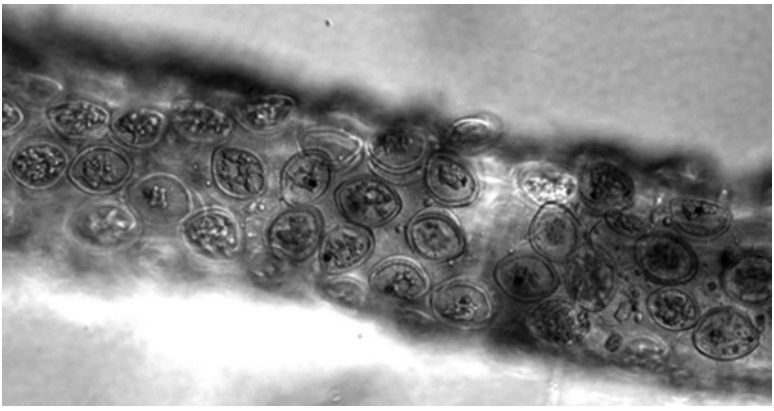

Figure 5. *Cladophora* hosting an epiphytic community dominated by adnate *Cocconeis pediculus*.

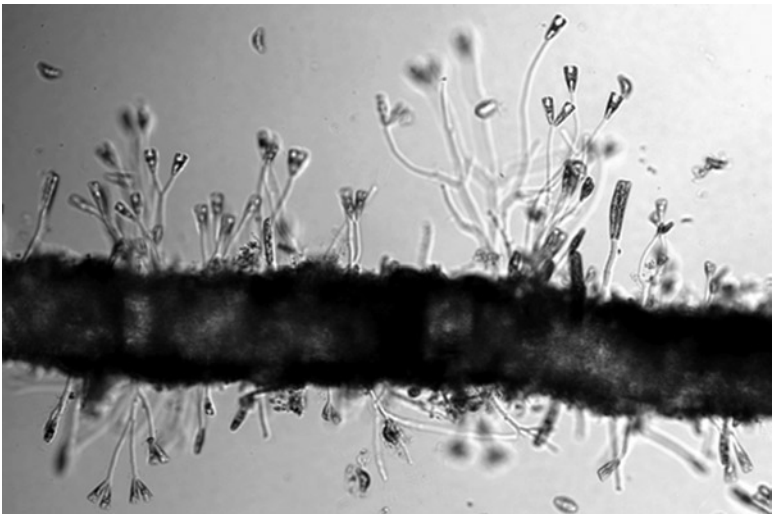

Figure 6. *Cladophora* hosting an epiphytic community dominated by stalked diatoms, *Gomphonema* and *Rhoicosphenia*.

Several researchers have studied the patchiness of epiphytic diatoms on plant hosts. Sullivan (1977) studied diatom epiphytes on *Ruppia* in a New Jersey salt marsh and found minimal differences in diatom community structure on leaves and internodes of *Ruppia*; however, Knapp and Lowe (2009) found much microhabitat specificity in their study of epiphytic diatoms on aquatic bryophytes in the Great Smoky Mountains National Park. The three streams that were studied were heavily shaded by riparian vegetation, and bryophytes supported a higher density and diversity of epiphytic algae than any other substratum in the streams. Mosses had significantly higher diatom densities on the adaxial leaf surface compared to the abaxial leaf surface; however, there was no difference in diatom density on either the adaxial or abaxial leaf surfaces of liverworts, which supported diatom densities statistically identical to the density observed on the abaxial surface of moss leaves. This research demonstrated that the morphology of mosses, comprised of leafy whorls, provided a greater level of protection from disturbance than the open, flat nature of leafy liverworts and further demonstrated that differences in microscale habitats, such as between mosses and liverworts, can result in differing diatom distribution and density that may be critical to stream structure and function.

6. Epilithon

Epilithic diatom communities (living on rocks) are most abundant on stream beds swept clear of sediment by continual current or in lentic littoral zones where wave action keeps shoreline stones free of sediment. In either case, these are normally

high-energy wave or current zones, and diatoms successfully occupying these habitats usually have adaptations for strong adhesion to the substrate. Species in the genera *Achnanthidium*, *Planothidium*, *Achnanthes*, *Gomphonema*, and *Rhoicosphenia* are common components of freshwater epilithon. Nutrient sources for epilithic communities are either the water column or from microbial regeneration from within the periphyton community (Stevenson and Glover, 1993).

The role of mineralogy in determining diatom distribution has not been widely studied but Totti et al. (2007) found no significant difference of diatom abundance and biomass on three substrates examined in the Adriatic Sea (marble, quartzite and slate). However, Lowe et al. (2007) found significant differences between diatom communities growing on wetwalls of 11 different bedrock types in the Great Smoky Mountains, USA. Epilithic diatoms growing on limestone minerals differed strongly from all other bedrock types. The authors concluded that bedrock-mediated differences in pH were probably responsible for the major differences.

7. Conclusions

Diatom communities are microbial analogs of larger plant communities. The community may contain low-growth adnate populations, taller stalk-forming or apically attached populations and highly motile populations that move within the complex. The community is externally regulated by the same top-down variables (disturbance and predation) and bottom-up variables (light and nutrient resources) as higher plant communities. Within the community, interactions such as competition among often hundreds of diatom populations further shape the structure and function of the community. To understand the patterns of structure and function of the diatom community, it is necessary to conduct experiments and observations at the appropriate scale. Tools such as microprobes, nuclear track autoradiography, confocal and electron microscopy have enabled researchers to study these microbial communities at the appropriate scale; however, there are still a myriad of unknowns about the diatom community. What is the adaptive significance of the vast variability in diatom shape and frustular structure? How and when do diatoms communicate with other members of their population? We know that they do find each other and can express sexual compatibility but many of the details remain a mystery. Is there recognition or chemical communication between diatom populations? If so, how and why does it occur? Hopefully, some of these questions will be answered in the future as researchers conduct experiments at diatom-relevant scales.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 313–332
DOI 10.1007/978-94-007-1327-7_14, © Springer Science+Business Media B.V. 2011

PATTERNS OF DIATOM DISTRIBUTION IN RELATION TO SALINITY

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1. Introduction

It became evident relatively early in diatom research that diatoms are greatly influenced by water salinity, and that marine and freshwater diatom floras are strikingly different. Ehrenberg (1836) was already classifying diatom species as either "Süsswasserthierchen" or "Seethierchen" (freshwater or marine animalcules). Numerous classifications of diatom species reflecting their relation to salinity were developed in the twentieth century starting from Kolbe's "Halobien system" (Kolbe, 1927; Hustedt, 1957; Simonsen, 1962; Proshkina-Lavrenko, 1953; Van der Werff and Huls, 1957–1974; Ziemann, 1971; others). Each classification was based on its author's experience with diatoms inhabiting fresh, saline, and intermediate salinity waters of a particular type (estuaries, coastal areas, and inland waters) and of a particular geographic area. Species included in various salinity categories and the exact positions of the boundaries between these categories, differed, therefore, among the classifications. The visualization of species distributions in the form of tables and graphs helped to find discontinuities, which were interpreted as biologically meaningful thresholds, and which were used to establish boundaries between halobien categories. Kolbe (1927, p. 112), for example, established three main categories of diatoms in relation to salinity: "euhalobien" (salinity optima in the 30–40 g/l range), "mesohalobien" (salinity optima in the 5–20 g/l range), and "oligohalobien" (salinity optima below 5 g/l). This latter category was in turn divided into three subcategories: "halophile" (tolerating some salts), "indifferente" (wide tolerance), and "halophobe" (tolerating only very low salinities).

In the paper devoted to the diatoms of the River Weser, Hustedt (1957) outlined his version of the "halobien system" and stated that in his experience the salinity 0.2 mg/l represents an important biological boundary for diatoms. He lowered, therefore, the "mesohalobien–oligohalobien" boundary from 5 to 0.2 g/l. Various "halobien" systems combined in different degrees the concepts of species optima and tolerances (ranges). In Simonsen's (1962) system, for example, three major salinity groups, "polyhalobien," "mesohalobien," and "oligohalobien," were based on species optima, while subgroups reflected species tolerances. Halobien classifications served as an indispensable tool in paleolimnology, studies of shoreline displacement, and coastal geomorphology (Denys and De Wolf, 1999). In the

late twentieth century, the quantitative assessment of diatom distributions along salinity gradients started to replace, however, authoritative halobien systems. Juggins (1992), for example, characterized species distributions in relation to salinity in the River Thames estuary using regression approaches. He also confirmed the existence of at least two salinity thresholds (around 0.2 and 5 g/l) that limit the distribution of diatom species.

Various studies of organisms other than diatoms indicated the existence of ecophysiological salinity thresholds. Perhaps, the most frequently cited phenomenon is the species diversity minimum at the salinity of 5–7 g/l described by Remane (1934, 1971). Such observations reinforced the opinion that diatoms also must have salinity thresholds. Remane explained that the diversity minimum occurs because most organisms found in brackish waters are either marine or freshwater by origin, and brackish water is a marginal portion of their distribution range: "They [brackish-water species] either come from freshwater and are 'limnogenous' or else from the sea 'thalassogenous' " (Remane, 1971, p. 117). This view has been passed on to diatoms, although Remane (1971, p. 84) himself pointed out that "Diatoms have large numbers of species everywhere." It is important to note that the Remane's phenomenon of a transitional species-poor zone is different from the concept of ecotone, which is a zone of sharp transition between two different habitats, and which is characterized by the increased species diversity due to the spatial mass effect, the enrichment by species from both adjacent habitats. The concepts of ecotone and ecocline (gradual change in species composition) are related to spatial boundaries between habitats and communities, while the reason of Remane's phenomenon is a physiological threshold that exists because of the separated evolutionary histories of marine and freshwater organisms.

The idea that most diatoms found in brackish water are either freshwater or marine species that managed to adapt to brackish conditions, or are immediate descendants of marine and freshwater organisms, became firmly established in diatom literature at least from 1950s. For instance, discussions on the origins of diatom floras of large isolated or semi-isolated brackish water bodies, such as the Caspian Sea, Black Sea, Aral Sea, and the Sea of Azov, invariably mentioned "species of freshwater origin," "marine species," and only a few species hypothetically evolved from ancestors that inhabited Paratethys, the large shallow sea that existed from Oligocene to Pliocene in Central Europe and western part of Asia (Karayeva and Makarova, 1973; Proshkina-Lavrenko, 1955, 1963a, b; Proshkina-Lavrenko and Makarova, 1968). The idea that all diatoms come from two different stocks, marine or freshwater, is also reflected in Simonsen's (1962) "halobien" system. In his system, the lower range of distributions of all "oligohalobien" (freshwater) species is the same as the lowest possible salinity of the freshwater, while the upper limit varies between subgroups, indicating that some species managed to adapt to brackish conditions. The subgroup "meioeuryhaline oligohalobien," for example, includes species with very narrow tolerances, which are confined to freshwaters, while species with wider tolerances can also be found in waters of higher salinities. Similarly, the upper limit of all "polyhalobien" species is the same, approximately

the maximum salinity of the sea, while the lower limits differ among subgroups. The finer circumscription of genera in 1970s only strengthened this view. Round and Sims (1981) emphasized that almost all diatom genera are either marine or freshwater, and only a few species are "leaking" to the opposite habitat. This opinion implied that there is a salinity threshold that can be crossed only after considerable physiological modifications took place, and that salinity preference, therefore, is a highly conserved character.

Mann (1999) compared the marine–freshwater salinity threshold to the Rubicon, meaning that it is a point of no return. The invasions were thought to happen predominantly in one direction, from marine to freshwaters, because diatoms are believed to be ancestrally marine (Sims et al., 2006). Mann (1999) pointed out, however, that several genera, especially among the raphid diatoms, are widely distributed in both habitats, and challenged the idea of only a few diatom invasions into freshwaters.

The existence of physiological salinity thresholds in diatoms is not only an interesting theoretical question, but also has practical implications. The salinity of rivers, lakes, and wetlands often changes as a result of human activities. When trying to establish water quality guidelines, environmental agencies are looking for scientific evidence of especially drastic shifts in Biota at certain salinities, which may represent such thresholds. But where exactly are these thresholds? How wide and deep is the Rubicon? What are the reasons for its existence? Recent progress in two areas of diatom research advances our understanding of diatom–salinity relationships. The first is the quantitative analysis of diatom distribution patterns and the second is the reconstruction of the evolutionary history of diatoms.

2. Quantitative Assessment of Diatom Distributions Along Salinity Gradients

2.1. DETECTING SALINITY THRESHOLDS

The existence of ecophysiological salinity thresholds could be confirmed by (1) a decrease of diatom species richness at certain salinities, (2) an increase of species turnover or β-diversity at certain salinities, (3) a decrease of the number of taxa with optima around these salinity values, or (4) larger than average salinity tolerances in diatoms found at such threshold salinities. Species optima and tolerances can be calculated from the parameters of regression models fitted to the data. In practice, the estimates of species distributions are most often calculated as average salinity values weighted by relative abundance of species ("WA optima") and standard deviations around optima weighted by species relative abundances ("WA tolerances," ter Braak and Barendregt, 1986).

It is well known that parameters of species distributions inferred from field surveys datasets only partially reflect physiological optima and tolerances. Ecological optima may be displaced in relation to the physiological optima as a result of competition. Estimated optima and tolerances also strongly depend on

the distribution of environmental characteristics in datasets and the calculation method (ter Braak and Looman, 1986). WA optima and tolerances, for example, are known to be shifted toward the center of the distribution of environmental characteristic, and distorted at the ends of a gradient where species distributions are truncated. They are supposed to be more realistic when the environmental characteristic is distributed evenly across the dataset, which is rarely the case in observational studies. If there is a sharp break in the distribution of an environmental characteristic in the dataset, a similar break will be observed in species optima calculated from these data. The larger the dataset, and the more evenly salinity values are distributed across it, the closer to real optima and tolerances the estimates should be. Since hypothetical diatom salinity barriers cited in the literature range from 0.2 to 18 g/l, it makes sense to examine any datasets that cover this range of salinities.

2.2. ESTUARIES AND COASTAL AREAS

Salinity affinities of diatoms have been used extensively in reconstructing sea levels and the geological history of coastal regions starting from investigations in the Baltic (Holst, 1899; Cleve, 1899). Even coarse classifications of diatoms into salinity categories were sufficiently informative for paleoreconstructions of salinity changes and events deduced from them, such as marine transgressions, regressions, land uplifts, tsunamis, and earthquakes (Denys and De Wolf, 1999). Transfer functions that were based on quantitatively estimated parameters of species distributions only recently became a standard tool for such studies (e.g., Campeau et al., 1999; Zong and Horton, 1999; Plater et al., 2000; Sawai et al., 2004; Horton et al., 2006). Here I review distributions of salinity optima and tolerances of coastal diatoms from a few studies where such information was available.

Wilderman (1984, 1987) studied benthic and planktonic diatoms of the River Severn estuary in Maryland, USA, where salinity ranged between 2 and 20 g/l. The samples were collected four times a year at 30 sites located along six transects. Wilderman calculated the WA optima, which she called "characteristic salinity values" for the 47 most abundant diatom species. These optima (Wilderman, 1984, Chapter 6, Table 1, p. 517) are plotted in their increasing order in Fig. 1a. Only one small break in the line formed by individual points at salinity values around 5–7 g/l can be interpreted as a "threshold." However, a similar break can be seen in the plot of observed salinity values (average per each of six transects and per sampling season, Fig. 1b, data from Chapter 3, fig. 2, p. 58). The lines on Fig. 1a, b follow the same pattern, which means that the discontinuity in the optima was caused by the break in the observed salinity distribution.

A detailed study of benthic diatom species distribution along the salinity gradient in the River Thames estuary was published by Juggins (1992). In this study, the range of observed salinity was 0.08–34 g/l. The plot of WA species optima and tolerances from Table 8.2 in Juggins (1992) shows some breakpoints

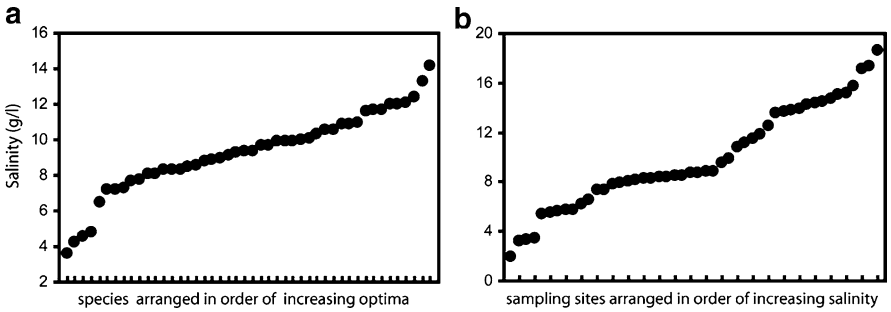

Figure 1. Distribution of the WA salinity optima for 47 diatom species (**a**) and observed salinities (**b**) in the estuary of River Severn, Maryland, USA (Data from Wilderman, 1984).

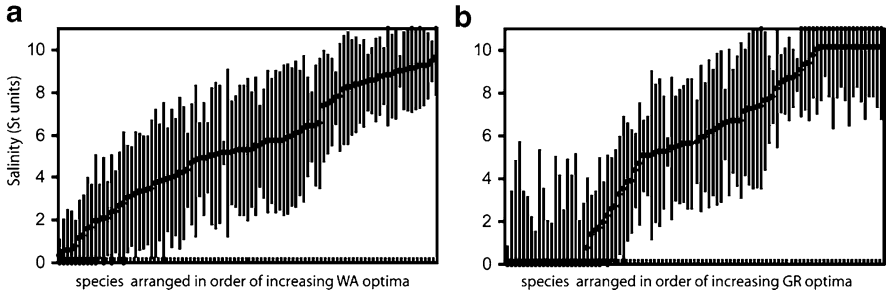

Figure 2. Distribution of diatom species optima and tolerances from the River Thames estuary (Juggins, 1992, Table 8.2). (**a**) WA optima and tolerances; (**b**) GR optima with WA tolerances. Salinity is expressed in St units, calculated as Ln(salinity, mg/l + 80).

in optima at salinities about 6–7 S_t units, which approximately corresponds to 0.5–1 g/l (Fig. 2a).

The line representing optima calculated from fitting a Gaussian logit regression (GR, Fig. 2b) abruptly flattens at 5 S_t units (~0.2 g/l), indicating that more species in the dataset have optima above this value than below. If the value of 0.2 g/l represented the boundary between "truly freshwater" and "brackish-water" diatoms, the expected pattern would be the opposite one: there should have been more freshwater than brackish-water species. Apparently, this breakpoint is a consequence of salinity distribution in the dataset used for optima calculation. The breakpoints at salinities around 1 and 10 S_t units (0.08 and 22 g/l) should be regarded as artifacts because species distributions are truncated at these values (Fig. 2b) and optima could not be inferred correctly from the parameters of the Gaussian logit curves.

Quinlan and Phlips (2007) compared distributions of various algal groups along the salinity gradient in the open estuary of Suwanee River, Florida. Their ordination plots showed that the diatom community composition was changing gradually with the increase of salinity.

Muylaert et al. (2009) studied phytoplankton species responses to salinity in the River Schelde estuary, Belgium, using an ordination technique and Generalized Additive Model (GAM) fitting. They concluded that changes in the composition of the phytoplankton, composed largely of diatoms, were continuous along the salinity gradient that stretched from 0.15 to 30 g/l. They also found an increase of alpha diversity (species richness) in the transitional zone between fresh and marine waters instead of the expected minimum. The spatial mass effect, which is the enrichment by species from adjacent freshwater and marine habitats, was suggested as a cause of the increased diversity in this transitional zone.

Huvane (2002) studied surface sediment diatoms at 17 sites in Florida Bay, Florida, where the average salinity per site varied between 7.7 and 33.7 g/l. WA optima from her Table 17.2 are plotted in Fig. 3a, while the observed average salinities are shown in Fig. 3b. Two lines follow the same pattern, indicating that salinity optima were evenly distributed, and any observed "breakpoints" are artifacts of the uneven distribution of the observed salinity in the dataset. No clear relationship between salinity and diatom diversity was observed in this study.

Gaiser et al. (2005) developed the diatom salinity transfer function for coastal wetlands in the Everglades, South Florida. In this dataset, the observed salinity had a bimodal distribution with one cluster of sites with salinities below 5 g/l, and another above 15 g/l. The plot of optima and tolerances (Fig. 4) reflects this bimodality with two long "tails" of species optima at the low and high ends of salinity spectrum.

Snoeijs and coauthors studied the ecology of benthic diatoms in the Baltic Sea using multivariate statistical analyses (Snoeijs, 1994, 1995, 1999; Busse and Snoeijs, 2002, 2003; Ulanova and Snoeijs, 2006) and modeling distributions of diatom species in relation to salinity by fitting hierarchical response models (Ulanova et al., 2009). Salinity in their study areas varied between 0.4% and 12% and was one of the most important factors in explaining patterns of diatom distribution. Although optima and tolerances were not published, the ordination

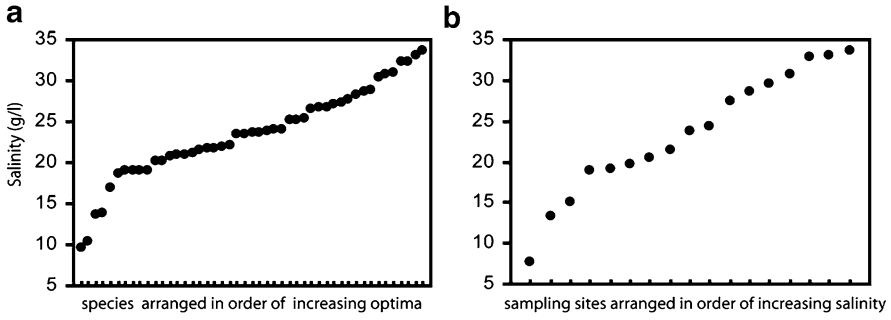

Figure 3. Distribution of salinity WA optima (**a**) and observed average salinities (**b**) in Florida Bay reported by Huvane (2002, Tables 17.1 and 17.2).

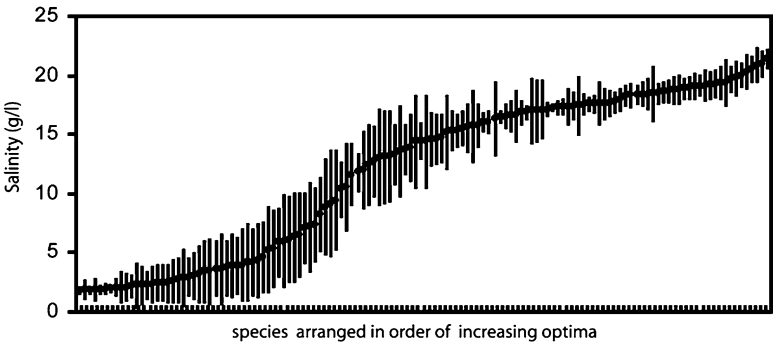

Figure 4. Distribution of salinity optima and tolerances of 132 diatom species from coastal wetlands, Everglades, Florida, reported by Gaiser et al. (2005, Appendix).

plots did not show any clear species clusters as would be expected if the species were indeed separated into salinity groups. No minima in species richness related to salinity were found, and occasional "species minima" were attributed to other factors, such as abundance and species richness of macroalgal hosts.

2.3. LAKES

Diatoms of lake sediments are often used to reconstruct past salinity fluctuations, which, in turn, can be used to infer climatic and hydrological changes. Salinity optima and tolerances of diatoms from surface sediments were quantified in numerous lake datasets (e.g., Fritz et al., 1991, 1993; Wilson et al., 1994, 1996; Roberts and McMinn, 1998; Reed, 1998; Sylvestre et al., 2001; Bloom et al., 2003). Fritz et al. (1993) used a set of 66 freshwater and saline lakes of the Northern Great Plains, North America to develop a transfer function for inferring salinity. Figure 5 shows distribution of WA optima and tolerances for 141 species that had relative abundance >1% in at least one sample (Fig. 5a, from Table 2 in Fritz et al., 1993) and observed salinity in 66 lakes (Fig. 5b, from Table 1 in Fritz et al., 1993). Both curves follow the same general pattern, which means again that salinity optima were evenly distributed. Tolerances did not exhibit any maxima or minima.

The diatom–salinity transfer function was developed by Cumming et al. (1995) for lakes of British Columbia, Canada. In the dataset of 111 lakes, average salinity ranged from 0.04 to 196 g/l. Distribution of observed WA optima (Fig. 6a) and salinity values expressed at log scale in the dataset was fairly even (Fig. 6b) with both lines following the same general pattern.

Wilson et al. (1996) used surface sediment samples from 219 freshwater and saline lakes located in Western North America to develop the salinity transfer function. Salinity ranged in this dataset from 0.02 to 620 g/l, and its distribution across the dataset was fairly even, except of the low number of observations at

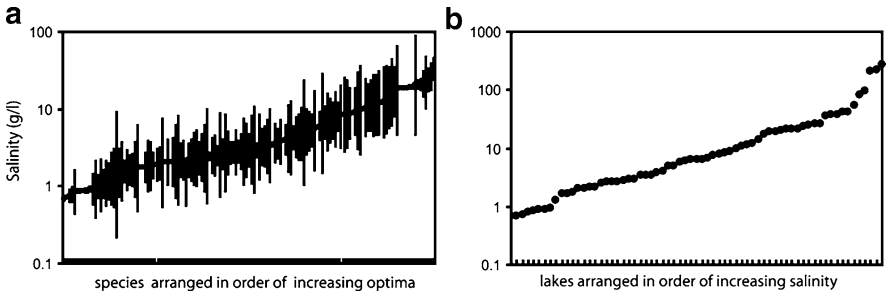

Figure 5. Distribution of salinity WA optima (**a**) and observed average salinities (**b**) in lakes of the Northern Great Plaines reported by Fritz et al. (1993, Tables 1 and 2).

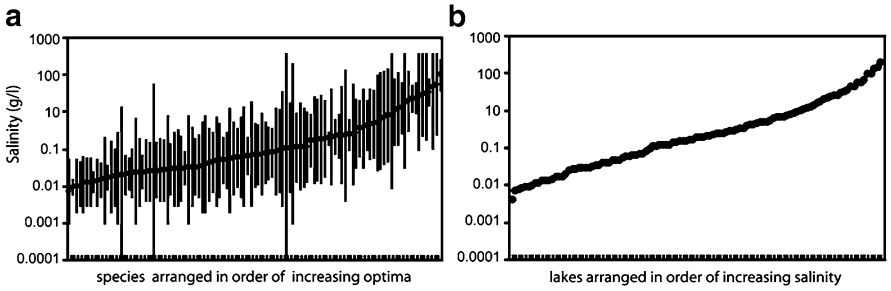

Figure 6. Distribution of salinity WA optima (**a**) and observed average salinities (**b**) in lakes of British Columbia reported by Cumming et al. (1995, Chapter 1.5 and Appendix A).

both ends of the spectrum. Species optima were also distributed evenly in the range from 0.1 to 10 g/l (Fig. 3a and b in Wilson et al., 1996). A small break at 10 g/l can hardly be interpreted as an indication of a physiological threshold, because only a few lakes have salinities above that value. There was no trend in the tolerances expressed at the log scale.

2.4. RIVERS

Potapova and Charles (2003) analyzed diatom distribution along salinity gradients in a large dataset of river benthic samples collected across USA for the USGS National Water Quality Assessment (NAWQA) program. The dataset covered the range of conductivity from 10 to 14,500 µS/cm, which corresponds to salinities of approximately 0.007–9.7 g/l. The greatest majority of observations fell in the range of 100–3,000 µS/cm (~0.07–2 g/l). A few high-conductivity sites were located in estuaries and in other areas influenced by a seawater influx. The median conductivity value was 334 µS/cm, which approximately corresponds to the threshold between oligo- and mesohalobous diatoms (0.2 g/l) established by Hustedt (1957),

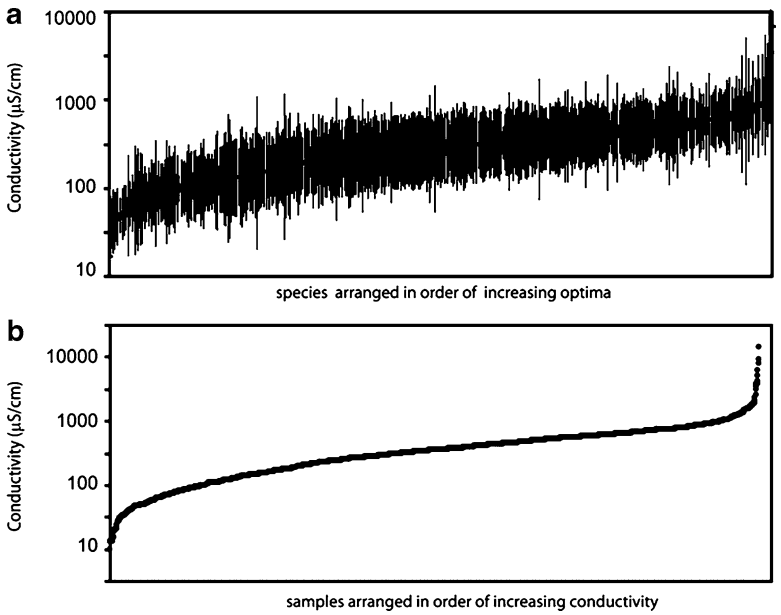

Figure 7. Distribution of WA optima and tolerances of diatom species (**a**) and observed conductivity (**b**) in the NAWQA dataset of 3,031 diatom samples collected from USA rivers.

which makes the dataset useful for exploring this threshold. Plots of species WA optima and tolerances (Fig. 7a) and of observed conductivity (Fig. 7b) follow the same pattern. No breaks suggesting thresholds are evident.

The same dataset was used to calculate WA optima and tolerances for genera instead of the species (Fig. 8). Apparently, genera have tolerance ranges not much wider than species and their optima are also continuously distributed. These optima should not be, of course, considered realistic for genera well represented in both inland and marine waters, such as *Cocconeis*, *Achnanthes*, *Nitzschia*, *Parlibellus*, and *Surirella*. The optima for several genera (*Actinella*, *Aneumastus*, *Anorthoneis*, *Campylostylus*, *Cosmioneis*, *Ellerbeckia*, *Haslea*, *Muelleria*, *Peronia*, *Proschkinia*, *Seminavis*, and *Tetracyclus*) are based only on a few observations. They are shown because their relative positions, in general, correspond to the common knowledge on the ecology of these diatoms. Optima of these genera are found mostly at the extremes of the conductivity gradient and the low numbers of occurrences reflect poor representation of samples from rivers of high and low mineral content in the dataset. It is noteworthy, however, that the difference in absolute values optima of the genera that are considered "freshwater," may be as high as an order of magnitude.

Modeling species distributions along an environmental gradient using WA approach assumes that species have unimodal types of responses, described by symmetrical bell-shaped curves. If this is not the case, and many species do have

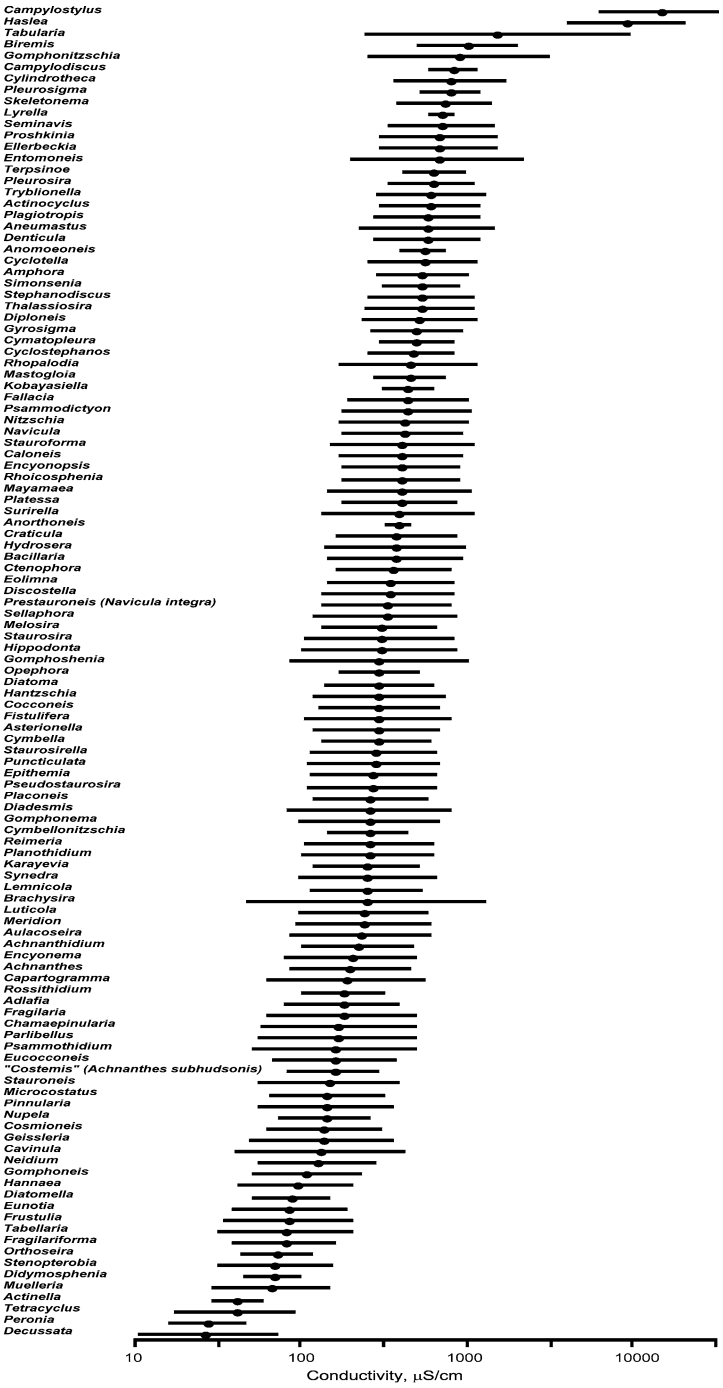

Figure 8. Distribution of WA optima and tolerances of 117 diatom genera in the NAWQA dataset of 3,031 diatom samples collected from rivers across USA.

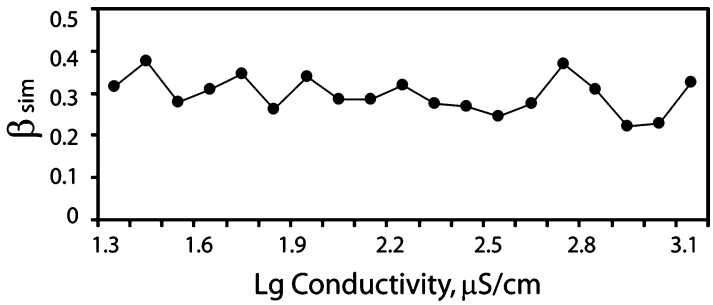

Figure 9. Diatom species turnover or β-diversity along conductivity gradient in the NAWQA river dataset. A total of 2966 samples from the NAWQA river dataset, which ranged in conductivity from 20 to 1,585 µS/cm, were included in the analysis that was based on species presence–absence. Each *point* represents the turnover of species composition (β_{sim}) between two adjacent conductivity categories.

a more complex-shaped response curve, then thresholds common for many species might be revealed by examining the response curves obtained by fitting regression models. The GAM modeling approach similar to that employed by Muylaert et al. (2009) was used with the US river data. Response curves for the most species were unimodal and were distributed evenly along the gradient of conductivity.

The species richness did not show any relation to conductivity in the NAWQA dataset. Species turnover was examined in this dataset using a common measure of β-diversity, β_{sim} (Koleff et al., 2003). Samples arranged along conductivity gradient were divided into 20 categories (Lg conductivity, µS/cm, 1.3–1.4, 1.4–1.5, etc.) and lists of species found in each category of samples were created. Species turnover between each pair of adjacent categories of samples was calculated as β_{sim} = min (b,c)/min (b,c) + a, where a = total number of species shared by two categories, and b and c – number of species unique for categories b and c, respectively. If a threshold existed around salinity 0.2 g/l, a peak of species turnover would be evident approximately in the middle of the observed conductivity range (in categories that correspond to Lg conductivity 2.0–2.2). Figure 9 shows that fluctuations of the β_{sim} metric were comparable over the whole range of observed conductivities, and therefore, no threshold could be detected.

2.5. NO EVIDENCE OF SALINITY THRESHOLDS FROM SURVEYS DATA

The quantitative assessment of species turnover along the salinity gradient, thus, does not reveal any thresholds or breakpoints, besides those that are artifacts of the uneven distribution of observed salinities in datasets. It appears that diatom species divide the available salinity spectrum occupying as many available niches as possible following a species-packing model (MacArthur, 1970). Diatom genera

also seem to be continuously distributed in respect to salinity. The species richness data do not show any trends along salinity gradients, but sometimes enrichment from two adjacent water masses is observed.

The idea of a physiological barrier dividing marine and freshwater diatoms originated from an observation that most genera are almost entirely either marine or freshwater, and only a very few are brackish water. The question, however, is what is meant by these categories. According to the Venice system (1959), the boundary between fresh and brackish water is 0.5 g/l (conductivity ~750 μS/cm), while the boundary between brackish (mixohaline) and marine (euhaline) water is 30 g/l. If we suppose that the freshwater species are those confined to salinity zone of 0–0.5 g/l, and that tolerance calculated as one standard deviation from the optimum adequately describes species range, then about only 65% of species and 52% of genera from the US rivers dataset should be considered freshwater (Fig. 7). The optima and tolerances in the NAWQA dataset are, however, skewed toward the freshwater part of the spectrum because most of the sampling localities were in freshwaters. Many species common in rivers are known to form populations in brackish waters, and if samples from brackish waters were included in the dataset, even fewer species and genera would appear as truly freshwater. Moreover, the true range of species distribution is surely wider that one unit of the standard deviation. Therefore, even fewer taxa are really confined to the part of the spectrum below the 0.5 g/l boundary. Datasets, including saline lakes and coastal areas, would not yield almost any freshwater species at all, since the upper limits of tolerances are almost always higher than 0.5 g/l (Figs. 2, 4–6), although many species represented in these datasets are commonly known as "freshwater" diatoms.

How many "marine" species and genera are confined to the area of the salinity spectrum above 30 g/l? Although the quantitative information on the optima and tolerances at the high end of the salinity spectrum is scarce, it appears that large populations of many representatives of the genera currently classified as "marine" are often found at salinities below 30 g/l. The examples include: *Coscinodiscus*, *Asteromphalus*, *Bacteriastrum*, *Biddulphia*, *Detonula*, *Ditylum*, *Guinardia*, *Lauderia*, *Leptocylindrus*, *Porosira*, *Rhizosolenia*, *Asterionellopsis*, *Ardissonea*, *Catacombas*, *Catenula*, *Cymatosira*, *Grammatophora*, *Licmophora*, *Rhabdonema*, *Striatella*, *Thalassionema*, *Thalassiothrix*, and others commonly found in inland seas and coastal areas with lowered salinities (e.g., Proshkina-Lavrenko, 1955, 1963a, b; Hällfors, 2004). In fact, most of the neritic and coastal benthic diatoms considered "marine," were shown in experiments to tolerate salinities lower than 34–35 g/l, typical for an open ocean (Eppley, 1977; Mizuno, 1992). Even if the boundary of marine and brackish waters is set to 18‰, still some representatives of the mentioned genera will be found living at the brackish side of this boundary. Lowering the boundary even further, to, say, 5–7 g/l, will leave only a small part of the salinity spectrum still designated as "brackish water." No wonder that only a few diatom taxa can be confined to such a narrow zone, as a majority of diatom species have wider tolerances. Stenohaline marine diatoms, on the other hand, are usually pelagic species. Their stenohalinity has

mostly been deduced from the field, but not experimental data. It is therefore not clear whether their distributions are limited by salinity only. Open ocean habitats are characterized, for example, by the greater mixed layer depths (MLD) and lower nutrient supply than coastal areas. Similarly, salinity might not be the only factor dividing "marine" and "freshwater" diatoms: the oceans are usually nitrogen limited and have much greater MLD compared to inland water bodies where phosphorus is more often limiting and MLD is shallow (Litchman et al., 2009).

It might be argued that the terms "marine," "brackish," and "freshwater" refer to species optima rather than their ranges of distribution. In this case, the proportion of marine taxa would be, of course, much higher than that of the other two groups. As can be seen in the examples for this chapter, the distribution of ecological optima closely follows the distribution of the observed salinities in the dataset. In an imaginary dataset randomly covering water bodies across the Earth, the distribution of optima would then be proportional to the distribution of observed salinities, that is, with a great majority of optima in the 30–40 g/l range, fewer below 0.5 g/l, and even fewer in between. Only a small proportion of water bodies on the Earth are hypersaline. As a result, relatively few diatoms adapted to salinity above 40 g/l. In hypersaline environment (above 75 g/l), a decline in both species richness (Ehrlich and Dor, 1985; Herbst and Blinn, 1998) and growth rates of the cultures (Clavero et al., 2000) has been demonstrated.

3. Salinity Barrier in Evolutionary History of Diatoms

Both the diatom fossil record and the high number of marine diatom genera point at the marine origin of diatoms, and indicate that only a few diatom lineages colonized freshwaters (Round and Sims, 1981). The phylogenetic reconstructions based on molecular data continue, however, to unravel a more complicated sequence of events than just a few invasions of inland water bodies. The phylogeny of Thalassiosirales reconstructed by Kaczmarska et al. (2005), who used the SSU rDNA data, showed, for example, that the freshwater representatives of Thalassiosirales are polyphyletic. Alverson et al. (2007) used a multigene approach and a dense taxon sampling to create a more robust phylogeny of the same group. Their work revealed that there were at least three lineages within Thalassiosirales that independently colonized freshwaters. Moreover, they found that at least three recolonizations of the marine habitat by the freshwater lineages took place. Recent studies of the phylogeny of araphid diatoms based on SSU rDNA data (Sato et al., 2008a, b, 2009) revealed at least three freshwater lineages: *Diatoma* + *Asterionella*, *Staurosira* (a sister to marine *Nanofrustulum*), and *Fragilaria* + *Fragilariforma* + *Ulnaria*. Moreover, the clade containing *Tabularia* can also be considered as a successful colonizer of inland waters because *T. fasciculata* is a common inhabitant of inland water bodies with an elevated ionic content.

Among raphid diatoms, there are multiple examples of lineages that span both marine and freshwater habitats including the large genera *Navicula* sensu stricto,

Nitzschia, *Surirella*, and *Amphora* (Mann, 1999). Phylogenetic reconstructions of the raphid group based on nuclear genes SSU rRNA, partial LSU rRNA, and the plastid gene *rbc*L (Bruder and Medlin, 2008) confirmed the monophyly of these genera and other lineages that contain both marine and freshwater representatives. For instance, the so-called freshwater monoraphid diatom lineage includes *Achnanthidium minutissimum* and *Planothidium lanceolatum* found in fresh and brackish waters, and *Pauliella taeniata*, a common planktic diatom of the northern seas.

As Mann (1999) noted, several genera of diatoms considered to be mainly marine (e.g., *Hantzschia*, *Achnanthes* sensu stricto) also contain species commonly found in terrestrial subarial habitats. Other examples of genera found in both terrestrial and coastal habitats are *Melosira* and *Cosmioneis*. In fact, the marine nature of these genera is relative: *Melosira*, *Hantzschia*, and *Achnanthes* are extremely common in coastal waters with the salinity lower than the typical marine, and often in the brackish range (0.5–30 g/l). On the other hand, the genus *Ellerbeckia* that has been considered terrestrial until recently has been shown to contain a marine species *E. sol* (Crawford and Sims, 2006). Interestingly, all of the occurrences of *E. arenaria* in the US NAWQA river dataset (Fig. 8), the Montana river dataset (L. Bahls, 2009 (personal communication)), and the EDDI lake dataset are from waters of relatively high conductivity (300–700 μS/cm). Although this species is found mainly on rock surfaces, and not in lakes and rivers, apparently it has adapted to a rather elevated salt content.

As has been shown by Medlin and Kaczmarska (2004) and later confirmed by Alverson et al. (2006), *Ellerbeckia* diverges at the base of the diatom phylogenetic tree, which brings up the question about the salinity preferences of the earliest diatoms. Two lines of evidence point to the marine ancestry of diatoms: the absence of freshwater diatoms in strata below the mid-Eocene, and the predominantly marine lineages at the base of the diatom molecular phylogenetic tree (Sims et al., 2006). However, at present, it is only possible to speculate what was the habitat of the earliest diatoms. Their robust thick-walled frustules seem to be adapted to a benthic habitat and a variable salinity typical for nearshore environments, but not to the open ocean (Round and Crawford, 1981; Nikolaev and Harwood, 2000). The recent discovery of diatoms in non-marine deposits of the Lower Cretaceous even points to a terrestrial or semiterrestrial habitat (Harwood et al., 2004). Perhaps, the ability to adapt to changing salinity is innate for diatoms as a group, and some lineages retained this ability better than others. Although the salinity of the world ocean is remarkably stable, there are indications that it was declined during the Early Cretaceous and the Miocene (Messinian) salinity "crises" (Hay et al., 2006). The absence of freshwater diatoms in the Cretacious and Paleocene deposits also does not prove that they did not exist before the Eocene since the fossil record can easily be incomplete. The finding of fairly modern-looking thalassiosiroid diatoms (*Cyclotella*, *Discostella*, and *Puncticulata*) in the mid-Eocene sediments (Wolfe and Siver, 2009) indicates that modern freshwater lineages had possibly originated much earlier than the previously available fossil record suggested.

In summary, no discontinuities in species turnover along the salinity gradient can be shown at present, and recent phylogenetic studies show that several diatom clades were able to shift their salinity affinities in both directions. Large diatom clades, thus, are predominantly marine or freshwater not because salinity represents an insurmountable evolutionary barrier, but because habitats with intermediate salinity are relatively ephemeral, unstable, and isolated in time and space. This is why relatively few diatoms evolved there. The marine lineages are apparently most numerous because of the spatial dominance and relative temporal stability of the ocean compared to the fresh and brackish water bodies.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 333–363
DOI 10.1007/978-94-007-1327-7_15, © Springer Science+Business Media B.V. 2011

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DIATOMS FROM HOT SPRINGS FROM KURIL AND SAKHALIN ISLANDS (FAR EAST, RUSSIA)

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1. Introduction

Hot springs have received a tremendous amount of attention recently, particularly as they relate to microbial organisms. They have been sites where interesting and useful DNA has been extracted and developed to a diverse set of academic and applied uses (Arnheim et al., 1990; Watson et al., 1992). Hot springs were one of the first sites of discovery of a third domain of life, the Archaea (Barns et al., 1994, 1996). Extreme environments such as hot springs have become more interesting for those who are seeking to discover life on places other than earth within our own solar system, and beyond. Thus, hot springs may represent analog habitats that life forms can withstand; what we humans perceive to be "extreme," i.e., very cold, hot, with different chemical compositions.

Much has been made of the prokaryotes in these extreme environments, particularly Cyanobacteria (e.g., Brock and Brock, 1967), but much less has been documented relative to the eukaryotes. Thermal resistance of thermophilic and mesothermic algal species is high, and they successfully live and thrive in water bodies with temperatures of 30–84°C. A group of eukaryotes that does seem to occupy a wide set of ecological niches are the diatoms, and species are known to occur in, on and around ice, as well as extremely acid and hot waters.

Hot springs have been recorded from all continents on earth except Antarctica. Surveys of diatoms from hot springs, though the number of studies is limited, have been accomplished in many of the regions of the world. We present in Table 1, a partial listing of the surveys of diatoms from hot springs around the world.

Hot springs also offer the opportunity to test hypotheses regarding the biogeography of microbial organisms. Kociolek and Spaulding (2000) detailed a number of families, genera, and species that appear to be localized in their

Table 1. Partial listing of hot springs by continent and country in which diatoms have been recorded.

Continent/country	Hot springs	References
Asia		
Russia	Kamchatka	Petersen, 1946
Russia	Kamchatka	Yoshitake et al., 2008
India	Vajreshwari	Gonzalves, 1947
India	Various	Thomas and Gonzalves, 1965a, b, c, d, e, f
Sunda Islands	Various	Hustedt, 1937–1938
Japan	Toyama Prefecture	Emoto and Yoneda, 1942
Japan	Hokkaido	Yoneda, 1962
Japan	Shizuoka Prefecture	Kobayashi, 1957a
Japan	Shiobara	Kobayashi, 1957b
Japan	Various	Molisch, 1926
Japan	Various	Owen et al., 2008
Japan	Isobe Hot Springs	Fukushima et al., 2002
Japan	Various	Villeneuve and Pienitz, 1998
Europe		
Czech Republic	Carlsbad	Corda, 1840; Sprenger, 1930
Czech Republic	Various	Bilý, 1934
Italy	Triponzo	Dell'Uomo, 1986
Iceland	Various	Krasske, 1938; Biebl and Kusel-Fetzmann, 1966; Owen et al, 2008; Villeneuve and Pienitz, 1998
Spitsbergen	Various	Krasske, 1938
France	Chaudesaigues	Famin, 1933a, b
Azores	Various	Brock and Brock, 1967
North America		
USA	Yellowstone, MT	Mann and Schlichting, 1967; Stockner, 1967; Hobbs et al., 2009
USA	Mt. Rainier, WA	Stockner, 1967
USA	Ohanapecosh Hot Springs, WA	Stockner, 1968
USA	Alhambra, MT	Fairchild and Sheridan, 1974
USA	Lassen National Park, CA	Anderson, 1935
USA	Blue Lake Warm Spring, UT	Kaczmarska and Rushforth, 1983
Canada	Various	Villeneuve and Pienitz, 1998
South America		
San Salvador	Various	Hustedt, 1953
Africa		
Israel	Lake Kinneret region	Dor, 1974
Kenya	Lakes Elmenteita, Baringo	Mpawenayo and Mathooko, 2004
Burundi	Various	Mpawenayo et al., 2005
Zambia	Various	Compère and Delmotte, 1988
Nambia	Various	Schoeman and Archibald, 1988
Australia		
New Zealand	Various	Cassie, 1989; Cassie and Cooper, 1989; Owen et al., 2008
Java, Bali, Sumatra	Various	Hustedt, 1937–1938

distributions (vs. being widely distributed). And while Vyverman et al. (2007) attempted to further document patterns of diatom distributions that do not support an "everything is everywhere" model, their analysis lacks appropriate comparisons to understand biogeographic patterns and processes. Hot springs offer the possibility to examine habitats that may have similar temperatures and water chemistries, but occur in very different geographic locales, and see if they harbor and support similar or different species of diatoms.

Villeneuve and Pienitz (1998) compared floras of hot springs from Canada, Iceland, and Japan, and found that although they had similar environmental conditions, the structure of the diatom communities were quite different, especially with regard to species. Likewise, Owen et al. (2008) compared hot springs in Iceland, New Zealand, and Kenya, and the dominant taxa present in these systems were all quite in terms of major groups represented. However, Hobbs et al. (2009) found *Pinnularia acoricola* Hustedt and *Eunotia exigua* (de Brébisson *ex* Kützing) Rabenhorst in the hot springs of an acid habitat and noted that these taxa had also been reported from similar environments around the world (e.g., DeNicola, 2000).

Studies of hot springs algae in the eastern part of Russia are few. And, at present, studies on algal species diversity in hydrothermal environments of the Kuril Islands are still preliminary. There are several publications detailing results of investigations of hot spring algal floras of Kunashir Island. In the publication by Tereshkova with coauthors (1973), the authors provide some information on the cultivation procedure of four species of thermophilic blue-green algae (*Coccopedia* sp., *Synechococcus* sp., *Phormidium ambiguum* Gomont, and *P. foveolarum* (Montagne) *Gomont*. Sentzova (1991) described two species new to science – *Galdieria partita* Sentzova and *G. maxima* Sentzova (division Rhodophyta) from thermal acidic springs. Gromov et al. (1991) described a new acidophilic species of golden algae *Ochromonas vulcania* Gromov, Nikitina et Mamkayeva, from fumaroles of the Mendeleev Volcano. In Nikitina's (2005) monographic survey, there are data on flora of blue-green algae of hydrothermal outlets of Goryachy Beach and the Mendeleev Volcano, and she reported 15 taxa from 3 classes, 4 orders, and 7 families. In a short publication, Nikulina (2007) presents preliminary information about algal flora of hot springs of Kurilsky Reserve located on Kunashir Island. Data of diatoms from hot springs on Kunashir Island were recorded for the first time by Nikulina (2007).

The representatives of blue-green algae in underwater hydrothermaes of Yankicha Island are known from Beljakova's papers (2000a, b, 2001), which report 14 taxa of blue-green algae of two classes, two orders, and four families.

In this paper, we present the results of a biotic survey of freshwater and brackish diatoms from hot springs in eastern Russia, notably the several islands from the Kuril Islands and Sakhalin Island, and compare our results with others investigating hot springs from around the globe. This represents the first documentation of diatoms from hot springs on Shiashkotan, Yankicha, and Sakhalin Islands.

2. Materials and Methods

2.1. DESCRIPTION OF SAMPLING SITES

2.1.1. Kuril Islands

Kunashir Island: In August 1999, we investigated two streams in the area of the former Alekhino Settlement, both of which empty into Alekhin Bay. The water temperature was 48.1°C and 26°C at the time of sampling. Substrates were sand with a few small and medium-sized stones. These springs belong to the northern group of Alekhin springs located on the coast of the Golovnin Volcano facing the Sea of Okhotsk. According to the published data, water of this group of springs is referred to as calcium-sodium, sulfate-chloride thermae, with pH = 4–5, T = 50–55°C (Markhinin and Stratula, 1977).

In the caldera of Golovnin Volcano, periphyton (benthic) samples were taken from a hot spring in the solfatara field, at the foot of the southern slope of Central-East cupola of the volcano in January 2007. There were five groups of solfataras with thermal spring outlets distinguished, and water temperature varied from 60°C to 103°C, water chemistry sharply differs even within a group: from circumneutral and alkaline (pH = 6–8.5) hydrocarbonate-sulfate and sodium-calcium to acidic (pH = 2–2.5) sulfate and sodium (Markhinin and Stratula, 1977).

Periphyton algae were also sampled in "Stolbovskiye" thermal springs and a stream flowing from one of them in August 1999 and April 2007. The studied springs are located on the western coast of the island, 2 km south of Stolbchaty Cape. In August 1999, the water temperature was 40°C in the hot spring and 24°C in the brook downstream; habitats included small- and medium-sized stones. Stolbovskiye springs belong to circumneutral (pH = 6.7–7.0) nitric, sodium chloride-sulfate thermae, and their water temperature reaches 82°C (Zharkov and Poberezhnaya, 2008).

Shiashkotan Island: We studied periphyton diatoms collected from the hydrothermal boil located on the sea shore Voskhodnaya Bay, within 15 m of the water edge, between Obvalny Cape and Bobrovy Island (the side facing the Sea of Okhotsk) in August 1999. The water temperature reached 71°C at the time of sampling; the habitat included small- and medium-sized stones.

Literature data only are known for the hot springs of Obvalny Cape located on the Pacific side of Shiashkotan Island, on the Makarovsky Isthmus. According to their water composition, the springs are identified as sodium chloride, with general mineralization of 13.3 g/L, water temperature of 60°C, and pH = 6.98 (Markhinin and Stratula, 1977).

Yankicha Island: Periphyton algae were sampled in hydrothermal spring baths at their origin and the effluent streams in Kraternaya Bay in August 1999. The water temperature of the springs varied from 50°C to 60°C at the time of sampling; substrates in the baths consisted of sand, and in streams, small, medium-sized and large stones on sandy bottoms. These thermal springs belong to the South-East solfatara field, their water temperature reaches 101°C, pH = 2.8–3.7. In their chemical composition, the springs are similar to the surrounding seawater; in hydrochemical

type, they are sodium chloride, with mineralization of 25–27 g/L (Markhinin and Stratula, 1977).

2.1.2. Sakhalin Island

Phytoplankton and periphyton algae were collected in hot springs located on the shore of Dagi Bay in the northeast coast of Sakhalin Island. The water temperature was 37°C in the springs and 40.6°C in reservoir at the time of sampling. Substrates in the stream included sand, small and medium stones, with stratum of silt. According to the literature, water of Dagi hydrothermal springs is characterized as being hydrocarbonate-sodium chloride in composition, alkaline (pH = 7–8), and water temperature varied from 20°C to 55°C (Karpunin et al., 1998; Zharkov, 2008).

2.2. SAMPLE PROCESSING

All samples were cleaned by the method of Swift (1967), and processed into permanent microscope slides. Light microscope observations were made with a Nikon "Alphaphot 2" and a Carl Zeiss "Axioskop 40." Diatom photos were taken at the "Biotechnology and Genetic Engineering" on the campus of Institute of Biology and Soil Sciences, Far East Branch of Russian Academy of Sciences (head V.P. Bulgakov). Permanent slides are housed at Institute of Biology and Soil Sciences, Far Eastern Branch of Russian Academy of Sciences, Vladivostok, Russia.

3. Results and Discussion

3.1. KURIL ISLANDS

The diatom flora of Kuril Islands hot springs and the watercourses formed by them is represented by 145 species (162 species, varieties and forms) of 3 classes: Coscinodiscophyceae, Fragilariophyceae, and Bacillariophyceae (Table 2). In the taxonomic structure of the flora, the best represented genera include *Pinnularia* with 13 taxa, *Nitzschia* with 12 taxa, and *Navicula* with 10 species and varieties.

Kunashir Island: Biofilms on stones in the sulphureous spring flowing on the coast of Alekhin Bay is dominated by the diatoms *Nitzschia capitellata*, *N. palea, Pinnularia acidophila*, and *P. acidojaponica*. The species *Diatoma vulgare, Eunotia implicata, Gomphonema parvulum*, and *Nitzschia nana* also have high abundance estimates. The algal flora of another hot spring in Alekhin Bay watershed has similar floristic composition; however, occurrence of the species is characterized as solitary or rare (Table 3).

The composition of diatom flora of the hot spring in caldera of Golovnin Volcano is extremely poor and is represented by 25 intraspecific taxa of diatoms from the classes Fragilariophyceae and Bacillariophyceae. The frequency of the algae was negligible, solitary to not rare, and only *Placoneis elginensis* was estimated as solitary to very frequent (Table 3).

Table 2. Taxonomic composition of hot springs algae of Kuril (Kunashir, Shiashkotan, and Yankicha) and Sakhalin Islands.

Class	Order	Family	Genera	Species	Varieties and forms	Percent
Kuril Islands						
Coscinodiscophyceae	4	6	6	11	12	7.4
Fragilariophyceae	4	5	13	22	27	16.7
Bacillariophyceae	9	20	33	112	123	75.9
Total	17	31	52	145	162	100
Sakhalin Island						
Coscinodiscophyceae	7	10	11	17	17	12.4
Fragilariophyceae	3	4	11	15	17	12.4
Bacillariophyceae	10	24	40	99	103	75.2
Total	20	38	62	125	137	100

A hot spring and a stream with its origin from the spring ("Stolbovskiye" group of springs) had similar structure of diatom communities in August 1999. Stone biofilms in the thermal source were represented by *Rhoicosphenia abbreviata* developed in mass, as well as by species with lower abundance – *Nitzschia palea*, *N. nana*, *Planothidium lanceolatum*, and *Synedra ulna* (Table 3). *Gomphonema parvulum*, *Synedra ulna*, *Planothidium lanceolatum*, and *Nitzschia nana* were dominant in the stream. Subdominant taxa were *Melosira varians*, *Rhoicosphenia abbreviata*, *Achnanthidium minutissima*, *Navicula cryptotenella*, and *Nitzschia palea.*

The hydrothermal springs on Stolbchaty Cape that we studied in April 2007 are characterized by algal communities dominated by the diatoms *Tryblionella apiculata* and *Nitzschia capitellata* in combination with the following subdominants: *Amphora veneta* f. *capitata*, *Caloneis molaris*, *Encyonema hebridicum*, *Navicula cryptotenella*, and *Nitzschia nana*.

Shiashkotan Island: In a hot spring on Shiashkotan Island, 44 taxa of diatoms have been recorded. *Hannaea arcus* var. *linearis* f. *recta* had the highest abundance estimate; it was very frequent from this locality (Table 3).

Yankicha Island: In thermal springs and in streams flowing from them, *Navicula elginensis* and *Nitzschia aurariae* developed in mass in stone fouling. In addition, populations of *Diatoma vulgare* and *Nitzschia thermaloides* were abundant (Table 3).

3.2. SAKHALIN ISLAND

The diatom flora of Sakhalin hot springs is represented by 125 species (131 species, varieties, and forms) of 3 classes: Coscinodiscophyceae, Fragilariophyceae,

Table 3. Species composition of algae of hot springs of Kuril Islands and Sakhalin Island.

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
	Bacillariophyta											
	Coscinodiscophyceae											
	Coscinodiscales											
	Coscinodiscaceae											
1.	*Bacterosira fragilis* (Gran) Gran	–	–	–	–	–	1	P	mh	–	–	a-a
	Heliopeltaceae											
2.	*Actinoptychus senarius* (Ehrenberg) Ehrenberg	–	–	–	–	–	1	–	–	–	–	–
	Thalassiosirales											
	Thalassiosiraceae											
3.	*Thalassiosira bramaputrae* (Ehrenberg) Håkansson et Locker	–	–	–	–	–	1	–	–	–	β	–
4.	*Th. eccentrica* (Ehrenberg) Cleve	–	–	–	–	–	1	P	mh	i	–	b
5.	*Th. gravida* Cleve	–	–	–	–	–	1	P	mh	i	–	b
6.	*Th. nativa* Sheshukova–Poretzkaya	–	–	–	–	–	1	–	–	–	–	–
7.	*Th. nordenskiöldii* Cleve	–	–	–	–	1–2	–	P	hl	–	–	b
	Stephanodiscaceae											
8.	*Cyclotella meneghiniana* Kützing	1	–	–	–	–	–	B-P	hl	alf	α-β	k
9.	*C. striata* (Kützing) Grunow	–	–	–	–	–	1	–	hl	–	–	–
	Paraliales											
	Paraliaceae											
10.	*Paralia sulcata* (Ehrenberg) Cleve	–	–	–	–	–	1–3	P	mh	–	–	b
	Melosirales											
	Melosiraceae											

(continued)

Table 3. (continued)

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
11.	*Melosira dickei* (Thwaites) Kützing	1	2–3	–	–	–	–	B-P	i	i	–	–
12.	*M. moniliformis* (O.F. Müller) Agardh	–	–	–	3	1–2[a]	–	B-P	hl	–	–	k
13.	*M. nummuloides* Agardh	–	–	–	–	1–2[a]	–	—	mh	–	–	–
14.	*M. varians* Agardh Aulacoseirales Aulacoseiraceae	1–4[a]	1	–	–	1	1[a]	B-P	i	alb	β	k
15.	*Aulacoseira ambigua* (Grunow) Simonsen.	–	–	–	–	–	1[a]	P	i	alf	α-β	k
16.	*A. distans* (Ehrenberg) Simonsen var. *distans*	–	1	–	–	–	–	B-P	i	acf	χ-o	b
17.	*A. distans* var. *lirata* (Ehrenberg) Bethge	–	–	–	–	1	–	B-P	–	–	–	–
18.	*A. granulata* (Ehrenberg) Simonsen	1	1–2	–	–	1	1	P	i	alf	β	k
19.	*A. italica* (Kützing) Simonsen	1	–	–	–	–	1	P	i	alf	o-β	k
20.	*A. subarctica* (O.F. Müller) Haworth Triceratiales Triceratiaceae	–	–	–	–	–	1–2	P	i	alb	α-β	k
21.	*Cerataulus turgidus* (Ehrenberg) Ehrenberg	–	–	–	–	–	1	B-P	–	–	–	–
22.	*Odontella aurita* (Lyngbye) Agardh Plagiogrammaceae	–	–	–	–	1	1	B-P	mh	alf	–	–
23.	*Dimeregramma fulvum* (Gregory) Ralfs	–	–	–	–	1	–	B	–	–	–	–
24.	*D. minor* (Gregory) Ralfs Chaetocerotales Chaetoceraceae	–	–	–	–	–	1	–	–	–	–	–

25.	*Chaetoceros* sp. Fragilariophyceae Fragilariales Fragilariaceae	–	–	–	–	–	1	–	–	–	–	–
26.	*Asterionella formosa* Hassall	–	1[a]	1	1	1–2	–	P	i	alf	o-β	k
27.	*Ctenophora pulchella* (Ralfs) Williams et Round	1	1–2	–	–	1	1	B-E	mh	alf	β-α	b
28.	*Fragilaria capucina* Desmazières var. *capucina*	1–2[a]	1[a]	–	–	–	–	B-P	i	alf	o-β	k
29.	*F. capucina* var. *gracilis* (Østrup) Hustedt	–	–	–	–	–	1	–	–	–	β-α	–
30.	*F. capucina* var. *rumpens* (Kützing) Lange-Bertalot	–	–	–	–	–	1	B	i	acf	o	k
31.	*F. crotonensis* Kitton	1–2[a]	1[a]	–	1	–	–	P	i	alf	o-β	b
32.	*F. nitzschioides* Grunov	–	–	–	–	1	–	B	i	i	–	k
33.	*F. vaucheriae* (Kützing) J.B. Petersen	1–2[a]	1[a]	–	1[a]	1–2	1	E	i	alf	β	k
34.	*Fragilariforma bicapitata* (A. Mayer) Williams et Round	–	–	–	–	–	1	B	hb	acf	o-β	b
35.	*F. virescens* (Ralfs) Williams et Round	1	–	–	–	–	–	B	hb	i	χ	a-a
36.	*Hannaea arcus* (Ehrenberg) Patrick var. *arcus*	–	1	1	–	1	1	B	i	alf	χ	a-a
37.	*H. arcus* var. *amphioxys* (Rabenhorst) Patrick	1	–	–	–	–	–	B	i	alf	χ-o	a-a
38.	*H. arcus* var. *linearis* (Holmboe) R. Ross	–	1	1	–	1	–	B	i	alf	χ	a-a
39.	*H. arcus* var. *linearis* f. *recta* (Cleve) Foged	1–3[a]	1[a]	–	5[a]	1–2[a]	–	B	i	alf	χ	a-a
40.	*Staurosira construens* (Ehrenberg) Grunow f. *subsalina*	–	–	1	–	–	2	B	i	alf	β	k
41.	*S. construens* f. *venter* (Ehrenberg) Bukhtiyarova	1	–	–	–	–	4	B	i	alf	o	k

(continued)

Table 3. (continued)

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
42.	*S. pinnata* (Ehrenberg) D. M. Williams and Round	–	–	–	–	–	2[a]	B	hl	alf	β–α	k
43.	*Synedra inaequalis* H. Kobayasi	–	1	–	–	–	1	B	–	–	–	–
44.	*S. ulna* (Nitzsch) Ehrenberg var. *ulna*	1–6[a]	1–3[a]	1	1[a]	1[a]	1–2	B	i	alf	β-α	k
45.	*S. ulna* var. *oxyrhynchus* (Kützing) Van Heurck	–	–	–	–	–	1	B	–	–	β-α	–
46.	*Tabularia fasciculata* (Agardh) Will. and Round	–	1	–	–	1–2	1	B-E	hl	–	–	k
	Diatomaceae											
47.	*Diatoma anceps* (Ehrenberg) Kirchner	1	1	–	–	1	–	B	hb	alf	o-χ	a-a
48.	*D. hiemale* (Lyngbye) Heiberg	1[a]	1–2	–	1	–	–	B	hb	i	χ	a-a
49.	*D. mesodon* (Ehrenberg) Kützing	1–2[a]	1	–	2	1[a]	–	B	hb	alf	χ	a-a
46.	*D. moniliforme* Kützing	–	–	–	–	1	–	B-P	hl	–	β-α	k
50.	*D. vulgare* Bory	1[a]	1–5[a]	2–3	2[a]	1–4[a]	1[a]	B-P	i	alb	β	b
51.	*Meridion circulare* (Greville) Agardh var. *circulare*	1	1	–	1	–	1	B	hb	alf	χ-o	k
52.	*M. circulare* var. *constrictum* (Ralfs) Van Heurck	1–2[a]	1	–	1	–	–	B	hb	alf	χ-o	k
	Tabellariales											
	Tabellariaceae											
53.	*Tabellaria fenestrata* (Lyngbye) Kützing	–	–	–	1	–	–	B-P	hb	acf	β	b
54.	*T. flocculosa* (Roth) Kützing	1	1	1	1	1	1[a]	B-P	hb	acf	o-χ	a-a
	Licmophorales											
	Licmophoraceae											

55.	*Licmophora communis* (Heiberg) Grunov Striatellales Striatellaceae	–	–	–	–	1	–	B	–	–	–	–
56.	*Grammatophora angulosa* Ehrenberg Rhabdonematales Rhabdonemataceae	–	–	–	1	–	–	B	–	–	–	–
57.	*Rhabdonema minutum* Kützing Bacillariophyceae Eunotiales Eunotiaceae	–	–	–	–	–	1	–	–	–	–	–
58.	*Eunotia arcus* Ehrenberg	–	1	–	–	–	–	B	hb	acf	o	k
59.	*E. bilunaris* (Ehrenberg) Mills var. *bilunaris*	2	1–2[a]	–	–	1	1	B	i	acf	β	k
60.	*E. bilunaris* var. *linearis* (Okuno) Lange-Bertalot and Nörpel	–	–	–	–	1	–	B	–	–	o	–
61.	*E. denticulata* (Brébisson) Rabenhorst	–	1	–	–	–	–	B	–	acf	–	–
62.	*E. exigua* (Brébisson) Rabenhorst	1	1–2	2[a]	–	–	–	B	i	acf	χ	k
63.	*E. glacialis* Meister	–	–	–	–	–	1	B	–	acf	ρ	k
64.	*E. implicata* Nörpel, Lange-Bertalot and Alles	1[a]	1–4[a]	–	–	–	–	B	–	–	–	–
65.	*E. muscicola* Krasske	1	—	–	–	–	–	B	–	acf	–	–
66.	*E. parallela* Ehrenberg var. *angusta* Grunov	–	–	–	–	–	1	B	–	–	–	–
67.	*E. pectinalis* (O.F. Müller) Rabenhorst	1	1	–	–	–	–	B	hb	acf	χ	k
68.	*E. praerupta* Ehrenberg	1	1	1	1	–	–	B	hb	acf	χ	k
69.	*E. tenella* (Grunow) Hustedt Anomoeoneidaceae	–	–	–	–	1	–	B	hb	acf	χ-o	k

(continued)

Table 3. (continued)

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
70.	*Anomoeoneis sphaerophora* (Ehrenberg) Pfitzer	–	–	–	–	–	1–5[a]	P-B	hl	alb	χ-β	k
	Mastogloiales											
	Mastogloiaceae											
71.	*Aneumastus tusculus* (Ehrenberg) D.G. Mann et Stickle	–	–	–	–	1	–	B-P	i	alf	o-χ	k
72.	*Mastogloia elliptica* (Agardh) Cleve	–	–	–	–	–	1	B	mh	alf	–	k
73.	*M. smithii* Thwaites ex W. Smith	1–2[a]	1	–	–	1	1	B	mh	alf	β	k
	Cymbellales											
	Rhoicospheniaceae											
74.	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot	2–6[a]	1–2	–	1	1[a]	1[a]	B	hl	alf	β	k
	Cymbellaceae											
75.	*Brebissonia boeckii* (Ehrenberg) O'Meara	–	–	–	–	1	–	B	mh	–	–	b
76.	*Cymbella affinis* Kützing	1	1	1	1[a]	1–2[a]	1	B	i	alf	o–β	b
77.	*C. cistula* (Ehrenberg) Kirchner	1	–	–	–	–	–	B	i	alf	β	b
78.	*C. gracilis* (Ehrenberg) Kützing	–	–	–	–	–	1	B	hb	i	β	a-a
79.	*C. lanceolata* (Ehrenberg) Van Heurck	1	–	–	–	–	–	B	i	alf	β	–
80.	*C. naviculiformis* Auerswald	1	–	–	–	–	–	B	i	i	o	k
81.	*C. pusilla* Grunov	1	–	–	–	–	–	B	i	alf	–	k
82.	*C. subleptoceros* (Ehrenberg) Kützing	–	–	–	1	–	–	B	–	–	–	–
83.	*C. tumida* (Brébisson) Van Heurck	1	1	–	–	1	–	B	i	alf	o	b
84.	*Encyonema hebrridicum* Grunow ex Cleve	4[a]	–	–	–	–	–	B	i	i	o	a-a

85.	*E. minutum* (Hilse ex Rabenhorst) D.G. Mann	1[a]	1	1	1–2[a]	1	1	B	i	i	o	k
86.	*E. silesiacum* (Bleisch in Rabenhorst) D.G. Mann	1–3[a]	1	–	1–2[a]	1	1	B	i	alf	α	k
87.	*Placoneis elginensis* (Gregory) E.J. Cox	1[a]	–	1–4[a]	–	2–6[a]	1	B	i	i	o-β	k
	Gomphonemataceae											
88.	*Didymosphenia geminata* (Lyngbye) M. Schmidt	–	–	–	–	–	1	B	i	i	χ	a-a
89.	*Gomphoneis olivaceum* (Hornemann) Dawson ex Ross et Sims	1–2[a]	1	–	1	1	1	B	i	alf	β	b
90.	*G. quadripunctatum* (Østrup) Dawson ex Ross et Sims	1–3[a]	1	–	–	–	1	B	i	i	–	b
91.	*Gomphonema acuminatum* Ehrenberg var. *acuminatum*	1	1	–	–	–	–	B	i	alf	β	b
92.	*G. acuminatum* var. *coronatum* (Ehrenberg) W. Smith	1	–	–	–	–	1	B	i	alf	β	b
93.	*G. angustatum* (Kützing) Rabenhorst	2–3[a]	1–2[a]	1	2[a]	1	1–6[a]	B	i	alf	o	b
94.	*G. angustum* Agardh	–	–	–	1	–	–	B	i	alf	o	b
95.	*G. clavatum* Ehrenberg	–	1	–	–	1	–	B	i	i	o	k
87.	*G. intricatum* Kützing var. *vibrio* (Ehrenberg) Cleve	1	–	–	–	–	–	B	i	–	–	b
96.	*G. parvulum* (Kützing) Kützing var. *parvulum*	2–6[a]	1–4[a]	1	2[a]	1–2	2–4[a]	B	i	alf	β	b
97.	*G. parvulum* var. *lagenula* (Kützing) Frenguelli	3[a]	1	–	–	–	–	B	i	alf	–	k
98.	*G. truncatum* Ehrenberg	–	1	–	1	1	–	B	i	alf	β	b
99.	*Reimeria sinuata* (Gregory) Kociolek et Stoermer	1–2	1	–	1	–	1–2	B	i	alf	β	b
	Achnanthales											
	Achnanthaceae											

(continued)

Table 3. (continued)

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
100.	*Achnanthes brevipes* Agardh var. *intermedia* (Kützing) Cleve	–	–	–	–	–	1	B	–	–	–	–
101.	*A. coarctata* (Brébisson) Grunow	–	–	–	1–2	–	1	B	i	i	o	a-a
102	*A. exigua* Grunow var. *exigua*	1–2[a]	–	–	–	–	1	B	i	alf	β	k
103.	*A. exigua* var. *capitata* Hustedt	1–3[a]	–	–	–	–	–	B	–	–	–	–
104.	*A. hungarica* Grunow	–	–	–	–	–	1–6[a]	B	mh	alf	o-α	k
105.	*A. lanceolata* Brébisson ex Kützing var. *lanceolata*	1–6[a]	1–2[a]	–	1	1	1	B	i	alf	χ-β	k
106.	*A. lanceolata* var. *elliptica* Cleve	1–2[a]	1	–	–	–	–	B	i	alf	–	a-a
107.	*A. lanceolata* var. *haynaldii* (Schaarschmidt) Cleve	1–3[a]	–	–	–	–	–	B	i	alf	χ-β	k
108.	*A. linearis* (W. Smith) Grunov Achnanthidiaceae	–	–	–	–	–	1	B	i	i	χ-o	k
109.	*Achnanthidium minutissima* (Kützing) Czarnecki	1–4[a]	1[a]	1[a]	1	1	1	B	i	i	o-β	b
110.	*Eucocconeis flexella* Kützing Cocconeidaceae	–	1	–	–	–	–	B	mh	i	o	a-a
111.	*Cocconeis disculus* (Schumann) Cleve	–	1	–	1–3[a]	1	1–2	B	i	–	–	–
112.	*C. pediculus* Ehrenberg	–	–	1	–	–	–	B	hl	alf	β	k
113.	*C. pinnata* Gregory	–	–	–	–	1–2	–	–	–	–	–	–
114.	*C. placentula* Ehrenberg var. *placentula*	–	1	–	1	–	1[a]	B	i	alf	β	b
115.	*C. placentula* var. *euglypta* (Ehrenberg) Grunow	1–3[a]	1[a]	1	1–2[a]	1[a]	1[a]	B	i	alf	–	b

116.	*C. placentula* var. *lineata* (Ehrenberg) Van Heurck	–	–	1	–	1	–	B	i	alf	–	b
117.	*C. scutellum* Ehrenberg	–	1	–	–	1–2	1[a]	B	hl	–	–	–
	Naviculales											
	Diadesmidaceae											
118.	*Luticula mutica* (Kützing) D.G. Mann	–	1	–	–	–	1	B	i	i	o-β	a-a
	Amphipleuraceae											
119.	*Frustulia ampipleuroides* (Grunow) A. Cleve-Euler	1	–	–	–	–	1[a]	B	hb	acf	–	a-a
120.	*F. rhomboides* (Ehrenberg) De Toni	1	1[a]	–	–	–	–	B	hb	acf	o-χ	a-a
121.	*F. vulgaris* Thwaits	1–3[a]	1	–	–	–	1	B	hb	alf	o	b
	Neidiaceae											
122.	*Neidium ampliatum* (Ehrenberg) Krammer	1	1	–	–	–	–	B	hb	i	o	k
123.	*N. bisulcatum* (Lagerst.) Cleve	–	–	–	–	–	1	B	hb	i	o-β	b
	Sellaphoraceae											
124.	*Fallacia pygmaea* (Kützing) Stickle	–	1	–	–	–	1	B	mh	alf	α	b
125.	*Sellaphora pupula* (Kützing) D.G. Mann	–	–	–	–	–	1	B	hl	i	o-χ	k
	Pinnulariaceae											
126.	*Caloneis bacillum* (Grunow) Cleve	–	1	–	1	–	–	B-P	i	alf	o	k
127.	*C. molaris* (Grunow) Krammer	1–4[a]	–	–	–	–	–	B	i	i	–	a-a
128.	*C. silicula* (Ehrenberg) Cleve var. *silicula*	1	1	–	–	–	1	B	i	alb	o	k
129.	*C. silicula* var. *truncatula* Grunow	–	–	–	–	1	–	B-P	i	alf	–	k
130.	*Caloneis–Scoliopleura*	–	–	–	–	–	1–3	–	–	–	–	–
131.	*Chamaepinnularia krookii* (Grunow) Lange-Bertalot and Krammer	–	–	–	–	–	1	B	–	–	–	–

(continued)

Table 3. (continued)

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
132.	*Pinnularia acidojaponica* Idei et Kobayasi	1[a]	1–6[a]	1–3[a]	1	1	1	B	–	acf	–	–
133.	*P. acidophila* Hofmann et Krammer	–	1–6	–	1	1	–	B	–	acf	–	–
134.	*P. alpina* W. Smith	–	–	–	–	–	2	B	i	–	–	a–a
135.	*P. borealis* Ehrenberg	1	1	–	–	1	2	B	i	i	χ	a-a
136.	*P. brebissonii* (Kützing) Rabenhorst	–	–	–	–	1	–	B	i	i	α-β	b
137.	*P. major* (Kützing) Rabenhorst	1	–	–	–	–	–	B	i	acf	β	b
138.	*P. microstauron* (Ehrenberg) Cleve	1–2[a]	–	–	–	–	–	B	i	i	o	b
139.	*P. neomajor* Krammer	1	–	–	–	–	–	B	–	acf	o-χ	–
140.	*P. obscura* Krasske	1[a]	1–3	–	–	–	1	B	–	–	–	–
141.	*P. parvulissima* Krammer	–	–	–	–	–	1	B	–	–	–	–
142.	*P. rhombarea* Krammer	1	–	–	–	–	–	B	–	–	–	–
143.	*P. rupestris* Hantzsch	–	–	–	–	–	1	B	–	acf	–	–
144.	*P. stomatophora* (Grunow) Cleve	1	–	–	–	–	–	B	–	–	–	–
145.	*P. subgibba* Krammer var. *undulata* Krammer	1[a]	–	–	–	–	–	B	–	–	o	–
146.	*P. subrupestris* Krammer	–	1	–	–	–	1	B	–	–	–	–
147.	*P. viridiformis* Krammer	1[a]	1	–	–	–	1[a]	B	–	–	–	–
148.	*P. viridiformis* Krammer morphotype 2	–	–	–	–	–	1	B	–	acf	–	–
149.	*P. viridis* (Nitzsch) Ehrenberg	–	1	–	–	–	–	B	i	i	β	b
	Diploneidaceae											
150.	*Diploneis elliptica* (Kützing) Cleve	1–2[a]	1	–	–	–	1	B	i	alf	o	k
151.	*D. interrupta* (Kützing) Cleve	–	–	–	–	–	1–2	B	mh	i	–	k
152.	*D. oblongella* (Naegeli ex Kützing) Ross	–	–	–	–	–	1	B	i	alf	o–α	k

153.	*D. ovalis* (Hilse) Cleve	1[a]	1	–	–	–	1	B	hl	alf	β	b
154.	*D. pseudovalis* Hustedt	–	–	–	–	–	1	B	mh	alf	–	b
155.	*D. smithii* (Brébisson) Cleve var. *smithii*	–	–	–	–	–	1	B	mh	alf	–	k
156.	*D. smithii* var. *rhombica* Mereschk. Naviculaceae	–	–	–	–	–	1	B	hl	–	–	–
157.	*Navicula avenacea* (Brébisson et Godey) Brébisson	1–3[a]	1	1	1	1	–	B	i	acf	β	–
158.	*N. capitata* Ehrenberg	1	1	–	–	1	–	B	hl	alf	β-α	k
159.	*N. capitata* var. *hungarica* (Grunow) R. Ross	1	1	–	1	–	–	B	hl	alf	β	b
160.	*N. cryptocephala* Kützing	1–2[a]	1–2[a]	1	1	1[a]	1[a]	B-P	hl	alf	α	k
161.	*N. cryptotenella* Lange-Bertalot	2–4[a]	1–2[a]	–	1[a]	1	1[a]	B	i	alf	β	k
162.	*N. digitoradiata* (Gregory) Ralfs	–	–	–	–	1	1–4[a]	B	hl	alf	–	k
163.	*N. directa* (W. Smith) Ralfs	–	–	–	–	1[a]	1	B-P	mh	–	–	–
164.	*N. elegans* W. Smith	–	–	–	–	–	1	B	hl	–	–	–
165.	*N. integra* (W. Smith) Ralfs	1–2	1	–	–	–	1	B	mh	i	χ-o	a-a
166.	*N. menisculus* Schumann	1	–	–	–	–	–	B	hl	alf	β-α	k
167.	*N. peregrina* (Ehrenberg) Kützing	–	–	–	–	–	1–2[a]	B	mh	alf	–	k
168.	*N. placentula* (Ehrenberg) Grunow	–	1	–	–	–	–	B	i	alf	β	k
169.	*N. protracta* Grunow	–	–	–	–	–	1	B	hl	i	χ-β	k
170.	*N. radiosa* Kützing	1	–	–	–	1	1	B	i	i	o-β	k
171.	*N. rhynchocephala* Kützing	–	–	–	–	–	1	B	hl	–	β-α	–
172.	*N. salinarum* Grunow	–	–	–	–	–	1	B	mh	–	–	–
173.	*N. slesvicensis* Grunow	1[a]	1[a]	–	–	–	1–2[a]	B	hl	i	β	k
174.	*N. tripunctata* (O.F. Müller) Bory	–	–	–	–	–	1	B	i	i	β	k
175.	*Petroneis marina* (Ralfs) D.G. Mann Cosmioneidaceae	–	–	–	–	–	1–2	B	eu	–	–	b
176.	*Cosmioneis pusilla* (W. Smith) D.G. Mann and Stickle Pleurosigmataceae	–	–	–	–	–	1	B	hl	i	o-β	k

(continued)

Table 3. (continued)

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
177.	*Gyrosigma acuminatum* (Kützing) Rabenhorst	1	–	–	–	–	–	B	i	alb	β	b
178.	*G. attenuatum* (Kützing) Rabenh. Stauroneidaceae	–	–	–	–	–	1	B-P	i	alf	χ	k
179.	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg	–	–	–	–	–	1	B	i	i	χ-o	k
180.	*Trachyneis aspera* (Ehrenberg) Cleve Thalassiophysales Catenulaceae	–	–	–	–	–	1	B-P	mh	–	–	–
181.	*Amphora copulata* (Kützing) Schoeman et Archibald	–	–	–	–	–	1–2	B	i	alf	–	k
182.	*A. holsatica* Hustedt	–	–	–	–	–	1	B	–	–	β-α	–
183.	*A. normanii* Rabenhorst	1–2[a]	1	–	–	1[a]	1	B	hb	alf	β-α	b
184.	*A. ovalis* (Kützing) Kützing.	1[a]	1	–	–	1[a]	1[a]	B	i	alb	o-β	k
185.	*A. pediculus* (Kützing) Grunow	1–2[a]	–	–	1	–	–	B	i	alb	β	k
186.	*A. veneta* Kützing f. *capitata* E.Y. Haworth Bacillariales Bacillariaceae	5[a]	1	–	–	–	–	B	i	alb	β	b
187.	*Bacillaria paradoxa* Gmelin	–	–	–	–	1	–	P	mh	alb	β	k
188.	*B. socialis* Grunov	–	–	–	–	–	1	B-P	mh	–	–	–
189.	*Denticula kuetzingii* Grunow	1	1	–	–	1	–	B	i	alf	β	b
190.	*Hantzschia amphioxys* (Ehrenberg) Grunow	1	1	–	–	1	1	B	i	alf	α	k
191.	*H. distinctepunctata* (Hustedt) Hustedt	–	2	–	–	–	–	B	i	–	–	k

192.	*H. marina* (Donkin) Cleve in Cleve and Grunov	–	1	–	–	–	–	B	hl	–	–	–
193.	*Neodenticula seminae* (Simonsen and Kanaya) Akiba and Yanagisawa	–	–	–	–	–	1	–	–	–	–	–
194.	*Nitzshia amphibia* Grunow	–	–	–	–	–	1–2	B-P	i	alf	o	k
195.	*N. aurariae* Cholnoky	–	–	–	–	2–6[a]	–	B	i	–	–	k
196.	*N. brevissima* Grunov	1	–	–	–	–	–	B	hl	i	o-β	k
197.	*N. capitellata* Hustedt	2–6[a]	1–6[a]	–	–	–	–	B	i	alb	o	k
198.	*N. communis* Rabenhorst	–	–	–	–	–	1	B-P	i	alf	o	k
199.	*N. compressa* (Bailey) Boyer var. *elongata* (Grunow) Lange-Bertalot	–	–	–	–	–	1	B	hl	–	–	k
200.	*N. dissipata* (Kützing) Grunow	1[a]	1[a]	–	–	1	1[a]	B	i	alf	o-β	b
201.	*N. fonticola* Grunov	1–2[a]	1–2[a]	–	1	1	1	B	i	alf	o	b
202.	*N. frustulum* (Kützing) Grunow	1–3[a]	–	–	–	–	1[a]	B	hl	alb	o	k
203.	*N. linearis* W. Smith	1–2[a]	1[a]	–	–	1	–	B	i	i	o	b
204.	*N. nana* Grunow	2–6[a]	1–4[a]	1[a]	1	1	1–3[a]	B	mh	–	–	b
205.	*N. palea* (Kützing) W. Smith	3–5[a]	1–6[a]	1	2[a]	1	–	B	i	i	α	k
206.	*N. paleacea* (Grunow) Grunow	2[a]	1[a]	1[a]	1[a]	–	–	P	i	alf	o-β	k
207.	*N. pellucida* Grunow	–	–	–	–	1	–	B	hl	–	–	k
208.	*N. recta* Hantsch	1	–	–	–	–	–	B	i	alf	α-β	b
209.	*N. sigma* (Kützing) W. Smith	–	–	–	–	–	1	B	–	–	–	k
210.	*N. thermaloides* Hustedt	–	–	1	–	4[a]	–	–	hl	–	–	–
211.	*N. vermicularis* (Kützing) Hantzsch	–	–	–	–	1	–	B	i	alf	β	k
212.	*Thalassionema nitzschioides* (Grunow) Van Heurck	–	–	–	–	–	1	B-P	–	–	–	–
213.	*Tryblionella apiculata* Gregory	1–6[a]	1	–	–	–	1–5[a]	B	mh	alf	β	k
214.	*T. granulata* (Grunow) D.G. Mann	–	–	–	–	–	1	B-P	–	–	–	–
215.	*T. hungarica* (Grunow) D.G. Mann	–	–	–	–	–	1	B-P	mh	alf	α-β	k
216.	*T. levidensis* W. Smith	–	1	–	–	–	1–2	B	hl	alf	α	b
217.	*T. marginulata* (Grunow) D.G. Mann	1	–	–	–	–	–	–	hl	–	–	–

(continued)

Table 3. (continued)

№	Algal taxa	Kunashir			Shiashkotan	Yankicha	Sakhalin	Ecological–geographical characteristic				
		Stolbchaty Cape	Alekhin Bay	Golovnin Volcano				B	H	pH	S	G
218.	*T. punctata* W. Smith Rhopalodiales Rhopalodiaceae	–	–	–	–	–	1	B	mh	–	–	k
219.	*Epithemia adnata* (Kützing) Brébisson	–	–	–	–	–	1	B	i	alb	β-α	k
220.	*Epithemia adnata* var. *porcellus* (Kützing) R. Ross	1	–	–	–	–	1	B	i	alb	β	k
221.	*E. sorex* Kützing	–	–	–	–	–	1	B	i	alf	o-α	k
222.	*Rhopalodia acuminata* Krammer	1–2[a]	1	–	–	–	1	B	hl	–	–	–
223.	*Rh. gibba* (Ehrenberg) O. Müller	–	–	–	–	–	1	B	i	alb	χ-o	k
224.	*Rh. gibberula* (Ehrenberg) O. Müller	–	–	–	–	–	1	B	mh	i	–	k
225.	*Rh. musculus* (Kützing) O. Müller	–	–	–	–	–	1	B-P	mh	alb	–	k
226.	*Rh. operculata* (Agardh) Hakansson	–	–	–	–	–	1	B	–	–	–	–
227.	*Rh. rupestris* (W. Smith) Krammer Surirellales Surirellaceae	–	1	–	–	–	–	B	–	–	o	a-a
228.	*Surirella angusta* Kützing	1	1	–	–	1	–	B	i	alf	β	k
229.	*S. brebissonii* Krammer et Lange-Bertalot	–	1	–	–	1	–	B	i	i	β	k
230.	*Surirella brebissonii* Krammer et Lange-Bertalot var. *kuetzingii* Krammer et Lange-Bertalot	–	–	–	–	–	2	B	–	–	β-α	–

231.	*S. minuta* Brébisson	1	–	–	–	–	–	B	i	alf	–	b
	Rhaphoneidales											
	Rhaphoneidaceae											
232.	*Delphineis surirella* (Ehrenberg) G. Andrews	–	–	–	–	–	1	–	–	–	–	–

To estimate the frequency of taxa occurrence at the stations, we used the six-point scale: 1 – solitary (one to five cells in the slide); 2 – rare (10–15 cells in the slide); 3 – not infrequent (25–30 cells in the slide); 4 – frequent (one cell in each row of the cover glass at magnification with immersion); 5 – very frequent (several cells under the same conditions); 6 – in bulk (several cells in each visual field under the same conditions) (Korde, 1956)

B (biotope): P – planktonic, B–P – benthic–planktonic, B – benthic, E – epiphytic, B–E – benthic–epiphytic. H (relation to salinity): eu – euhalobic, mh – mesohalobic, hl – halophilous, hb – halophobic, i – indifferent. pH (relation to pH of water): alf – alkaliphilous, alb – alkalibiontic, acf – acidophilous, i – indifferent. S (relation to saprobity of water): χ – xenosaprobous, χ-o – xeno-oligosaprobous, o-χ – oligo-xenosaprobous, χ-β – xeno-betamesosaprobous, o – oligosaprobous, o-β – oligo-betamesosaprobous, β – betamesosaprobous, o-α – oligo-alphamesosaprobous, β-α – beta-alphamesosaprobous, α-β – alpha-betamesosaprobous, α – alphamesosaprobous, ρ – polysaprobous. "–" – data absent. G (geographical distribution): a-a – arctic-alpine, b – boreal, k – cosmopolitan

[a]High guarantee of occurrence of cells in an alive status

and Bacillariophyceae (Table 2). The genera *Navicula*, *Pinnularia*, and *Nitzschia* have a leading place in diatom flora and contain 13, 9, and 8 taxa, respectively.

Diatom communities of periphyton and plankton have similar composition of species, but *Gomphonema angustatum*, *Anomoeoneis sphaerophora*, and *Tryblionella apiculata were* prevalent in periphyton samples and *Achnanthes hungarica* was relatively abundant in the plankton samples. The algal flora of Sakhalin hydrothermal springs is represented by common rheophilic diatoms, indifferent to salinity (e.g., *Hannaea arcus*, *Gomphonema parvulum*, *Didymosphenia geminata*, *Synedra ulna*, *Encyonema silesiacum*, *Planothidium lanceolatum*), but the number of maritime and brackish taxa is considerable (Table 3).

4. General Observations and Conclusions

Ten Sakhalin and Kuril Islands hot springs and reservoirs were investigated and 232 species, varieties, and forms of diatoms were discovered in them. Diatoms *Synedra ulna*, *Achnanthes hungarica*, *Planothidium lanceolatum*, *Rhoicosphenia abbreviata*, *Gomphonema angustatum*, *G. parvulum*, *Navicula slesvicensis*, *Pinnularia acidophila*, *P. acidojaponica*, *Anomoeoneis sphaerophora*, *Nitzschia capitellata*, *N. nana*, *N. palea*, and *Tryblionella apiculata* were dominant or more abundant in communities in different sampling sites with temperature from 24°C to 71°C.

Diatom communities investigated springs and reservoirs included from 23 (25) to 101 species (107 intraspecific taxa) (Table 3). The largest number of taxa is recorded for diatom communities in springs and reservoirs with water temperature from 37°C to 60°C (75–107 taxa). Hot springs with temperature above or below this interval had less number of taxa – from 25 to 59. Algal taxa with high guarantee of occurrence of cells in an alive status were recorded. An alive alga accounted a third part from all compositions of hot springs algal flora and represents 77 species, varieties, and forms (Table 3), and all frequent and bulk algae were composed in this number.

Kamchatka Peninsula is located geographically close to the Kuril and Sakhalin Islands, and also has a many hot springs. The algal flora of Kamchatka hot springs was investigated over a 100 years, by many authors: Schmidt, 1875–1959, 1885; Gutwinski, 1891; Cleve, 1894–1895; Elenkin, 1914; Petersen, 1946. Results of recent studies of diatoms from Kamtschatka hot springs described in the paper of Japanese and Russian authors (Yoshitake et al., 2008). Yoshitake with coauthors investigated algal flora of five hot springs from Kamchatka with water temperature from 18.0°C to 71.5°C and recorded 39 alive intraspecific taxa. Seven of them are dominant in the spring's algal communities: *Achnantes exigua*, *Amphora veneta*, *Caloneis bacillum*, *Neidium ampliatum*, *Nitzschia amphibia*, *N. frustulum*, and *Pinnularia marchica* Ilka Schönfelder.

Hot springs algal floras of Kamchatka and Kuril alongwith the Sakhalin Islands have great number of common species, but different dominants or more abundant in communities species. There were alive diatoms in common with hot

Table 4. Distribution of hot springs algae of Kuril and Sakhalin Islands into ecological and geographical groups.

	Kuril Islands		Sakhalin	
Ecological–geographical group	**Number of taxa**	**Percent**	**Number of taxa**	**Percent**
Biotop				
Benthic	131	80.9	99	72.3
Planktonic	7	4.3	8	5.8
Benthic–planktonic	17	10.5	17	12.4
Epiphytic	1	0.6	1	0.7
Benthic–epiphytic	2	1.2	2	1.5
No data	4	2.5	10	7.3
Total	162	100	137	100
Relation to salinity of water				
Euhalobic	–	–	1	0.7
Mesohalobic	12	7.4	25	18.3
Halophilic	23	14.2	20	14.6
Indifferent	88	54.3	54	39.4
Halophobic	17	10.5	8	5.8
No data	22	13.6	29	21.2
Total	162	100	137	100
Relation to pH of water				
Alkalibiontic	11	6.8	11	8.0
Acidophilous	18	11.1	50	36.5
Indifferent	27	16.7	23	16.8
Acidophilous	71	43.8	9	6.6
No data	35	21.6	44	32.1
Total	162	100	137	100
Relation to saprobity of water				
Xenosaprobous (χ, χ-o, o-χ)	21	13.0	11	8.0
Oligosaprobous (χ-β, o, o-β)	39	24.1	26	19.0
Beta-mesosaprobous (β-o, o-α, β, β-α)	44	27.2	35	25.6
Alphamesosaprobous (α-β, β-ρ, α, α-ρ)	9	5.5	8	5.8
Polysaprobous (ρ–α, ρ)	–	–	1	0.7
No data	49	30.2	56	40.9
Total	162	100	137	100
Geographical distribution				
Cosmopolitan	66	40.7	60	43.8
Boreal	43	26.5	27	19.7
Arctic-alpine	20	12.4	11	8.0
No data	33	20.4	39	28.5
Total	162	100	137	100

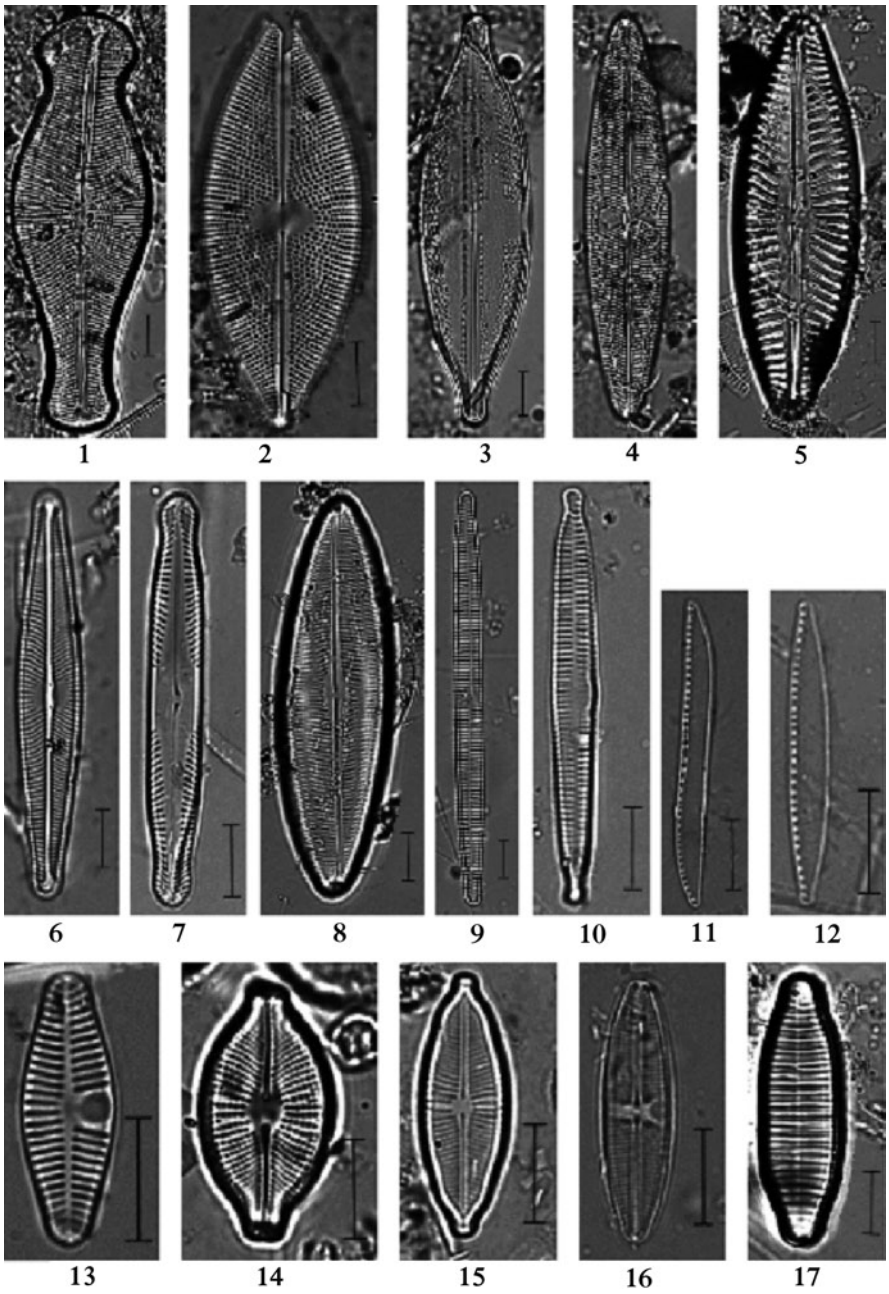
springs from Kamchatka, Kuril, and Sakhalin Islands: *Melosira varians*, *Hannaea arcus* var. *linearis* f. *recta*, *Diatoma mesodon*, *Eunotia bilunaris*, *Rhoicosphenia abbreviata*, *Encyonema silesiacum*, *Gomphonema parvulum*, *Achnanthes lanceolata*, *Cocconeis placentula*, *Frustulia rhomboides*, *Navicula cryptocephala*, *Nitzshia frustulum*, *N. nana*, and *N. palea.* It should be noted that all these species are typical representatives of coldwater springs, rivers, and lakes of Kuril and Sakhalin Islands (Nikulina, 2002, 2004, 2005, 2008).

Our research results indicate that with rise in temperature of water of reservoirs above 70°C, the diatom species composition becomes impoverished. However, a few species remain in the elevated temperatures and actively develop and even form masses. Bascially, it would appear that the vegetation of thermal springs consists of algae of cold waters which have adapted to high temperatures. Diatoms such as *Pinnularia acidojaponica*, *P. acidophila*, and *Nitzschia thermaloides* are true thermophilic species or characteristic representatives of hot waters. They were not registered in Kuril and Sakhalin rivers and streams with cold water.

The diatom flora analysis of the thermal springs from these three Kuril Islands has shown that information on algae being confined to particular habitats is known for 158 taxa or 97.5% of the general number of species, varieties, and forms recorded in the studied area. Most of the algae present are benthic and benthic–planktonic species, i.e., 80.9% and 10.5%, respectively (Table 4). Data on salinity preferences are known for 140 species, varieties, and forms (86.4% of the general number) of algae. The algae indifferent to salinity changes (they comprise 54.3%) and halophilic (13.3% of the total number of taxa) are the most numerous groups (Table 4). Data on pH preferences are known for 78.4% of species, varieties, and forms of algae recorded here, among those alkaliphilic species prevail (43.8%). Indicators of water saprobity are known for 113 taxa of algae or 69.8% of the general number of taxa in the algal flora of the studied area. Oligosaprobous and betamesosaprobous are the most abundant groups; they comprise 24.0% and 27.2%, respectively, of the taxa present. The share of the rest of the known saprobic groups makes up 18.5% (Table 4). Geographical distribution is known for 129 species, varieties, and forms, which comprises 79.6% of general number of algae. The share of widely distributed or cosmopolitan species is 40.7%, boreal 24.4%, and arctic-alpine 12.4% (Table 4).

Sakhalin hot springs diatom flora is characterized by prevalent benthic, indifferent to salinity, acidophilous and betamesosaprobous species (Figs. 1–17).

Figures 1–17. Figure 1 *Didymosphenia geminata* (Lyngbye) M. Schmidt. Figure 2. *Petroneis marina* (Ralfs) D.G. Mann. Figure 3. *Anomoeoneis sphaerophora* (Ehrenberg) Pfitzer. Figure 4. *Trachyneis aspera* (Ehrenberg) Cleve. Figure 5. *Pinnularia alpina* W. Smith. Figure 6. *Navicula avenacea* (Brébisson et Godey) Brébisson. Figure 7. *Pinnularia acidojaponica* Idei et Kobayasi. Figure 8. *Scolioneis* sp. or *Caloneis* sp. Figure 9. *Synedra ulna* (Nitzsch) Ehrenberg. Figure 10. *Hannaea arcus* var. *linearis* f. *recta* (Cleve) Foged. Figure 11. *Nitzcshia nana* Grunow. Figure 12. *N. palea* (Kützing) W. Smith. Figure 13. *Achnanthes lanceolata* Brébisson ex Kützing. Figure 14. *Cosmioneis pusilla* (W. Smith) D.G. Mann & Stickle. Figure 15. *Navicula integra* (W. Smith) Ralfs. Figure 16. *Achnanthes hungarica* Grunow. Figure 17. *Diatoma vulgare* Bory. Scale bars = 10 µm.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5. Acknowledgments

The authors are very thankful to Dr. N.A. Eremenko (State Nature Reserve "Kurilsky") and Dr. S.V. Shedko (Institute of Biology and Soil Sciences FEB RAS) for the help with sampling the algae, Dr. M.V. Cherepanova (Institute of Biology and Soil Sciences FEB RAS) and A.V. Artemova (Institute of Oceanology FEB RAS) for consultation of identification of marine diatoms, and Dr. R.V. Zharkov (Institute of Marine Geology and Geophysics FEB RAS) for putting the information about the chemistry of thermal springs at our disposal.

The work was supported by International Program Division of the U.S. National Science Foundation, grant numbers DEB-9400821, DEB-9505031, and DEB-00716556(Prof. Theodore W. Pietsch, principal investigator) and supported in part by grant Far East Branch of Russian Academy of Science 09-III-A-06-179 (Dr. Tatiana V. Nikulina, principal investigator).

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 365–384
DOI 10.1007/978-94-007-1327-7_16, © Springer Science+Business Media B.V. 2011

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LIVING ON THE PH EDGE: DIATOM ASSEMBLAGES OF LOW-PH LAKES IN WESTERN POMERANIA (NW POLAND)

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1. Introduction

To survive in extreme, i.e. stressed, environments, organisms must evolve adaptations to at least one stress-causing factor. Low water pH (below, or much below, 7.0) results from a water body lacking or losing the capacity to buffer the presence or input of acid(s) (e.g. Oliver and Kelso, 1983) and is a cause of a major environmental stress. To overcome acid stress requires specific metabolic and physiological adaptations that relatively few species only have been able to develop (Gross, 2000; Toulmond, 1991). Therefore, acidic and acidified water bodies are characterised by low biological diversity (Stokes, 1986 and the references therein).

Acidification of freshwater reservoirs is regarded as a serious environmental problem (e.g. Dixit and Smol, 1989 and the references therein; Battarbee et al., 1999 and the references therein; van Dam and Mertens, 2006). Although occurring in the past (e.g. Battarbee et al., 1999; Smol, 2002), it has become exacerbated by relatively recent human activities (e.g. Flower and Battarbee, 1983; Guilizzoni et al., 1992). However, prevalence of a low pH in a water body can be a natural state. The low water pH in naturally acidic water bodies (NAWB) is brought about naturally by high concentrations of organic acids (Collier et al., 1990; Outridge et al., 1989), like in lakes and pools situated within a peatbog or ombrotrophic mire (e.g. Wheeler, 1993). Such water bodies are usually shallow, poor in plant nutrients, with water stained dark due to exposure to peat, and with high dissolved organic matter content (Schalles and Shure, 1989). The fringe vegetation is typically dominated by bog mosses (*Sphagnum* spp.) in association with a low number of other species. Due to the specific nature of their communities and because of many important ecosystem services they provide (e.g. water retention, carbon sink, wildlife refuge; Barkham, 1993), such ecosystems account for the value of wetlands, increasingly threatened by man's various activities (Joosten and Clarke, 2002).

Knowledge of aquatic communities in NAWBs provides information of both scientific and applied interest. Research on NAWB communities helps to elucidate questions of physiological tolerance of and adaptation to acid stress, to explain small- (lake gradient; Rauch et al., 2006) and large-scale (e.g. regional; McNicol et al., 1998; Snucins et al., 2001; Kwandrans, 2007) effects, including differences in biological diversity, and to form predictions as to how non-acidic habitats and the communities they support might change when stressed by anthropogenic acidification. More broadly stated, studies on NAWB aquatic communities contribute to developing a conceptual framework in which to consider natural versus anthropogenic acidification as a means of assisting management of valuable wetland habitats such as peatlands.

Diatoms have a long history of use as pH indicators, their utility being proven in reconstruction of the timing and extent of lake acidification in the past and in research on NAWB (Battarbee et al., 1999; Buczko, 2006; Johansen, 1999; Kwandrans, 2007). This work is a part of a comprehensive study undertaken to collect information on taxonomic composition and structure of NAWB diatom assemblages as exemplified by peatbog pools and lakes in Western Pomerania (NW Poland), a region known for abundance of such natural wetlands. This information is the requisite first step towards building a taxonomic and ecological reference database to be used as a management tool in conservation and protection of the wetland habitats. In this work, we explore the composition and structure of diatom assemblages in two NAWBs and compare them with those of a non-NAWB in the area.

2. Area of Study

The study was carried out in three water bodies located in Western Pomerania (NW Poland; Fig. 1); the two NAWBs were Lake Kąpielowe (LK) and Lake Żółwia Błoć (ZB), Lake Piaski (LP)being a non-NAWB. The general characteristics of the lakes are summarised in Table 1.

Lake Piaski (LP), although non-acidic itself, is surrounded by plant assemblages representing a peatbog succession series which includes also species typical of alkalitrophic habitats, particularly at the interface with the open lake water. The peatbog in the vicinity of the lake is a floristic reserve.

Lake Kąpielowe (LK) is one of two small lakes that emerged after inundation of carriers in a former peat dig; it is located within an extensive mid-forest raised bog at the final stage of plant succession. The aquatic vegetation is formed by small patches of water lily associations (*Nymphaetum candidae* and *Nupharo-Nymphaetum albae*); vegetation complexes encroaching onto the lake are characteristic of oligotrophic systems undergoing terrestrialisation (*Caricetum lasicarpae*, *Eriophoro angustifolio-Sphagnetum*, *Ledo-Sphagnetum*, *Sphagnetum pinetosum* and *Sphagnetum betuletosum*).

Lake Żółwia Błoć (ZB) is surrounded by a carpet bog of the *Eriophoro angustifolio-Sphagnetum recurvi* type as well as by *Sphagnum* moss associations

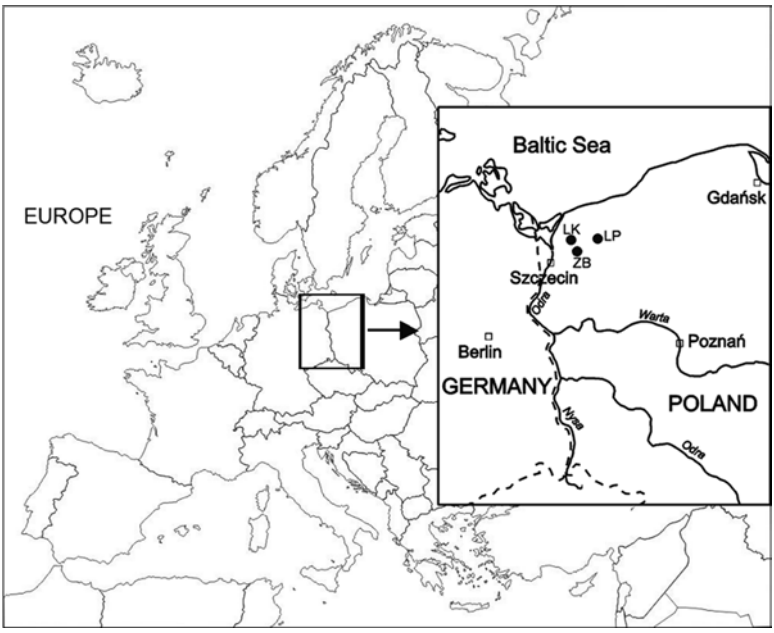

Figure 1. Location of the water bodies studied.

Table 1. General characteristics of the lakes studied.

Lake	Geographic location		Area (ha)	Sampling periods	Conservation status
	Latitude	Longitude			
Piaski (LP)	53°41′35″N	15°27′11″E	4.1	April, July, September, November 2003	Floristic reserve
Kąpielowe (LK)	53°42.868′N	14°57.593′E	1.8	April, July, September, November 2003	None
Żółwia Błoć (ZB)	53°38.113′N	014°52.559′E	1.5	April, July, September, November 2003	Planned peatbog reserve

(dominated by *S. cuspidatum*) typical of the initial stage of bog terrestrialisation; it is a site of a planned peatbog reserve.

3. Materials and Methods

The lakes were surveyed seasonally (spring, summer, autumn) in 2003 (Table 1). During each survey, water for diatom analyses was collected from the littoral of each lake into 50-ml bottles; bryophytic diatoms were isolated from water squeezed out from a moss clump into a container.

Table 2. Ranges of major environmental parameters recorded in the lakes under study.

Lake	Temp (°C)	Conductivity (µS/cm)	pH	Dissolved organic C (mg/dm^3)	Nitrate N (mg/dm^3)	Ammonia N (mg/dm^3)	Dissolved P (µg/dm^3)	Dissolved Si (mg/dm^3)
LP	6.0–18.1	74–386	4.0–8.2	0.1–13.5	0.009–0.03	0.01–0.32	6–36	2.94–7.99
LK	6.1–21.0	81–173	3.8–4.8	2.3–11.5	0.009–0.06	0.02–0.51	2–40	1.20–6.28
ZB	6.0–21.5	20–96	3.6–4.6	4.5–12.9	0.009–0.06	0.02–0.58	10–60	0.03–2.98

Each collection was accompanied by measurements of relevant environmental parameters, including water pH, temperature, conductivity and dissolved oxygen content (WTW probes); water samples were also collected for laboratory assays, i.e. dissolved organic carbon (IR CO_2 detection after catalytic combustion; TOC 500, Shimadzu), nitrate nitrogen (ion-exchange chromatography; Shimadzu), dissolved phosphorus (photometry with molybdenum blue after digestion of filtered samples; CARY 300, Varian), ammonia nitrogen (photometry using indophenol technique; Flow Solution III, Perstorp) and dissolved silica (heteropoly acid photometry with molybdenum blue; UV 2101 photometer, Shimadzu).

In the laboratory, diatoms from water and moss water samples were mounted in a standard manner (Battarbee, 1986), viewed with light and SEM microscopy, and identified. On each slide examined, more than 300 diatom valves were counted.

Environmental preferences of diatom species were based on the relevant database contained in the OMNIDIA ver.3 software (Lecointe et al., 1993, 1999).

Numerical treatment of environmental and diatom data (all diatom species included) involved a number of multivariate techniques: the principal components analysis (PCA) was conducted on non-transformed environmental data (listed in Table 2) to identify the factors responsible for differences in the abiotic regimes of the lake. The analysis was performed using the Statistica v.6 software (Statistica Poland). The ENV module of the PRIMER v. 6 software package (Carr, 1996; Clarke and Warwick, 1994) was used to identify the environmental parameters which formed the strongest correlations with the structure of diatom communities in each lake. The degree of dissimilarity between the taxonomic composition of diatom floras in the three lakes was assessed by means of the PRIMER's non-metric multidimensional scaling (MDS) module. The analysis was performed on the presence–absence and log-transformed relative abundance data of those taxa supplying >3% to the total valve count in a sample (Kwandrans and Eloranta, 2005). The diatom taxa primarily responsible for the dissimilarities were identified using the PRIMER's SIMPER module.

4. Results

In terms of the environmental parameters of interest for this study (summarised in Table 2), LP proved markedly different from both LK and ZB, mainly in having wider pH and conductivity ranges. While the pH range in the non-NAWB

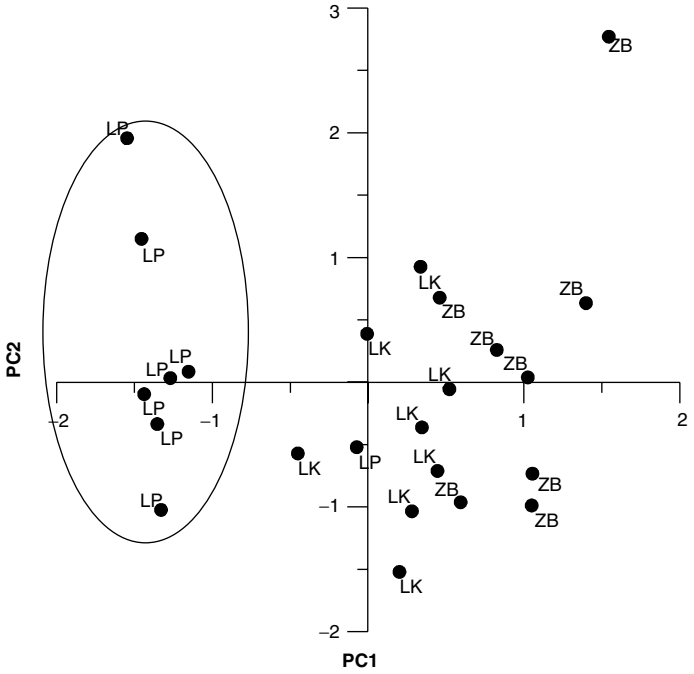

Figure 2. PCA plot (note the separation of LP along the first PCA axis).

extended well into the alkaline part of the values on most sampling occasions, it was consistently below 5.0 in the two NAWBs. The upper limit of the conductivity range was much higher in LP than in either LK or ZB; the lower conductivity limit was similar in LP and LK, but the entire ZB conductivity range was located much lower on the scale. ZB featured the widest dissolved P range, its dissolved Si contents, however, being much lower than those in LK or LP. Consistently with those findings, the PCA plot (Fig. 2) shows a clear separation of LP from both LK and ZB along the first principal component axis (ca. 34% of total variance explained; Table 3), formed primarily by a combination of conductivity, pH and dissolved Si. Conductivity and pH had the highest loadings (eigenvectors) on the first principal component axis and are therefore regarded as being a major cause of habitat differences between NAWBs and non-NAWBs in this study. The second principal component explained 20.63% of the total variance and involved mainly ammonia N (Table 3), thus reflecting primarily the strong seasonal variability of that parameter in all the three lakes.

The diatom floras of the three lakes comprised a total of 225 taxa (Table 4).

The diatom flora in the non-NAWB consisted of a total of 216 taxa. The two NAWBs, taken together, supported a total of 19 taxa, but differed somewhat in the taxonomic richness (15 and 11 taxa in LK and ZB, respectively). The taxonomic richness of the LP flora varied widely from season to season (from 71 taxa in November to 126 in September) due to temporal succession, the variability

Table 3. Eigenvalues and eigenvectors of environmental variables produced by PCA.

Principal component	Eigenvalue	% Total variance explained	Contributing environmental variables (eigenvectors in parentheses)
PC1	3.0449	33.84	Conductivity (−0.9175); pH (−0.82937); dissolved Si (−0.7145)
PC2	1.8571	20.63	NH_4–N (0.7809)

Table 4. List of diatom taxa identified in the lakes studied.

Lake	Diatom taxa
LP	*Achnanthes conspicua*, *A. exigua*, *A. linearioides*, *Achnanthidium* sp., *A. minutissimum*, *A. petersenii*, *A. ziegleri*, *Adlafia* sp., *A. bryophila*, *Amphlipleura pellucida*, *Amphora calumetica*, *A. copulata*, *A. inariensis*, *A. pediculus*, *Aneumastus tusculus*, *Asterionella* sp., *Brachysira neoexilis*, *Caloneis* sp., *C. lancettula*, *C. tenuis*, *Chamaepinnularia gandrupii*, *C. mediocris*, *Cocconeis neodiminuta*, *C. placentula* var. *placentula*, *Craticula cuspidata*, *Cyclotella cyclopuncta*, *C. ocellata*, *C. radiosa*, *Cymatopleura solea*, *Cymbella affinis*, *C. aspera*, *C.* cf. *cuspidata*, *C.* cf. *cymbiformis*, *C.* cf. *parva*, *C. excisa*, *C. helmckei*, *C. laevis*, *C. lanceolata*, *C. langebertalotii*, *C. neoleptoceros*, *C. neocistula*, *C. parva*, *C. proxima*, *Cymbopleura ehrenbergii*, *C. lata*, *C. naviculiformis*, *Denticula tenuis*, *Diploneis oculata*, *Encyonema* sp., *E. caespitosum*, *E. minutum*, *E. silesiacum*, *E. ventricosum*, *Encyonopsis cesatii*, *E. microcephala*, *Eolimna minima*, *Epithemia adnata*, *E. sorex*, *Eucocconeis flexella*, *Eunotia* sp., *E. arcubus*, *E. arcus*, *E. bilunaris* var. *bilunaris*, *E. bilunaris* var. *linearis*, *E. bilunaris* var. *mucophila*, *E. botuliformis*, *E.* cf. *intermedia*, *E.* cf. *minor*, *E. exigua*, *E. glacialifalsa*, *E. implicata*, *E. minor*, *E. paludosa*, *E. rhomboidea*, *E. seminulum*, *E. steineckei*, *Fragilaria* sp., *F. amphicephala*, *F. arcus*, *F. biceps*, *F. brevistriata*, *F. capucina* var. *capucina*, *F. capucina* var. *distans*, *F. capucina* var. *gracilis*, *F.* cf. *austriaca*, *F.* cf. *brevistriata*, *F.* cf. *construens*, *F.* cf. *dilatata*, *F.* cf. *gracilis*, *F.* cf. *polonica*, *F.* cf. *tenera*, *F.* cf. *ulna*, *F. construens*, *F. construens* f. *venter*, *F. crotonensis*, *F. danica*, *F. delicatissima*, *F. dilatata*, *F. lapponica*, *F. nanana*, *F. parasitica*, *F. pinnata*, *F. tenera*, *F. ulna*, *Frustulia saxonica*, *Geissleria acceptata*, *G. schoenfeldii*, *Gomphonema* sp., *G. acuminatum*, *G. angustatum*, *G. angusticephalum*, *G. auritum*, *G. brebissonii*, *G. calcareum*, *G. capitatum*, *G.* cf. *exilissimum*, *G.* cf. *parvulum*, *G.* cf. *pumilum*, *G. clavatum*, *G. constrictum*, *G. coronatum*, *G. dichotomum*, *G. hebridense*, *G. insigne*, *G. insigniforme*, *G. lateripunctatum*, *G. minutum*, *G. parvulum* var. *parvulum* f. *parvulum*, *G. pumilum*, *G. sarcophagus*, *G. truncatum*, *G. vibrio*, *Karayevia clevei*, *Kobayasiella parasubtilissima*, *Mastogloia lacustris*, *Navicula absoluta*, *N. aquaedurae*, *N. cari*, *N.* cf. *libonensis*, *N. concentrica*, *N. cryptotenella*, *N. cryptotenelloides*, *N. densilineolata*, *N. diluviana*, *N. geisslerae*, *N. hofmanniae*, *N. laterostrata*, *N. obdurata*, *N. oblonga*, *N. oligotraphenta*, *N. praeterita*, *N. pseudoventralis*, *N. radiosa*, *N. raederiae*, *N. reichardtiana*, *N. seibigiana*, *N. seminulum*, *N. subalpina*, *N. tripunctata*, *N. utermoehlii*, *N. veneta*, *N. verecundiae*, *N. vitrea*, *N. wiesneri*, *N. wildii*, *Neidium ampliatum*, *N. dubium*, *Nitzschia* sp., *N. acidoclinata*, *N. amphibia* f. *amphibia*, *N. angustata*, *N.* cf. *lacuum*, *N. denticula*, *N. dissipata*, *N. gracilis*, *N. heufleriana*, *N. lacuum*, *N. palea*, *N. paleaeformis*, *N. perminuta*, *N. radicula*, *N. recta*, *N. rectirobusta*, *N. regula*, *N. sublinearis*, *N. subtilis*, *N. tenuis*, *Pinnularia* sp., *P. cleveiformis*, *P. microstauron*, *P. nodosa*, *P. notabilis*, *P. stomatophora* var. *stomatophora*, *P. subcapitata* var. *elongata*, *Placoneis placentula*, *Planothidium delicatulum*, *P. frequentissimum*, *P. nodosum*, *P. rostratum*, *Punctastriata* sp., *P. linearis*, *Rhopalodia gibba*, *R. parallela*, *R. ventricosa*, *Rossithidium petersenii*, *Sellaphora* cf. *pupula*, *S. laevissima*, *S. stroemii*, *Stauroneis* sp., *Stauroneis kriegeri*, *S. leguminopsis*, *S. siberica*, *Staurosira zeilleri* var. *eliptica*, *Tabellaria fenestrata*, *T. flocculosa*

(continued)

Table 4. (continued)

Lake	Diatom taxa
LK	*Aulacoseira granulata*, *Eunotia bilunaris* var. *bilunaris*, *E. bilunaris* var. *mucophila*, *E. exigua*, *E. genuflexa*, *E. paludosa*, *Frustulia* cf. *crassinervia*, *F. saxonica*, *Nitzschia gracilis*, *N. paleaeformis*, *Pinnularia* sp., *P. rupestris*, *P. subcapitata* var. *elongata*, *Pinnularia subgibba*, *Tabellaria flocculosa*
ZB	*Cocconeis pediculus*, *Eunotia bilunaris* var. *bilunaris*, *E. exigua*, *E. genuflexa*, *E. incisa* var. *incisa*, *E. meisteri*, *E. paludosa*, *Frustulia* cf. *crassinervia*, *F. crassinervia*, *F. saxonica*, *Pinnularia subcapitata* var. *elongata*

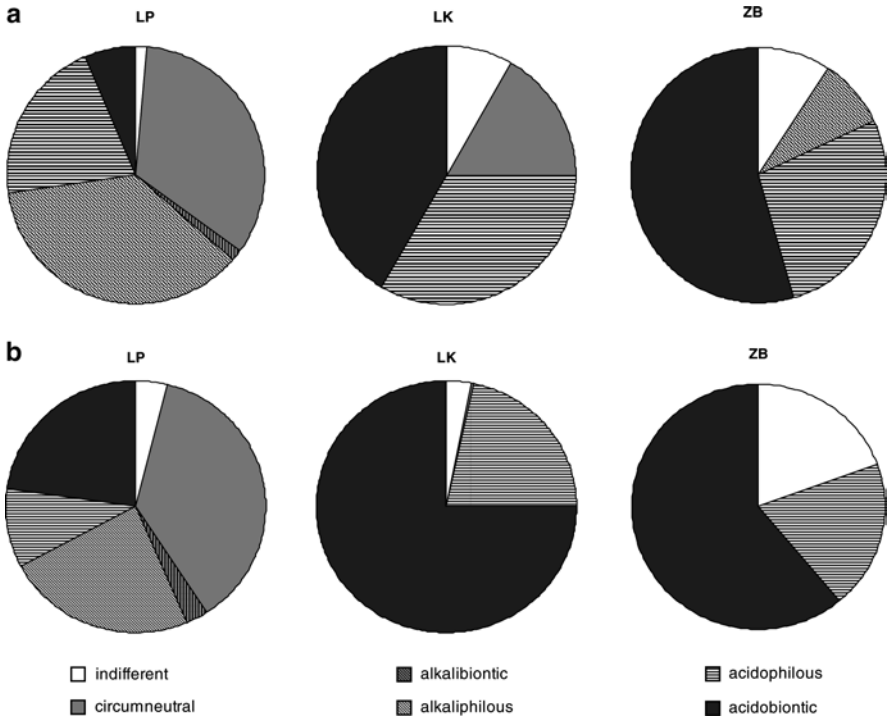

Figure 3. Composition of diatom floras in the lakes studied (pH adaptation type): (**a**) Number of taxa; (**b**) relative abundance (relative abundances of circumneutral taxa in LK and alkaliphilous in ZB were too low to be visible on the graphs).

contrasting with the consistently low, season-independent number of species in LK and, particularly, ZB (from 5 taxa in September and November to 8 in July) (A. Witkowski et al., 2005).

The LP diatoms were, overall, dominated by *Achnanthidium minutissimum*, a common neutrophilous species, although, due to bryophytes fringing the lake, acidophilous, and even acidobiontic, taxa were present as well (Fig. 3).

Table 5. Correlations (multivariate Spearman's correlation coefficients; BIOENV procedure) between diatom assemblage structure and environmental variables.

Lake	Variable	Correlation coefficient
LP	Conductivity	0.588
	Conductivity + dissolved silica + dissolved organic carbon	0.564
	Dissolved silica + dissolved organic carbon + pH	0.542
LK	pH + conductivity	0.730
	Dissolved organic carbon + pH + conductivity	0.706
	Dissolved silica + dissolved organic carbon + pH + conductivity	0.705
ZB	Dissolved phosphorus + ammonia nitrogen + dissolved organic carbon	0.543
	Dissolved phosphorus + ammonia nitrogen + nitrate nitrogen + dissolved organic carbon + conductivity	0.539

The dominants in LK and ZB were typical pH-adapted species: *Eunotia paludosa* and *Nitzschia paleaeformis* in LK and *Frustulia saxonica* and *E. bilunaris* var. *bilunaris* in ZB.

Thus, in addition to the differences between their abiotic environments and taxonomic richness, the three lakes differed markedly in terms of their dominants and pH adaptation of their diatom floras (Fig. 3). The LP assemblage was dominated – both in terms of taxonomic richness and relative abundance – by circumneutral (33.8% of the total number of taxa and 37.0% relative abundance) and alkaliphilous (36.4% and 23.5%, respectively) taxa, which were, however, accompanied by a sizable admixture (6.5% and 22.7%, respectively) of acidobiontic diatoms (Fig. 3). The two NAWBs turned out to differ in the nature of pH adaptation of their diatom assemblages: while the diatom assemblage in LK showed a strong domination (41.7% and 74.9%) of acidobionts with small admixtures of species representing other pH adaptation types, the diatom assemblage in ZB was heavily (54.5% and 61.2%) dominated by acidobionts, but a relatively high proportion of the number of taxa (9.1%) and relative abundance (19.3%) was accounted for by diatoms indifferent to water pH (Fig. 3).

Diatom composition in each of the lakes was found to be fairly highly correlated with environmental variables (Table 5). While conductivity was the major variable underlying the assemblage structure in LP, pH and conductivity were most important in LK; in ZB, the most acidic of the lakes, a combination of dissolved phosphorus, ammonia nitrogen, and dissolved organic matter proved most important for the diatom assemblage structure.

The degree of dissimilarity between the diatom floras of the three lakes is shown in Fig. 4 and Table 6.

The diatom assemblage in LP was clearly divergent from the assemblages in LK and ZB: the similarity of the LP diatom flora with either of the NAWBs did not exceed 12% (Table 5), the low similarity being brought about by the vast difference in the number of taxa, including the non-pH adapted ones. Taken together, Tables 4 and 6 show a limited overlap between the diatom assemblages in the three

Table 6. Level (%) of taxonomic dissimilarity between the assemblages studied, including taxa and environmental variables responsible for dissimilarity (as revealed by SIMPER and ENV, respectively).

Assemblage combinations	% Overall dissimilarity	No. of taxa shared	Taxa mainly responsible for overall dissimilarity (% contribution to dissimilarity)
LP × LK	88.35	10	*Nitzschia paleaeformis* (4.05), *Eunotia paludosa* (3.90), *Frustulia saxonica* (2.90), *Pinnularia subcapitata* (2.79), *Eunotia genuflexa* (2.62), *Achnanthidium minutissimum* (2.15), *Eunotia bilunaris* (2.10), *Eunotia minor* (1.86), *Nitzschia amphibia* (1.67), *Eunotia steineckei* (1.60), *Planothidium nodosum* (1.55), *Epithemia adnata* (1.21), *Navicula cari* (1.19), *Gomphonema brebissonii* (1.11), *Cocconeis placentula* (1.08), *Navicula radiosa* (1.06), *Navicula seibigiana* (1.02), *Nitzschia acidoclinata* (1.02), *Encyonopsis microcephala* (1.02)
LP × ZB	90.85	5	*Frustulia saxonica* (6.13), *Eunotia paludosa* (4.05), *Eunotia bilunaris* (4.00), *Pinnularia subcapitata* (2.67), *Eunotia genuflexa* (2.61), *Achnanthidium minutissimum* (2.11), *Eunotia minor* (1.82), *Frustulia crassinervia* (1.80), *Nitzschia amphibia* (1.64), *Eunotia steineckei* (1.58), *Planothidium nodosum* (1.53), *Epithemia adnata* (1.19), *Navicula cari* (1.17), *Gomphonema brebissonii* (1.09), *Cocconeis placentula* (1.06), *Navicula radiosa* (1.04), *Nitzschia acidoclinata* (1.00), *Navicula seibigiana* (1.00), *Encyonopsis microcephala* (1.00)
LK × ZB	61.10	7	*Eunotia paludosa* (16.66), *Frustulia saxonica* (14.54), *Nitzschia paleaeformis* (13.30), *Eunotia bilunaris* (12.37), *Eunotia genuflexa* (10.64)
LP × (LK + ZB)	92.53	10	*Eunotia paludosa* (16.39), *Frustulia saxonica* (13.12), *Achnanthidium minutissimum* (11.58), *Nitzschia paleaeformis* (7.65), *Pinnularia subcapitata* (5.93), *Eunotia bilunaris* (5.51)

lakes, which further accentuates the distinction between our examples of NAWB and non-NAWB diatom floras. However, the two NAWBs were not overly similar in their diatom assemblages, either (Tables 4 and 6): they shared a few diatom taxa (seven), and the dissimilarity between them was due mainly to *Eunotia paludosa*, *Frustulia saxonica*, *Nitzschia paleaeformis*, *E. bilunaris*, and *E. genuflexa* (Table 6). Interestingly, the ZB diatom flora comprised *Cocconeis pediculus*, an alkaliphilous and eutraphenic species (van Dam et al., 1994). The species occurred at a low relative abundance (0.04%), which did not affect the overall pattern.

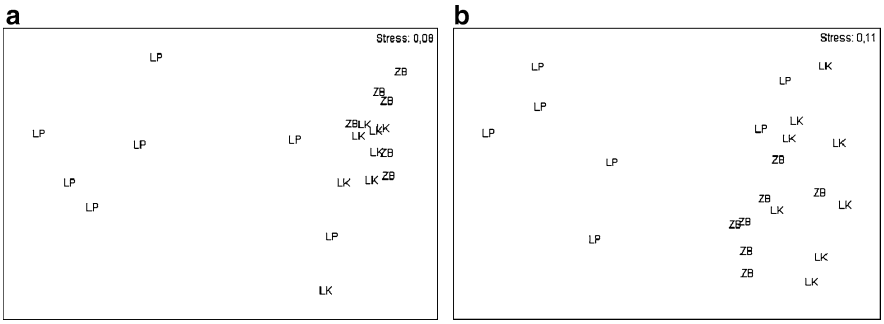

Figure 4. MDS plots on presence–absence (**a**) and relative abundances (**b**) of diatom taxa in the three lakes.

5. Discussion

The three lakes surveyed in this study turned out to differ markedly in their abiotic regimes. The water pH in the two NAWBs was consistently below 5.0, while the pH range in the non-NAWB extended well into the alkaline part of the pH scale (Table 2). This in itself could be a predictor of a substantial non-NAWB versus NAWB difference in diatom assemblages, as pH of about 4.7–5.6 is critical for aquatic biota due to the loss of the bicarbonate buffering system in the lake; it is the pH range that marks the maximum changes in phytoplankton communities (Dixit and Smol, 1989). Another pH threshold suggested as important for aquatic organisms spans the range of 3.5–4.0, below which many species are not capable of maintaining a population (DeNicola, 2000). As can be seen from Table 2, the NAWBs surveyed in this study – but not the non-NAWB – did experience pH values <4.0. Another environmental variable important for differentiating the diatom assemblages studied in this work was conductivity, generally taken as reflecting the dissolved cation content, notably Ca^{+} and Mg^{+} (Kwandrans, 2007). In this regard, ZB with its narrower conductivity range and lower conductivity values was markedly different from both LP and LK (Table 2). Thus, the set of lakes surveyed in this study was interesting in that they followed a certain gradient along the environmental axis. The gradient highlighted not only the differences between the non-NAWB and the two NAWBs, but also those between the two NAWBs themselves. LP, the least acid-stressed lake, occupies one end of the gradient; LK, an NAWB formed as a result of anthropogenic activities, takes an intermediate position, while ZB, a fully natural peatbog reservoir with high acidity, lower conductivity, higher upper limit of dissolved organic C and higher dissolved P contents and much lower dissolved Si contents (cf. Table 2) is situated at the opposite end of the gradient. It would be interesting to find out whether the diatom assemblages of the three lakes conformed to this gradient.

The diatom assemblages studied featured genera and lower rank taxa reported from peatbog reservoirs in central Europe (e.g. Buczko, 2006; Kwandrans, 2007; Matuła, 1995; Wojtal et al., 1999). Interestingly, the taxa that featured

prominently in the assemblages examined in this study, e.g. *Eunotia paludosa* and *Pinnularia subcapitata* var. *elongata*, were recorded as dominants in Hungarian peatbogs studied by Buczko (2006). In terms of taxonomic richness, however, the Western Pomeranian assemblages seem to be impoverished in comparison with other similar areas.

Because of the manner the samples were collected from each site (littoral water and fringe mosses), the diatom taxa identified in the samples analysed in this study represented planktonic (e.g. *Asterionella*, *Cyclotella*), benthic (e.g. *Achnanthes*, *Eunotia*, *Navicula*, *Nitzschia*) and periphytic (e.g. stalked forms *Gomphonema*, *Cymbella*) modes of life. In her study of diatoms found in Hungarian peatbogs, Buczko (2006) reported a high number of aerophytic forms, much less common in the materials collected from the three Western Pomeranian low-pH habitats. The difference can be explained by the fact that the sites studied by Buczko (2006), despite supporting bryophytes (including *Sphagnum*), were undergoing desiccation, hence the prominence of aerophytic diatoms. Our sites were located in fully aquatic habitats, for which reason their diatom floras were primarily aquatic. Moreover, in contrast to results reported by Buczko (2006), our data showed a higher number of typically bryophytic taxa (e.g. *Eunotia bilunaris* var. *bilunaris*, *E. bilunaris* var. *mucophila*, *E. genuflexa*, *E. incisa*, *E. steineckei*, *Fragilaria gracilis*, *Frustulia crassinervia*, *F. saxonica*, *Nitzschia gracilis*, *N. paleaeformis* and *Tabellaria flocculosa*) (cf. Table 4), emphasising the importance of *Sphagnum* mosses in supporting diatom diversity in peatbog habitats (Buczko, 2006; Pouličková et al., 2002).

In her review of diatoms *versus* water pH studies conducted by the mid-1980s, Stokes (1986) pointed out that, as pH decreases, the species richness becomes reduced and the species composition undergoes profound changes. The depressed species richness in acid and acidified aquatic environments has since been reported in a number of studies and reviews (e.g. Dixit and Smol, 1989; Matuła, 1995; Pouličková et al., 2002). Palaeolimnological evidence, too, shows reduced numbers of species in acidified environments (Ginn et al., 2007). This study confirmed the general pattern of a low taxonomic richness in waterborne and bryophyte-associated diatom assemblages inhabiting low-pH habitats. The importance of water pH for taxonomic richness of diatom communities is well illustrated in Fig. 5, which shows a clear distinction between the species-poor assemblages in LK and ZB on the one hand and the taxonomically much richer, albeit variable, assemblage in LP, on the other hand.

Although the reduction in species richness has been ascribed to the stress caused by excess hydrogen ions in the water, other – perhaps secondary – causes of the effect have been hinted at as well, e.g. lowered concentration of nutrients, change in chemical form of nutrients or alterations in grazing pressure (Stokes, 1986). This study seems to have elucidated a particular role of low dissolved Si supply (cf. Table 2): the lowest dissolved Si contents in ZB were accompanied by the lowest taxonomic richness characteristics of that site.

Analysis of the structure (composition and relative abundances) of the diatom assemblages studied showed that they did conform to the gradient (as illustrated by the MDS graphs in Fig. 4) revealed earlier by the environmental

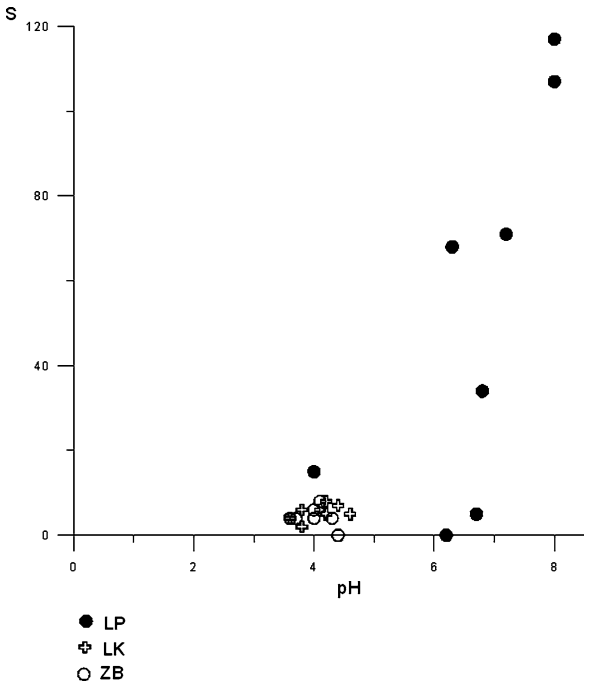

Figure 5. Number of taxa (S) versus water pH in the three lakes.

variables. Again, the non-NAWB (LP) with its highest number of taxa and the assemblage dominated by circumneutral diatoms is situated at one end of the gradient. LK, an NAWB which emerged as an outcome of anthropogenic activity (peat cutting) showed an intermediate number of taxa, most of which were acidobiontic–acidophilous, but were accompanied by other pH adaptation types as well. The opposite end of the gradient was taken by ZB, an NAWB with the lowest number of taxa and the strongest domination of acidobionts–acidophiles. Thus, as revealed by this study, the region of Western Pomerania in Poland supports a mosaic of low-pH lakes inhabited by a variety of diatom assemblages. Although characterised by a reduced taxonomic richness, they, however, support sets of unique taxa (see below).

It has to be remembered that the decrease in species richness with decreasing water pH is an effect visible at the community and ecosystem level, while the few species actually living in an acid-stressed system are acid tolerant and should be able to function efficiently at low pH (Stokes, 1986). At the aquatic ecosystem level, however, low diversity implies mainly loss of resilience, and low-resilience communities are increasingly more susceptible to stressors other than excess hydrogen ions (Stokes, 1986; Wittebolle et al., 2009). In this context, it is important to emphasise that the taxa which did occur in the NAWBs explored in this

study represented highly stenotopic forms and could be regarded as having a high indicative potential (Krammer and Lange-Bertalot, 1986, 1988, 1991a, b). They are also important for conservation reasons.

An example is provided by the genus *Eunotia*, typical of oligotrophic, low-mineral aquatic habitats, rare in Europe (Krammer and Lange-Bertalot, 1991a; Lange-Bertalot, 1996). Moreover, members of the genus show a marked preference towards acid waters, being mainly acidobiontic or acidophilous (e.g. Kwandrans, 2007). It is estimated that the majority (even up to 80%) of European representatives of the genus are endangered to a various degree, extremely rare or probably extinct (Lange-Bertalot, 1996). Some authors (e.g. Siemińska, 1992) reported *Eunotia meisteri* as endangered in Poland, *E. rhomboidea* being considered rare (Siemińska, 2006). In this study, the two species proved relatively frequent. While as few as 7.5% of the *Eunotia* taxa are currently not threatened, further 12.5% are classified as not endangered, but their occurrence is too poorly known for any unambiguous classification. Another example of a unique taxon is *Frustulia crassinervia*, referred to by Lange-Bertalot (2001) as regressive in Europe due to anthropogenic degradation of its natural habitats, i.e. peatbogs. Interestingly, *F. crassinervia* was, in our samples, prominent both in terms of relative abundance and frequency of occurrence (A. Witkowski et al., 2005). Our study then supplied valuable information on the distribution of taxa having a high conservation value on the European scale.

As frequently pointed out, low-pH effects on the diatom flora depend on the cause of acidity (organic vs mineral acids; Stokes, 1986; Kwandrans, 2007). In water bodies stressed by anthropogenic acidification, low pH is usually brought about by the presence of mineral (sulphuric and nitric) acids. Ecosystems of such lakes, including diatom communities, are exposed to strong adverse effects accompanying acidity, e.g. mobilisation of toxic metals (Stokes, 1986; Gross, 2000). Our study concerned brown-water lakes in which the low pH is due to the presence of humic (organic) acids. In such lakes, adverse effects mentioned are much less pronounced, if at all, mainly on account of toxic metal complexation (Yakovlev, 2001). Therefore, ecosystems of such lakes have inbuilt detoxification mechanisms, and effects of potential additional acidification (e.g. anthropogenic) should be expected to be less acute. On the other hand, while anthropogenic acidification is reversible (i.e. anthropogenically acidified water bodies can be restored and can recover from acid stress, as evidenced by changes in composition of diatom communities following application of restoration measures; Kwandrans, 2007; Kwandrans and Eloranta, 2003; McNicol et al., 1998; Smol, 2002; Yan et al., 2003), deacidification is not possible in NAWBs because of the inherently low pH of their water and constant supply of humic acids from the peatbog. This renders living communities of such lakes, including diatom assemblages, more vulnerable to effects of degradation and, because of their unique nature, makes such lakes ideal candidates for conservation and protection.

6. Acknowledgements

This study was supported by the Polish Ministry for Science and Higher Education grant No. 3 P04 F01923. We thank Dr. W. Kowalski, Drs. Ilka and Jörg Schönfelder, Genowefa Daniszewska-Kowalczyk and Agnieszka Kierzek for assistance at various stages of the work. We are grateful to Dr. Rosalina Stancheva for helpful comments on an earlier version of the manuscript.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 385–400
DOI 10.1007/978-94-007-1327-7_17, © Springer Science+Business Media B.V. 2011

THE DIATOM ALGAE OF LAKE KINNERET, ISRAEL

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1. Introduction

Lake Kinneret lies 210 m below Mediterranean Sea level (MSL) in the northern part of the East African Rift Valley. It is the only freshwater lake in Israel and one of the few natural bodies of freshwater in the Middle East (Pollingher, 1986). The lake acquired its present configuration about 20,000 years ago. Earlier, during the Warm period, a part of the present territory was occupied by the Lisan Lake (Begin et al., 1974). Lake Kinneret is a warm monomictic lake. Full homothermy occurs between December and February and a steady thermocline develops after April. During the stratification period (from May to December), the epilimnion (16–22 m thick, temperature range 15–30°C) is rich in oxygen and poor in nutrients. The hypolimnion is completely devoid of oxygen and rich in sulfides and ammonia. The concentration of nitrogen in the lake water depends mostly on the external input from the Jordan River and is regulated by the pattern and intensity of floods. The concentration of silica is about 10 mg/L, and the concentration of dissolved phosphorus rarely exceeds 10 μg/L (Serruya, 1975; Pollingher et al., 1986).

Discussion of Lake Kinneret diatom algae should include fossil, modern planktonic, and modern benthic assemblages.

2. Fossil Diatoms of Lake Kinneret

The fossil diatoms of Lake Kinneret were first studied in the 1980s (Pollingher et al., 1986). Sediment samples were collected in the southern part of the lake with a 4.8-m core and dated by the ^{14}C method. The dating showed that the sample represented about 5,000 years of Lake Kinneret history, with five chronological periods clearly distinguishable on the basis of different diatom assemblages.

During the period corresponding to the lower part of the core, from the bottom to 3.4-m depth (about 5,000–3,000 years ago, respectively), diatom abundance in the lake was found to be up to 50×10^6 cells/g dry sediment. The diatoms

were probably the main constituents of the lake's total phytoplankton biomass. Diatom assemblage was dominated by large *Cyclotella* cf. *notata* occurring together with *Cyclotella ocellata*, *Cyclotella kuetzingiana*, and a few large *Stephanodiscus* cf. *astraea.* Small benthic forms, *Fragilaria* spp. and *Amphora pediculus*, were present in low abundance.

This floristic composition of the planktonic assemblages was definitely different from that of present Lake Kinneret phytoplankton, and most of the dominant species became extinct at the period's end. The high abundance of large centric diatoms points to oligotrophic conditions in the lake during the corresponding period.

Higher in the core, at a depth of 3.4–2.9 m (~3,000–2,700 years ago, respectively), diatom abundance decreased to *Cyclotella–Stephanodiscus* complex joined by *Melosira agassizii* – the algae that usually develop in rather eutrophic waters. This may be correlated with the dense human settlement and intensive agricultural activity around the lake during the period (Stiller et al., 1984).

In the next core part of 2.9–2.4 m (~2,700–2,400 years ago, respectively), the planktonic diatoms completely disappeared from Lake Kinneret. A few benthic forms, as well as some euryhaline species, were present sporadically. The event that led to the diatom disappearance after a very high abundance period has not yet been identified. Probably, the cause was climatic changes and/or tectonic activity.

In the 2.4–0.4 m part (~2,400–1,600 years ago, respectively), diatom abundance varied from rare to 15×10^6 cells/g dry sediment. The assemblage was dominated by small benthic species, *Fragilaria construens*, *Fragilaria brevistriata*, *Achnanthes minutissima*, *Amphora pediculus*, and *Cocconeis diminuta*, accompanied by some large forms: *Navicula*, *Caloneis*, and *Surirella*. All these forms are known among the present benthic flora of the lake. *Cyclotella ocellata* and *Cyclotella kuetzingiana* have also been found in the lake. *Aulacoseira granulata* has appeared in the lake, but is rare.

During the period corresponding to the upper part of the core, from the 0.4–0 m depth (~last 1,500 years), *Aulacoseira granulata* became dominant in long chains, occurring together with *Cyclotella ocellata*, *Cyclotella kuetzingiana*, *Fragilaria*, *Achnanthes*, etc. It seems that in this period, the limnological conditions and phytoplankton association of the lake were quite similar to those in the present time.

3. Planktonic Living Diatoms of Lake Kinneret

3.1. THE HISTORY OF TAXONOMIC STUDIES OF LAKE KINNERET DIATOMS

The first list of Lake Kinneret diatoms was published in 1873 by Petit (1883) and included 40 planktonic, benthic, and epiphytic species. In 1893, Barrois organized an expedition to Lake Kinneret and distributed the collected material among several biologists for species identification (Barrois, 1894). Unfortunately, this team did not include a phycologist, and dinoflagellate *Ceratium hirundinella* was the only identified algal species. In 1912, another expedition was organized

by Annandale. The expedition results also determined zoological life forms only; however, it was mentioned that "the latter live to a large extent on the minute algae that are extremely abundant" (Annandale, 1913). Studies of Lake Kinneret algal flora were renewed several decades later, when Rayss (1944, 1951) described 30 algal species, including 3 species of diatoms. Komarovsky (Kimor) described the *Fragilaria crotonensis* bloom that occurred in the lake in 1948 (Komarovsky, 1951). In 1959, the same author published a list of 83 Lake Kinneret phytoplankton species, including 17 species of diatoms including *Cyclotella catenata*, *Melosira (Aulacosira) granulata*, *Fragilaria crotonensis*, *Synedra affinis*, *Synedra ulna*, and *Navicula* sp. A similar but more limited list was published in 1961 by Yashouv and Alhunis (Yashouv and Alhunis, 1961). In 1965, Komarovsky and Pollingher published a booklet describing 150 species of Lake Kinneret planktonic algae, including diatoms (Kimor and Pollingher, 1965).

In 1969, the regular Lake Kinneret monitoring program was started. Within the limits of this program, phytoplankton composition was detected weekly at several depths at a permanent sampling station in the central, deeper part of the lake. Chlorophyll concentration and water chemical parameters were detected simultaneously with phytoplankton sampling – as part of the monitoring program. The algal biomass (wet weight) was calculated based on the calculation of each species' biovolume from appropriate geometric formulae (Hillebrand et al., 1999). The monitoring program has continued for 40 years and is still continued today, making Lake Kinneret one of most studied lakes in the world, with a considerable long-term database.

After the establishment of the monitoring program, the main aim of algological studies shifted from species composition to ecosystem productivity. In addition, because the most characteristic feature of the Lake Kinneret ecosystem until the last 15 years was the annual winter-spring bloom of the dinoflagellate *Peridinium gatunense*, the majority of phycological studies focused on this alga. However, in 1978, Pollingher published a new list of 222 Lake Kinneret algal species belonging to 8 divisions (Pollingher, 1978). The diatoms reported were among the most numerous taxa of algae in the lake, ranking third according to the number of species present (Table 1). In 1995, Ehrlich published the "Atlas of the Inland-Water Diatom Flora of Israel" (Ehrlich, 1995). To date, this atlas is the most detailed study of Israeli diatoms, presenting taxonomical and ecological descriptions of species from a number of habitats, including Lake Kinneret. One year later, Meyer and Håkansson (1996) described a new Kinneret planktonic diatom species – *Cyclotella polymorpha.* This species has not been reported for other water bodies. In 2000, Nevo and Wasser (2000) published the "Biodiversity of Cyanoprokaryotes, *Algae* and Fungi of *Israel*" *study series*. This series included some information about Lake Kinneret diatoms. In 2005, the project "On-line Photocatalog of Lake Kinneret Phytoplankton" was begun by Alster and Zohary. This catalog can be viewed at: http://planktonnet.awi.de//index.php?thematicid=2051#search. It contains high-quality, light-microscopic photographs, and taxonomical and ecological information about the lake planktonic algae. The future plan is to expand the catalog by making the taxonomic and ecological descriptions more detailed and to include benthic algae.

Table 1. Diatoms found in Lake Kinneret phytoplankton (Pollingher, 1978).

Centrales
Atteya zachariasi
Aulacoseira granulate
Aulacoseira granulata var. *angustissima*
Cyclotella kuzinginiana
Cyclotella meneghiniana
Cyclotella stelligera
Melosira arenaria
Melosira italic
Stephanodiscus hantzschii
Pennales
Amphora ovalis
Bacillaria paradoxa
Cocconeis pediculus
Cocconeis placentula
Cymatopleura elliptica
Cymatopleura solea
Cymatopleura solea var. *apiculata*
Cymbella affinis
Cymbella cymbiformis
Cymbella Helvetica
Epithemia muelleri
Epithemia zebra
Fragilaria construens var. *subsalina*
Fragilaria crotonensis
Gomphonema parvulum
Gomphonema ventricosum
Gyrosigma acuminatum
Gyrosigma attenuatum
Gyrosigma spencerii var. *nodifera*
Navicula cryptocephala
Navicula ryncocephala
Nitzschia acicularis
Nitzschia holsatica
Nitzschia hungarica
Nitzschia stagnorum
Nitzschia stigmoidea
Pleurosigma angulatum var. *strigosum*
Rhoicosphaenia curvata
Stauroneis anceps
Surirella capronii
Surirella ovalis
Surirella robusta
Surirella robusta var. *splendid*
Surirella spiralis
Surirella tenera var. *nervosa*
Synedra ulna
Synedra ulna var. *acus*
Synedra ulna var. *affinis*
Synedra ulna var. *fasciculate*
Synedra ulna var. *oxyrhynchys* fa. *mediocontracta*
Synedra ulna var. *ramesi*

3.2. THE PRESENT PLANKTONIC DIATOM ASSEMBLAGE IN LAKE KINNERET

As was mentioned above, the modern diatom flora of Lake Kinneret started to form 1,600–2,400 years ago, when the main species became *Aulacoseira granulata*, *Cyclotella* spp, *Fragilaria*, and *Achnanthes*. These algae are present in the lake until today; however, from the start of regular phytoplankton studies at the lake in 1968, the phytoplankton assemblage has changed considerably. The modern history of Kinneret phytoplankton can be divided into two periods (Berman et al., 1995; Zohary, 2004). The period from 1968 to 1993 was quite stable, characterized by a typical annual pattern revolving around a winter-spring bloom of the dinoflagellate *Peridinium gatunense*. In addition to dinoflagellate blooms, the diatoms *Aulacoseira granulata*, accompanied by *Cyclotella polymorpha*, formed blooms in the winters of 1978, 1982, 1983, 1988, and 1991; *Synedra (Fragillaria) rumpens* appeared in high numbers in summer 1976; and *Anemones vitera* proliferated in fall 1982 (Pollingher, 1986; Berman et al., 1998). The second period started in 1994 and still continues today. It is characterized by annual pattern loss, random alternation of "*Peridinium* bloom years" and "no *Peridinium* years," and increased variability of blooming algae and magnitude of non-*Peridinium* blooms (Zohary, 2004). Generally, the succession of planktonic diatom assemblages in Lake Kinneret during regular observation periods has been followed. In the 1970s, diatoms comprised only a minor component of the lake phytoplankton, but since the early 1980s, their relative biomass has gradually increased, with conspicuous winter peaks in excess of 100 g m^{-2} occurring in some of the years. As with the *Peridinium* blooms, these winter diatom peaks were attributed to a single species, *Aulacoseira granulata.* While winter *Aulacoseira granulata* blooms were recorded in the 1980s, they intensified in more recent years and contributed to the altered annual patterns in the years since 1995. Until 1994, the peak of *Peridinium* biomass tended to be lower in *Aulacoseira* bloom years. Since 1995, this relationship has no longer held, and years with winter *Aulacoseira granulata* blooms were followed by either extensive *Peridinium* blooms (1995, 1998), low *Peridinium* blooms (1999), or *Peridinium* bloom absence (1997, 2001). The small peaks in diatom biomass at other times of the year after 1994 were, as in previous years, attributed to *Cyclotella polymorpha* and *Synedra* spp. Other diatom species occurred in the lake at low biomass density periods (Berman et al., 1995; Zohary, 2004).

3.3. THE EPILITHIC DIATOMS IN THE LITTORAL ZONE OF LAKE KINNERET

The littoral zone in lakes is defined as the area of shallow water until the compensation depth – the depth at which the rate of photosynthesis equals the rate of respiration (Wetzel, 1975). The littoral zone is very important because it acts as a mediator between the terrestrial and the aquatic ecosystems (Wetzel, 1975). In most lakes, the littoral zone can be defined via the border of the submerged

macrophyte growth area (Howard-Williams and Lenton, 1975). In lakes where there is no submerged macrophytic flora, the littoral zone is defined as the area up to the maximal depth till which epilithic algae grow. The littoral zone of Lake Kinneret was, therefore, defined as the area between the surface and a depth of 3–5 m, which encompasses approximately 5% of the lake area (Gasith, 1991). When the size of a lake increases, its area increases faster than the length of its beach. The littoral area in large lakes is, therefore, less important than the open water. Gasith (1991) hypothesized that in large lakes, the littoral zone can be a limiting factor for the entire ecosystem because of the various vital functions of the littoral resources that serve the entire lake.

In aquatic environments, algae are usually divided into different groups according to their physical distribution: phytoplankton, i.e., microscopic algae that are passively transported by the currents; metaphyton, i.e., macroscopic algae that grow in the water or below its surface (macrophytic algae or seaweeds); and periphyton, i.e., algae that grow on submerged surfaces or float attached to these surfaces. The latter algae are divided into subgroups: epiphytic periphyton, i.e., algae that grow on water plants; epizooic periphyton, i.e., algae that grow on live aquatic animals; epilithic periphyton, i.e., algae that grow on the surface of rocks and stones at the bottom of a lake or river; epipelic periphyton, i.e., algae that grow on loose sediment; and epipsammic periphyton, i.e., algae that grow or stay within a sediment (Wetzel, 1975; Robinson et al., 1997a, b).

The epilithic (attached to stones) algae constitute the major part of the periphyton in the wave-swept littoral zone. Their species composition usually differs strongly from that of the phytoplankton since it is dominated by species capable of attachment to the substrate. Like the lake's phytoplankton, the epilithic community exhibits pronounced seasonality in response to changes in nutrients and temperature (Wehr and Sheath, 2002).

The epilithic algae play a major role in gross production in the lake and are a food resource for water creatures such as invertebrates (Lamberti and Moore, 1984; Roemer et al., 1984; Rosemond, 1994; Scheffer et al., 1997) and fish (Power et al., 1985; Sala and Boudouresque, 1997).

The importance of the epilithic algae as a resource for organic material in the littoral area increases in lakes in which there is no submerged macrophytic flora (Round, 1973; Loeb et al., 1983).

There are relatively few detailed descriptions of the community structure of epilithic algae (Kelly et al., 1998; Winter and Duthie, 2000). The epilithic communities in lakes undergo vast and rapid changes in their biomass and distribution, which makes it difficult to evaluate their biomass and predict their photosynthetic activity (Likens et al., 1985; Cattaneo et al., 1993; Rosemond, 1994). Various factors affect their development, including light regimes (Steinman et al., 1991), water temperature (Howard-Williams and Allanson, 1981; Auer et al., 1983; Roos, 1983), mixing and wave activity (Peterson et al., 1990), nutrient availability (Fairchild et al., 1985), sea-level changes (Gasith and Gafny, 1990), type of substrate (Robinson, 1983), erosion (Koseff et al., 1993), and intra/interspecies competition (Jenkerson and Hickman, 1986).

In Lake Kinneret, the littoral zone has been defined as ranging from 3 to 5 m (Por, 1968; Dor, 1974; Round, 1978), and it encompasses about 5% of the lake area (Gasith, 1991). The lake has an area of 170 km^2 and the littoral zone is 2 km^2 at high water level (−209 m) (Gafny and Gasith, 2000), but there are estimates as high as 14 km^2 for the littoral zone at low water levels (Dor, 1970).

The littoral zone of Lake Kinneret has almost no submerged macrophyte flora, hence, most of benthic primary production is due to periphytic algae. However, in contrast to the phytoplankton, which has been monitored regularly since 1969, there are only a few studies on the benthic algal flora in this lake. Dor (1970, 1971, 1974) defined the epilithic algal species, Gafny and Gasith (2000) studied their biomass and primary production rates, and Yehoshua (2002) studied the ecophysiology and function of the epilithic algae in Lake Kinneret.

Gafny and Gasith (2000) found that epilithon development in Lake Kinneret was strongly affected by water-level fluctuations. During winter and spring, the water level rises and newly inundated rocky areas are colonized by epilithon. During summer and fall, the water level falls, with a concomitant reduction in the availability of rocky areas. The epilithic algae in the littoral of Lake Kinneret are characterized by seasonal dynamics that include changes in the assemblages of species, total biomass, and primary production. The average biomass of epilithic algae is highest at the end of winter, with chlorophyll *a* concentrations of 6 μg cm^{-2} stone, and low at the end of summer, with chlorophyll *a* concentrations of only 1.9 μg cm^{-2} stone surface (Yehoshua, 2002).

The biomass of epilithic algae per unit area resembles the average yearly values per square meter that were measured for phytoplankton in the epilimnion, with a total production of 1,765 ton C year^{-1} (Yehoshua, 2002).

The total phytoplankton production in Lake Kinneret per year is 110,000 ton C (Berman et al., 1995). It may, therefore, be assumed that the epilithic algae make a significant contribution, at least in the local aspect of the littoral.

Yehoshua et al. (2008) observed 54 different algal species, 22 of which were diatoms. *Gomphonema* sp. was the dominant alga on stony substrates and was found throughout the year. *Cymbella* sp., *Navicula* spp., and *Nitzschia* sp. were present for 10, 9, and 7 months, respectively (Table 2). Of the nonfilamentous epilithic algae, diatoms (mostly *Gomphonema* sp., *Cymbella* sp., *Navicula* spp., and *Nitzschia* spp.) were found on the stones at a high frequency, contributing about 80% of the total number of cells per unit area throughout the year. The Cyanobacteria (mainly *Lyngbya* sp.) and the Chlorophyta (mostly *Tetraedron minimum*) made a small contribution to the annual cell counts (approximately 18% and 2%, respectively) (Yehoshua et al., 2008). Similar to previous studies (Dor, 1970, 1971), we found that the phytobenthic community of Lake Kinneret consisted mostly of diatoms and Cyanobacteria. The number of Cyanobacteria species was relatively high, but their contribution to total algal biomass was low. In contrast, there were fewer diatom species but their contribution to community biomass was high, as noted previously by Rahat and Dor (1968) and Dor (1970, 1971). Round (1978) reported that diatoms were responsible for approximately 90% of the primary production of the phytobenthos in Lake Kinneret, while the

Table 2. List of diatom epilithic taxa and their monthly recording over a 12-month period.

S. No.	Organism	Duration (months of presence)
	Diatoms	
1.	*Gomphonema* sp.	12
2.	*Cymbella* sp.	10
3.	*Navicula* spp.	10
4.	*Nitzschia* spp.	8
5.	*Cocconeis* sp.	6
6.	*Achnanthes* sp.	5
7.	*Synedra ulna*	5
8.	*Amphora* sp.	5
9.	*Cyclotella polymorpha*	4
10.	*Diatoma* sp.	4
11.	*Surirella* sp.	4
12.	*Synedra affinis*	3
13.	*Anomoeoneis* spp.	3
14.	*Epithemia* sp.	2
15.	*Fragilaria* sp.	2
16.	*Gomphoneis* sp.	2
17.	*Amphipleura* sp.	2
18.	*Bacillaria* sp.	1
19.	*Denticula* sp.	1
20.	*Gyrosigma* sp.	1
21.	*Pinnularia* sp.	1
22.	*Synedra rumpens*	1

contribution of Cyanobacteria and Chlorophyta was low. Dor (1974) found that although Chlorophyta were the most important component of the Lake Kinneret phytoplankton community, representing approximately 55% of the total number of species, they constituted only 13% of the phytobenthic species. We observed that Cyanobacteria constituted the majority (58%) of the lake phytobenthic species, although only about 17% of the lake phytoplankton species belong to this group. The proportion of diatoms in phytoplankton and phytobenthos is similar, at ~26% (Dor, 1974).

There may be some interchange between planktonic and epilithic algal populations in Lake Kinneret. Some of the epilithic diatoms may detach from the stony substrate and pass into the water column; conversely, some planktonic species may sink and continue to grow as part of the benthic community. We note that all Chlorophyta species (except *Cladophora* and *Spirogyra*) reported by us as being in the phytobenthos, were also found in the phytoplankton (Dor, 1974). Some of these phytoplanktonic species might have sunk to the bottom and survived in the sediment, while others (such as *Cosmarium* and *Oocystis*) could have grown actively as epiphytes.

To study the depth profile of the epilithic algal populations, we used an incubation system made of PVC cylinders of 1 in. diameter, cut into 25 pieces, each 8.5 cm long. These cylinders were threaded onto a 1-cm-diameter stainless steel rod

attached at the bottom to a heavy 50 × 50-cm^2 stainless steel plate. With the upper tube at the water surface, the base was at a depth of ~2 m. The cylinders were abraded with sandpaper to encourage algal growth. The system was incubated in the littoral zone for 1–2 months, which sufficed for the development of epilithic algal communities (Castenholz, 1960; Sládečkova, 1962). The cylinders together with the algae that had grown on them were then placed into 50-mL tubes containing 40-mL filtered lake water (GF/F Whatman), and fixed with Lugol. The algae were then carefully scraped off the cylinder. The samples were examined with an Olympus AHBT3 Vanox Research Microscope at 40 × 10 magnification. The algae were identified to genera or species level using the manuals of Bourrelly (1966, 1968, 1970), Kimor and Pollingher (1965), and Prescott (1979). Ten replicates of each sample were counted and identified using a hemocytometer.

We also observed depth-dependent changes in the populations of epilithic algae. Diatoms were dominant in the uppermost layer of the water column, at depths of 0.25–0.7 m and at light intensities of 200–560 μmol quanta m^{-2} s^{-1}, in winter and summer, respectively, dominated by *Gomphonema* sp., *Nitzschia* spp., *Navicula* spp., *Cymbella* sp., *Cocconeis* sp., *Achnanthes* sp., and *Synedra ulna* (Nitzsch) Ehrenberg. Chlorophyta, especially *Cladophora glomerata*, were dominant at depths of 1.2–1.6 m (at a light intensity of 30–200 μmol quanta m^{-2} s^{-1}). The Cyanobacteria species (*Chroococcus* sp. and *Lyngbya* sp.) were also abundant at all depths and seasons (Yehoshua et al., 2008).

Diatoms are prevalent in the uppermost water layer because they have efficient mechanisms to deal with supraoptimal irradiance levels, mainly by means of an enhanced xanthophyll cycle that allows the dissipation of excess light energy as harmless thermal radiation (Lavaud et al., 2003). Nevertheless, this may not, by itself, be a sufficient explanation for their dominance, since, several chlorophytes also have efficient xanthophyll cycles, and even some cyanophytes manage to thrive under intense light. However, in addition, diatoms have a greater capacity to adhere to stony substrates than filamentous Chlorophyta and, thus, are less affected by high turbulence in the upper water layers (Stockner and Armstrong, 1971; Loeb and Reuter, 1981; Aloi et al., 1988; Roberts and Boylen, 1988). Previous studies show that both diatoms and filamentous Chlorophyta such as *Cladophora glomerata* prefer the high light intensity conditions at shallow depths (Lowe et al., 1982; Sheath and Morison, 1982; Stevenson and Stoermer, 1982; Yehoshua, 2002). The appearance of *Cladophora* in the deeper water (1.5 m) suggests that this species might be less tolerant to high light intensity than diatoms, and more susceptible to both desiccation and wave-generated abrasion.

4. The Species Composition of Epiphytes on *Cladophora glomerata* Kütz. (Chlorophyta) in Lake Kinneret

The filamentous alga, *Cladophora glomerata*, which extensively covered stones in the Lake Kinneret littoral, is known as a widely distributed species, occurring in freshwater lakes, rivers, and shallow sea regions (Whitton, 1970; Dodds and

Gudder, 1992; Sheath and Cole, 1992). This alga is important, since its cell biovolume is much greater than that of nonfilamentous algae (Lowe and Pan, 1996). In addition, the filaments of *Cladophora* create a benign microenvironment for small epiphytic algae and invertebrates, thus increasing the functional surface area of the littoral (Dodds, 1991a, b).

As can be seen in Fig. 1, the entire surface of the *Cladophora* filaments is covered by epiphytes, mostly the diatoms *Gomphonema* sp. and *Cocconeis* sp., in more than one layer. We suggest that there is a mutualistic symbiosis between the chlorophyte and the epiphytes. The latter shield the *Cladophora glomerata* filaments from photodynamic damage caused by high irradiance, while they gain a surface for attachment several times larger than that of the bare stones. One wonders whether *C. glomerata* may provide some additional advantage to the epiphytes, such as increased nutrient supply or some hitherto undetected growth stimulant (Marks and Power, 2001).

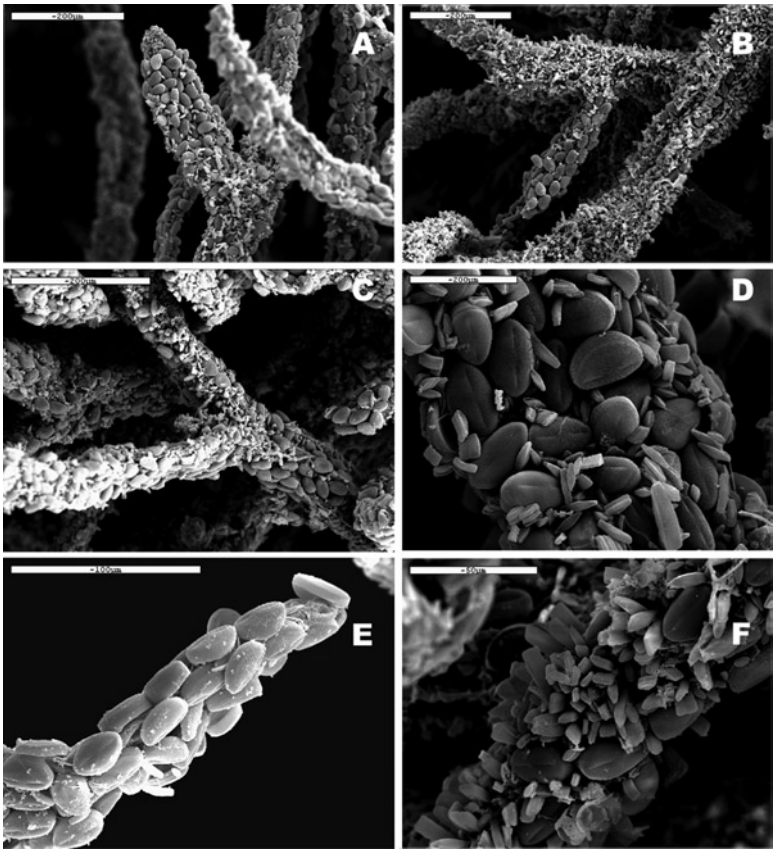

Figure 1. Scanning electron micrographs of *Cladophora glomerata* and epiphytes, mostly the diatoms *Gomphonema* sp. *and Cocconeis* sp., in Lake Kinneret.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 401–417
DOI 10.1007/978-94-007-1327-7_18, © Springer Science+Business Media B.V. 2011

RHEOPHILE APENNINE DIATOMS AND THEIR USE AS BIOINDICATORS OF WATER QUALITY

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1. Introduction

The Apennines are a majestic mountain chain extending 1,350 km along the Italian peninsula and divided in three main sections: northern, central, and southern. The highest peaks are in the central Apennines, where are present some remarkable massifs: the Mts. Sibillini with Mt. Vettore (2,476 m); the Monti della Laga with Pizzo di Sevo (2,419 m) and Mt. Gorzano (2,458 m); and finally, the Gran Sasso with Corno Grande (2,912 m) and the Maiella with Mt. Amaro (2,793 m).

This contribution refers, above all, to the rivers of the central Apennines which originate on the slopes of these mountains and enter the Adriatic Sea. They cut into prevalently limestone terrains. The expression "rheophile diatoms" generically refers to all the species encountered in these watercourses, which are short (100 km on an average), on steep slopes and thus really populated in the great majority by rheophile forms.

Generalizing widely, the hydrographic basins of a typical Apennine watercourse can be divided into three main stretches (Dell'Uomo, 1991): upper, middle, and lower, in each of which, using an adaptation and simplification of the longitudinal zonation of watercourses proposed by Illies and Botosaneanu (1963), various biological zones can be met with.

The upper, or Apennine stretch lies above 500–400 m a.s.l. according to geographical features of the territory, and includes both mountain springs (*crenon*) and torrents (*epirhithron*), where current is fastest in the whole length of the stretch. Waters are cold and well oxygenated and their temperature is almost constant in all seasons (8–10°C). The terrains are decidedly calcareous. The river bed is covered with large stones and sometimes locally with rocks.

The middle or pre-Apennine stretch ranges from 500 to 400 m down to 100 m a.s.l., flowing through a hilly zone with mainly sandstone, marl, and clayey terrains. Stones, pebbles, and gravel cover the bed. The uppermost part of this stretch corresponds to the *metarhithron* zone, and the lowermost to the *hyporhithron* zone.

The lower, or sub-Apennine stretch, identifiable as the *potamon* zone, lies below 100 m a.s.l. and flows over its own alluvial deposits through what has almost become a plain. The bed is covered in gravel of increasingly smaller grain size, and then in sand and silt.

The central Apennine watercourses have a relatively high bicarbonate concentration in the water and a basic pH mostly shifting between 7.5 and 8.5.

2. An Outline of Freshwater Diatom Ecology

Among the ecological factors affecting the growth, distribution, and seasonal variations of freshwater diatoms, in addition to light indispensable for photosynthesis and silica necessary for the formation of the cell walls, are the speed of current, temperature, pH, dissolved oxygen, and the chemical composition of the water.

2.1. PHYSICOCHEMICAL PARAMETERS

2.1.1. Current Speed

This is without doubt a factor affecting greatly the diatom distribution, because it conditions numerous aspects of their metabolism, such as respiration and absorption of dissolved compounds. In addition, the current continually renews nutrients and carries away products of catabolism, thus limiting autotoxic effects. Obviously, the rheophile environment involves a whole series of adaptations enabling the organisms to adhere to the substrate.

2.1.2. Temperature

This is one of most difficult parameters to correlate directly with diatoms, because its variations influence a whole series of other chemical and biological factors. In any case, these algae show a sharp preference for cool spring and autumn waters and seem intolerant of temperatures over 40°C, except for brief periods.

2.1.3. pH (Hydrogen Ion Concentration)

Diatom sensitivity to pH has been used for various classifications; here is proposed the following:

- Alkalibionte forms require alkaline waters.
- Alkaliphile forms prefer alkaline waters.
- Neutrophile to indifferent forms: the former prefer a pH around 7, the latter live in a wide range of pH values.
- Acidophile forms prefer acid environments.
- Acidobionte forms require acid waters, such as that in peat bogs, where pH values between 4 and 5 are often found. They are not present in Apennine watercourses.

2.1.4. Dissolved Oxygen

On the basis of dissolved oxygen, Van Dam et al. (1994) divide the diatoms into five classes:

- Species that require very high oxygenation of the water body (100% of the saturation value)

– Species that require, in any case, a good quantity of oxygen (saturation > 75%)
– Species that develop in environments with a moderate quantity of oxygen (saturation > 50%)
– Species for which a fairly low quantity of oxygen is sufficient (saturation > 30%)
– Species able to survive in the presence of a minimum quantity of oxygen (saturation value about 10%)

2.2. CHEMICAL COMPOSITION

2.2.1. Nutrients

The sensitivity (affinity/tolerance) of aquatic organisms to nutrients (above all, phosphates, nitrates, and ammonium salts) has given rise to the formulation of what is called, in its various facets, the trophic system. Over the years, numerous authors have contributed to this system, but with explicit reference to diatoms; Hofmann (1994), Van Dam et al. (1994), and Rott et al. (1999) are among those who have brought, recently, important contributions. Summarizing and simplifying the various proposals, freshwater diatoms can be classified into:

– Species characteristic of hypotrophic (or ultraoligotrophic) environments
– Species characteristic of oligotrophic environments
– Species characteristic of mesotrophic environments
– Species characteristic of eutrophic environments
– Species characteristic of hypertrophic environments
– Usually, these levels are defined on the basis of the concentration of total phosphorus, assuming for the first level a value lower than 4 $\mu g\ l^{-1}$; this value increases progressively in the successive levels, to exceed, in the last one, 100 $\mu g\ l^{-1}$.

2.2.2. Organic Compounds

At the beginning of the last century, Kolkwitz and Marsson (1902, 1908, 1909) identified a clear relationship between aquatic organisms and dissolved organic matter. Thus, was born the system of saprobic organisms, or saprobic system which, with successive contributions (including, among others, Liebmann, 1962; Fjerdingstad, 1964, 1965; Caspers and Karbe, 1966; Sládeček, 1973, 1986; Lange-Bertalot, 1979; Rott et al., 1997), led to the division of aquatic organisms into various levels, or degrees, that manifest increasing affinity/tolerance for dissolved organic matter. It contains nutritional elements, important chemical mediators via oligodynamic action, such as vitamin B12, and factors of antibiotic action produced, above all, by bacteria that act heterospecifically on all levels of the trophic chain. Diatoms are very well integrated into the system of saprobic organisms and can be classified into the five levels proposed by Sládeček (1973):

– Xenosaprobic diatoms do not tolerate organic matter, except in minimal quantities.
– Oligosaprobic diatoms tolerate only small quantities of organic matter.
– β-Mesosaprobic diatoms grow well in the presence of a moderate quantity of organic matter, which is completely degraded by bacteria.

– α-Mesosaprobic diatoms populate environments with a high quantity of organic matter, the demolition of which is not total because of the insufficiency of available oxygen.
– Polysaprobic diatoms tolerate very strong organic pollution, where the reductive processes prevail over the oxidative ones with formation of toxic and reductive compounds; few diatoms survive in these extreme conditions.

The main parameters of reference for defining saprobic levels are BOD_5, which increases from the first (<2 mg l^{-1}) to the fifth (>10 mg l^{-1}), and dissolved oxygen that gradually diminishes, while the bacterial load increases considerably.

2.2.3. Dissolved Salts

Diatom ability to adapt to variations of saline concentration, and in particular to chlorides, can be very limited (stenohaline forms) or decidedly ample (euryhaline forms). In rivers, the former are found above all near springs, while the latter prefer to populate zones near the mouth, where the degree of salinity is very variable. Among the authors who have worked to develop a system of salinity or halobic system referred expressly to diatoms are Kolbe (1927), Hustedt (1956), Ziemann (1982), and Van Dam et al. (1994). Dell'Uomo (2004) proposed this revised classification of diatoms correlated above all to chlorides:

– Halophobe diatoms are forms that require a chloride concentration below 20 mg l^{-1}.
– Oligohalobe exigent diatoms prefer a moderate quantity of chlorides, from 20 to 50 mg l^{-1}.
– Oligohalobe tolerant diatoms show their optimal development at salinity values between 50 and 200 mg l^{-1}.
– Halophile diatoms are stimulated by a relatively high chloride concentration, 200–500 mg l^{-1}.
– β-Mesohalobe diatoms are found in oligobrackish water, with salinity that oscillates between 0.5 and 5 g l^{-1}.
– α-Mesohalobe diatoms, they occur in brackish water with salinity from 5 to 20 (−30) g l^{-1}.
– Euhalobe diatoms are typically marine forms, salinity between 30 and 40 g l^{-1}.
– Polyhalobe (or hyperhalobe) diatoms tolerate values of salinity >40 g l^{-1}.

Only diatoms belonging to the first five levels were found in the Apennine rivers.

3. Diatoms of the Apennine Watercourses

To date, about 450 taxa have been identified in Apennine watercourses. It should be noted that this contribution does not consider the zone close to the mouth where the freshwater mixes with saltwater. Table 1 reports only a partial list of the Apennine diatoms with the most interesting species from the ecological point

Table 1. Some of the most frequent and/or ecologically interesting diatoms of the Apennine rivers, with their synthetic indication for the biological water quality: I – excellent, II – good, III – mediocre, IV – bad, V – very bad.

Taxa and authors	Code	Water quality
Achnanthidium biasolettianum (Grunow) Lange-Bertatlot	ADBI	I–II
Achnanthidium bioretii (Germain) Edlun	ABRT	I–II
Achnanthidium minutissimum (Kützing) Czarnecki	ADMI	I–II
Achnanthidium subatomoides (Hust.) Monnier, Lange-Bertalot et Ector	ADSO	I–II
Adlafia minuscula (Grunow) Lange-Bertalot	ADMS	II–III
Amphora inariensis Krammer	AINA	I–II
Amphora montana Krasske	AMMO	II
Amphora normanii Rabenhorst	ANOR	I
Amphora pediculus (Kützing) Grunow	APED	I–II
Amphora thumensis (Mayer) Cleve et Euler	ATHU	I–II
Amphora veneta Kützing	AVEN	IV–V
Anomoeoneis sphaerophora (Ehrenberg) Pfitzer	ASPH	IV
Aulacoseira italica (Ehrenberg) Simonsen	AUIT	I–II
Bacillaria paxillifera (O.F. Müller) Hendey	BPAX	IV
Brachysira vitrea (Grunow) Ross	BVIT	I–II
Caloneis alpestris (Grunow) Cleve	CAPS	I
Caloneis amphisbaena (Bory) Cleve	CAMP	IV
Caloneis bacillum (Grunow) Cleve	CBAC	I–II
Campylodiscus hibernicus Ehrenberg	CHIB	I
Cavinula cocconeiformis (Gregory ex Greville) Mann et Stickle	CCOC	I–II
Cocconeis disculus (Schumann) Cleve	CDIS	I–II
Cocconeis euglypta Ehrenberg	CEUG	I–II
Cocconeis pseudothumensis Reichardt	COPS	I–II
Craticula accomoda (Hustedt) Mann	CRAC	V
Craticula cuspidata (Kützing) Mann	CRCU	III–IV
Craticula halophila (Grunow ex Van Heurck) Mann	CHAL	IV–V
Craticula molestiformis (Hustedt) Lange-Bertalot	CMLF	IV–V
Cyclostephanos dubius (Fricke) Round	CDUB	III–IV
Cyclotella meneghiniana Kützing	CMEN	IV
Cyclotella ocellata Pantocsek	COCE	I–II
Cymatoplaura solea (Brébisson) W.Smith	CSOL	III
Cymatopleura elliptica (Brébisson) W.Smith	CELL	II–III
Cymbella aspera (Ehrenberg) Peragallo	CASP	I
Cymbella helvetica Kützing	CHEL	I
Cymbella parva (W. Smith) Kirchner	CPAR	II
Cymbella tumida (Brébisson) Van Heurck	CTUM	I
Cymbopleura cuspidata (Kützing) Krammer	CBUC	I–II
Delicata delicatula(Kützing) Krammer	DDEL	I
Denticula tenuis Kützing	DTEN	I-II
Diadesmis gallica (W. Smith)	DGAL	I-II
Diatoma hyemalis (Roth) Heiberg	DHIE	I
Diatoma mesodon (Ehrenberg) Kützing	DMES	I-II

(continued)

Table 1. (continued)

Taxa and authors	Code	Water quality
Diatoma vulgaris Bory	DVUL	III
Diploneis elliptica (Kützing) Cleve	DELL	I-II
Diploneis oblongella (Naegeli) Cleve et Euler	DOBL	I-II
Diploneis ovalis (Hilse) Cleve	DOVA	I-II
Diploneis puella (Schumann) Cleve	DPUE	I-II
Discostella pseudostelligera (Hustedt) Houk et Klee	DPST	I-II
Discostella stelligera (Cleve Grunow) Houk et Klee	DSTE	I-II
Ellerbeckia arenaria (Moore) Crawford	EARE	I-II
Encyonema minutum (Hilse) D.G. Mann	ENMI	II
Encyonopsis aequalis (W. Smith) Krammer	EAQL	I-II
Encyonopsis caesatii (Rabenhorst) Krammer	ECES	I
Entomoneis paludosa(W.Smith) Reimer	EPAL	IV-V
Eolimna minima (Grunow) Lange-Bertalot	EOMI	IV
Eolimnna subminuscula (Manguin) Moser, Lange-Bertalot et Metzeltin	ESBM	IV-V
Epithemia adnata (Kützing) Brébisson	EADN	I-II
Epithemia argus (Ehrenberg) Kützing	EARG	I-II
Epithemia turgida Kützing	ETUR	I-II
Eucocconeis flexella (Kützing) Brun	EUFL	I
Eunotia arcus Ehrenberg	EARC	I-II
Eunotia bilunaris (Ehrenberg) Mills	EBIL	I-II
Fallacia lenzii (Hustedt) Lange-Bertalot	FLEN	I-II
Fallacia pygmaea (Kützing) Stickle et Mann	FPYG	IV
Fistulifera saprophila (Lange-Bertalot et Bonik) Lange-Bertalot	FSAP	IV-V
Fragilaria arcus (Ehrenberg) Cleve	FARC	I
Fragilaria capucina Desmazières	FCAP	II
Fragilaria virescens Ralfs	FVIR	I
Frustulia vulgaris (Thwaites) De Toni	FVUL	II-III
Geissleria decussis (Oestrup) Lange-Bertalot et Metzeltin	GDEC	I-II
Gomphonema acuminatum Ehrenberg	GACU	II-III
Gomphonema angustatum (Kützing) Rabenhorst	GANG	II-III
Gomphonema angustum Agardh	GANT	I-II
Gomphonema augur Ehrenberg	GAUG	II-III
Gomphonema clavatum Ehrenberg	GCLA	I-II
Gomphonema italicum Kützing	GITA	II-III
Gomphonema olivaceum (Hornemann) Brébisson	GOLI	II
Gomphonema pumilum (Grunow) Reichardt et Lange-Bertalot	GPUM	I-II
Gomphonema tergestinum Fricke	GTER	I-II
Gyrosigma acuminatum (Kützing) Rabenhorst	GYAC	III
Gyrosigma attenuatum (Kützing) Rabenhorst	GYAT	III
Hippodonta capitata (Ehrenberg) Lange-Bertalot et Metzeltin	HCAP	II–III
Karayevia clevei (Grunow) Bukhtiyarova	KCLE	I–II
Karayevia ploeonensis (Hustedt) Bukthiyarova	KAPL	II
Lemnicola hungarica (Grunow) Round et Basson	LHUN	IV
Luticola goeppertiana (Bleisch) D.G. Mann	LGOE	IV–V

(continued)

Table 1. (continued)

Taxa and authors	Code	Water quality
Luticola ventricosa (Kützing) D.G. Mann	LVEN	IV–V
Mastogloia smithii Thwaites	MSMI	IV
Mayamaea atomus (Kützing) Lange-Bertalot	MAAT	IV–V
Mayamaea permitis (Hustedt) Bruder et Medlin	MPMI	IV–V
Melosira varians Agardh	MVAR	III
Meridion circulare (Greville) C.A. Agardh	MCIR	I–II
Navicula cincta (Ehrenberg) Ralfs	NCIN	IV–V
Navicula cryptocephala Kützing	NCRY	III–IV
Navocula cryptotenella Lange-Bertalot	NCTE	I–II
Navicula gregaria Donkin	NGRE	IV
Navicula jakovljevicii Hustedt	NJAK	I–II
Navicula lanceolata (Agardh) Ehrenberg	NLAN	III–IV
Navicula leptostriata Jorgensen	NLST	I
Navicula menisculus Schumann	NMEN	III–IV
Navicula oblonga Kützing	NOBL	II
Navicula phyllepta Kützing	NPHY	IV–V
Navicula radiosa Kützing	NRAD	I–II
Navicula recens (Lange-Bertalot) Lange-Bertalot	NRCS	III–IV
Navicula reichardtiana Lange-Bertalot	NRCH	II–III
Navicula reinhardtii (Grunow) Grunow	NREI	II
Navicula schroeterii Meister	NSHR	III–IV
Navicula splendicula Van Landingham	NSPD	II
Navicula striolata (Grunow) Lange-Bertalot	NSTL	I–II
Navicula trivialis Lange-Bertalot	NTRV	IV
Navicula veneta Kützing	NVEN	IV–V
Navicula viridula (Kützing) Ehrenberg	NVIR	III
Naviculadicta seminulum (Grunow) Lange-Bertalot	NVDS	IV–V
Neidium ampliatum (Ehrenberg) Krammer	NEAM	I–II
Neidium affine (Ehrenberg) Pfitzer	NEAF	I
Neidium alpinum Hustedt	NALP	I
Nitzschia acicularis (Kützing) W. Smith	NACI	IV
Nitzschia acidoclinata Lange-Bertalot	NACD	I–II
Nitzschia amphibia Grunow	NAMP	III–IV
Nitzschia capitellata Hustedt	NCPL	V
Nitzschia clausii Hantzsch	NCLA	IV
Nitzschia communis Rabenhorst	NCOM	III–IV
Nitzschia dissipata (Kützing) Grunow	NDIS	II–III
Nitzschia dubia W.M. Smith	NDUB	III–IV
Nitzschia filiformis (W.M. Smith) Van Heurck	NFIL	IV
Nitzschia fonticola Grunow	NFON	II–III
Nitzschia inconspicua Grunow	NINC	III–IV
Nitzschia linearis (Agardh) W.M. Smith	NLIN	III–IV
Nitzschia pusilla (Kützing) Grunow	NIPU	IV

(continued)

Table 1. (continued)

Taxa and authors	Code	Water quality
Nitzschia sigma (Kützing) W.M. Smith	NSIG	IV
Nitzschia sigmoidea (Nitzch) W.M. Smith	NSIO	III–IV
Nitzschia sociabilis Hustedt	NSOC	III–IV
Nitzschia vitrea Normann	NIVI	IV–V
Nupela lapidosa (Lange-Bertalot) Lange-Bertalot	NULA	I
Pinnularia viridis (Nitzsch) Ehrenberg	PVIR	III
Placoneis clementis (Grunow) Cox	PCLT	II–III
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	PLFR	I–II
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	PTLA	I
Platessa montana (Kraske) Lange-Bertalot	PTMO	I
Pleurosira laevis (Ehrenberg) Compère	PLEV	IV
Puncticulata radiosa (Lemmermann) Håkansson	PRAD	I–II
Reimeria sinuata (Gregory) Kociolek et Stoermer	RSIN	I–II
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	RABB	II–III
Rhopalodia gibba (Ehrenberg) O.F.Müller	RGIB	II
Rossithidium petersenii (Hustedt) Round et Bukhtiyarova	RPET	I
Sellaphora bacillum (Ehrenberg) D.G. Mann	SEBA	I–II
Sellaphora pupula (Kützing) Mereschkowski	SPUP	III–IV
Seminavis strigosa (Hustedt) Danielidis et Economou-Amilli	SMST	V
Stauroneis phoenicenteron (Nitz.) Ehrenberg	SPHO	I–II
Stauroneis smithii Grunow	SSMI	I–II
Stephanodiscus hantzschii Grunow	SHAN	IV
Surirella brebissonii Krammer et Lange-Bertalot	SBRE	I–II
Surirella capronii Brébisson et Kitton	SUCA	III
Surirella helvetica Brun	SHEL	I–II
Surirella ovalis Brébisson	SOVI	II
Surirella spiralis Kützing	SSPI	I
Tabellaria flocculosa (Roth) Kützing	TFLO	I–II
Thalassiosira weissflogii (Grunow) Fryxell et Hasle	TWEI	III–IV
Tryblionella apiculata Gregory	TAPI	III–IV
Tryblionella calida (Grunow) D.G. Mann	TCAL	IV
Ulnaria capitata (Ehrenberg) Compère	UCAP	II

of view, and/or present with greater abundance in the rivers studied. The denominations here adopted follow first of all Krammer and Lange-Bertalot (1986–1991), but also Round et al. (1990), Krammer (2002), and Lange-Bertalot (1995–2004, 2000–2003) for recent names.

Figure 1 presents the ecograms of all the diatoms found in the rivers of the central Apennine with reference to the parameters of pH, oxygen, trophism, saprobity, and salinity. From the first ecogram, it can be deduced that the great majority of the species is alkaliphile, in line with the pH values of these waters, which flow over prevalently calcareous substrata and have a moderately basic pH. Some examples of these are *Diatoma hyemalis*, *Amphipleura pellucida*,

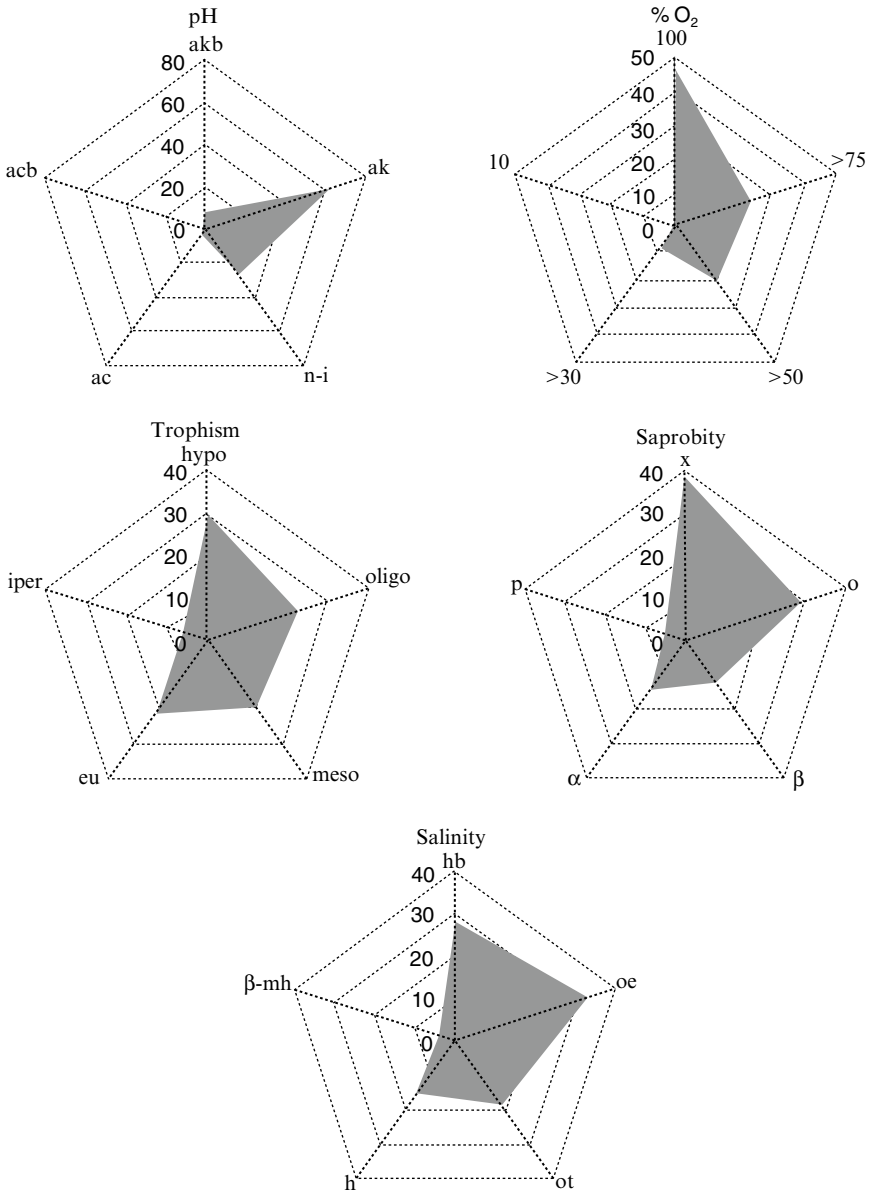

Figure 1. Ecograms of all the atoms observed in the Apennine rivers. pH: akb – alkalibionite species. % O_2: 100% – high, >75% – fairly high, >50% – moderate, >30% – low, 10% – very low. Trophism (species characteristic of): *hypo* hypotrophic environments, *oligi* oligotrophic environments, *meso* mesotrophic environments, *eu* eutrophic environments. Saprobity: *x* xenosaprobic species, *o* oligosaprobic species, *β* β-mesosaprobic species, *α* α-mesosaprobic species. Salinity: *hb* halophobe species, *oe* oligohalobe exigent species, *h* halophile species, *β-mH* β-mesohalobe species.

Cymatopleura solea, *C. elliptica*, *Epithemia argus*, *Ellerbeckia arenaria*, *Melosira varians*, and *Navicula oblonga*. There are also some alkalibionte forms, as *Anomoeoneis sphaerophora*, *Campylodiscus hibernicus*, *Diatoma vulgaris*, *Gyrosigma acuminatum*, *Navicula striolata*, and *Rhopalodia gibba*. Some forms considered acidophile rarely occur, such as *Achnanthidium subatomoides*, *Eunotia bilunaris*, *Navicula leptostriata*, *Neidium alpinum*, and *Nupela lapidosa*.

Considering dissolved oxygen, there are many diatoms that require the saturation value, among them: *Achnanthidium biasolettianum*, *A. minutissimum*, *Amphora normanii*, *Caloneis alpestris*, *Cymbella aspera*, *C. helvetica*, *Denticula tenuis*, *Diatoma hyemalis*, *Eucocconeis flexella*, *Fragilaria arcus*, and many others. Few and limited to the lower section of the watercourses, instead, are the forms that live in very poorly oxygenated environments, such as *Craticula accomoda*, *Cyclotella meneghiniana*, *Eolimna subminuscula, Fistulifera saprophila*, *Luticola goeppertiana*, *Mayamaea permitis*, *Naviculadicta seminulum*, *Nitzschia acicularis*, and *Eolimna minima*.

The trophic spectrum shows the prevalence of forms that require minimal or small quantities of nutrients, among them: *Achnanthidium bioretii*, *Cavinula cocconeiformis*, *Cocconeis pseudothumensis*, *Cymbopleura cuspidata*, *Denticula elegans*, *Diatoma mesodon*, *Diploneis elliptica*, *Epithemia argus*, *Gomphonema angustum*, *Meridion circulare*, *Navicula jakovljevicii*, *Planothidium lanceolatum*, *and Surirella spiralis*. Very well represented are also all the species that populate mesotrophic and eutrophic environments, while there is a reduced presence of those that prefer hypertrophic environments. Among the latter, there are various species of the *Nitzschia* genus, such as *N. capitellata*, *N. communis*, *N. sigmoidea*, *N. Tubicola*, and *N. umbonata*.

The saprobic spectrum highlights the dominance of xenosaprobic and oligosaprobic species, characteristic of the spring environments and mountain torrents of the watercourses studied. *Diatoma hyemalis*, *Eucocconeis flexella*, *Denticula tenuis*, *Diploneis ovalis*, *Fragilaria arcus*, *Stauroneis phoenicenteron*, and *Surirella spiralis* are some of these. At the extreme opposite, the polysabrobic forms, found for the most part in the lower section, are not numerous; among them: *Craticula accomoda*, *C. molestiformis*, *Luticola goeppertiana*, *Mayamaea atomus*, and few others.

The halobic spectrum sketches in broad strokes the same trend as the saprobic one; in this case as well, there is a sharp prevalence of forms that require low mineralization of the water body and that are found in the higher section.

Examples, among others, are: *Adlafia minuscula*, *Fallacia lenzii*, *Fragilaria virescens*, *Gomphonema clavatum*, *Platessa montana*, and *Tabellaria flocculosa*. However, there is also a small contingent of species that prefer fairly mineralized waters (β-mesohalobe forms), such as *Bacillaria paxillifera*, *Craticula halophila*, *Entomoneis paludosa*, *Mastogloia smithii*, *Navicula gregaria*, *N. veneta*, *Nitzschia filiformis*, and *Pleurosira laevis*. From the biogeographical point of view, of note are some interesting species rarely reported in the rest of the Italian hydrographic network, such as *Amphora thumensis*, *Cocconeis pseudothumensis*, and *Navicula*

jakovljevicii. They are found prevalently in cold, oligotrophic waters. In particular, *Navicula jakovljevicii* is a species from the Balkan region (Hustedt, 1945) and considered invasive for western Europe.

4. Diatoms as Indicators of the Biological Quality of Flowing Waters

All freshwater algae are sensitive to environmental conditions and are able to record the various events that occur in a water body. Among the algae, however, diatoms are the most useful for monitoring running waters. They are present in essentially all river environments, do not give particular problems for sampling, and react in a brief lapse of time (2–4 weeks) to changes in water quality. In addition, their taxonomy and autecology are largely well known. Thus, they afford an elevated degree of precision in assessing the trophic status and the degree of pollution of a water body.

Based on these premises, and further to all the researches carried out in the last decades above all, but not exclusively, in the Apennine rivers, a diatom-based eutrophication/pollution index, named EPI-D, was proposed as a pilot method for diatom-based monitoring of Italian watercourses (Dell'Uomo, 1996, 2004; Dell'Uomo et al., 1999; Torrisi and Dell'Uomo, 2001). This is an integrated index that takes into account the sensitivity of diatoms to nutrients, organic matter, and mineral salts dissolved in the water, especially chlorides. Therefore, its aim is to detect both phenomena of organic and mineral pollution, and eutrophication. Following Round (1981), the term eutrophication is here considered as the phenomenon of natural or man-made increase in nutrients, while pollution is intended as all agents, except nutrients, which are not produced under natural conditions. The EPI-D index uses Zelinka and Marvan formula (1961):

$$\text{EPI} - \text{D} = \sum a.r.i \,/ \sum a.r. \tag{1}$$

being the summary extended to all the diatoms of the site investigated. In this formula, "*a*" is the relative abundance of each species; "*i*" the sensitivity of the species to eutrophication and/or pollution, otherwise its ecological message; and "*r*" (reliability) is the validity of the message. The method used for attributing to all freshwater diatoms the values of "*i*" and "*r*" has been explained in the works above cited, where are also reported these values for the rheophile Apennine diatoms. The results provided by the EPI-D go from 20 to 1 according to the decreasing quality of the water, and lead to five main classes of water quality: excellent, good, mediocre, bad, and very bad. After its first proposal, the EPI-D was tested in various Apennine rivers with very good performances (Scuri et al., 2006; Torrisi and Dell'Uomo, 2006; Torrisi et al., 2008, 2010). A synthetic indication expressed by each species in terms of the biological quality of the water is given in Table 1. Figure 2 gives an example of a canonical correspondence analysis that took into consideration over 100 samples of diatoms collected from various environments of different Apennine watercourses. The 42 species reported are among the most

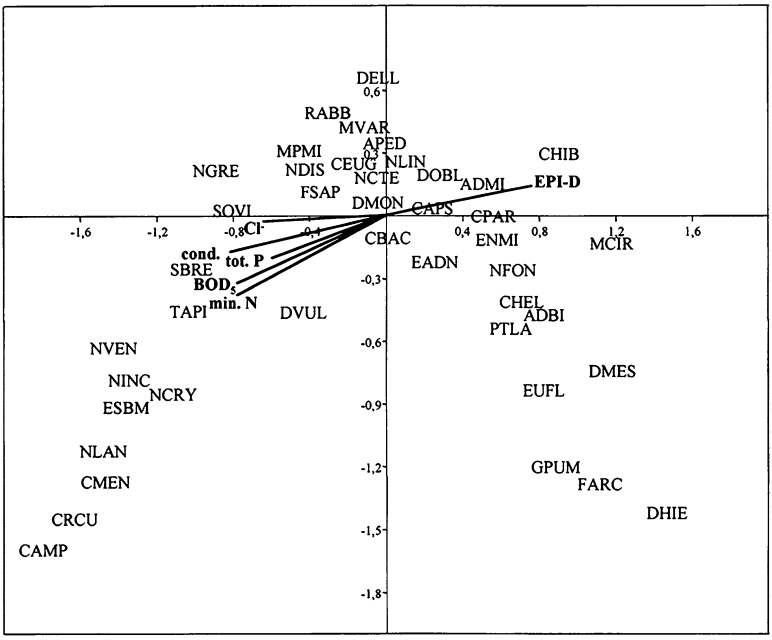

Figure 2. Canonical correspondence analysis among the EPI-D, the main physicochemical parameters which indicate eutrophication, organic and mineral pollution, and some of the most significant diatoms. ADBI *Achnanthidium biasolettianum*, ADMI *Achnanthidium minutissimum*, APED *Amphora pediculus*, CAMP *Caloneis amphisbaena*, CAPS *Caloneis alpestris*, CBAC *Caloneis bacillum*, CEUG *Cocconeis euglypta*, CHEL *Cymbella helvetica*, CHIB *Campylodiscus hibernicus*, CMEN *Cyclotella memeghiniana*, CPAR *Cymbella parva*, CRCU *Craticula cuspidata*, DELL *Diploneis elliptica*, DHIE *Diatoma hyemalis*, DMES *Diatoma mesodon*, DMON *Diatoma moniliformis*, DOBL *Diploneis oblongella*, DVUL *Diatoma vulgaris*, EADN *Epithemia adnata*, ENMI *Encyonema minutum*, ESBM *Eolimna subminuscula*, EUFL *Eucocconeis flexella*, FARC *Flagilaria arcus*, FSAP *Fistulifera saprophila*, GPUM *Gomphonema pumilum*, MCIR *Meridion circulare*, MPMI *Mayamaea permitis*, MVAR *Melosira varians*, NCTE *Navicula cryptocephala*, NDIS *Nitzschia dissipata*, NGRE *Navicula gregaria*, NFON *Nitzschia fonticola*, NINC *Nitzschia incospicua*, NLAN *Navicula lanceolata*, NLIN *Nitzschia linearis*, NVEN *Navicula veneta*, PTLA *Planothidium lanceolatum*, RABB *Rhoicosphenia abbreviata*, SBRE *Surirella brebissonii*, SOVI *Surirella ovalis*, TAPI *Tryblionella apiculata*. *Cond.* conductivity (μS cm^{-1} 20°C), Cl^- chlorides (mg l^{-1}), *tot. P* total phosphorous (mg l^{-1}), *min. N* mineral nitrogen (mg l^{-1}).

frequent, abundant, and ecologically significant. They are represented with the codes drawn from the OMNIDIA software (Lecointe et al., 1993).

The CCA shows a strong inverse correlation between the EPI-D and the main chemical parameters that indicate eutrophication, and organic and mineral pollution. Diatoms characteristic of environments of good or excellent quality are all located on the right of the graph and are positively correlated with growing values of the EPI-D. On the left side, instead, in direct correlation with the chemical parameters, which increase downstream, there are the species that tolerate degraded environments.

The EPI-D, developed primarily for the Apennine watercourses, has proven quite useful for rivers of other hydroecoregions in Italy and Europe, as testified by numerous applications conducted to date, which have yielded strongly positive judgments. Among others, in Italy: Battegazzore et al. (2004), Bona et al. (2007), Beltrami et al. (2009); in Europe: Gomà et al. (2004), Ács et al. (2004), Zgrundo and Bogaczewicz-Adamczak (2004), Rimet et al. (2005), Martín et al. (2010). In particular, Hlúbiková et al. (2007), after a detailed study conducted in influenced and noninfluenced sites of watercourses in the Slovak Republic, judged the EPI-D among the most suitable indices for assessing the ecological status of Slovak rivers, because it "correlates significantly with the relevant chemical and environmental variables and reflects the full range of pressures."

5. Conclusions

The number of diatoms found to date in the running waters of the central Apennine appears fairly high, if one considers that the rivers are short, rapid, and with little variety of environments.

As the pH and oxygen are concerned, the alkaliphile and oxyphile forms dominate sharply. In the saprobic spectrum, there is the prevalence of the xenosaprobic and oligosaprobic forms. The halophobe and oligohalobe species dominate in the halobic spectrum. A more uniform distribution is found in the various levels of the trophic spectrum, except for the last level (the hypertrophic one) which is very poorly represented. This highlights that the central Apennine watercourses are till now, as a whole, in a good condition.

In the upper torrential stretches, some species such as *Diatoma hyemalis* and *Eucocconeis flexella* occur; they are extremely sensitive to organic and mineral pollution and thus risk disappearing as anthropogenic pressure increases upstream.

From the biogeographical point of view, there are some interesting species rarely reported in the rest of the Italian hydrographic network, such as *Amphora thumensis*, *Cocconeis pseudothumensis*, and *Navicula jakovljevicii*.

Knowledge of the ecological characteristics of the individual species, furthered with all the observations conducted during recent decades on Apennine watercourses, has made it possible to develop an index (EPI-D) for biological monitoring of rivers that has proven to be of great usefulness and reliability, as testified by the numerous applications till now carried out in Italy and Europe.

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PART 4:
MARINE ECOLOGY

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Woelfel
Rüdiger
Schumann
Karsten
Lee
Villac
Kaczmarska
Bargu
Smith
Ozhan

Biodata of **Carolin Schlie, Jana Woelfel, Franziska Rüdiger, Rhena Schumann,** and **Ulf Karsten,** authors of "***Ecophysiological Performance of Benthic Diatoms from Arctic Waters***."

Carolin Schlie is a biologist interested in microalgae, especially taxonomy and ecology of Cyanobacteria and diatoms. In 2009, she finished her diploma thesis on molecular and morphological taxonomy of Cyanobacteria (*Chroococcales*) in shallow waters (Darß-Zingster Boddenchain, Baltic Sea) at the Institute of Biological Sciences at the University of Rostock, Germany. Since 2010, she is doing her Ph.D. on dark survival strategies of benthic diatoms from polar waters. This research includes, for example, analysis by HPLC as well as microscopic and molecular techniques.

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Jana Woelfel has finished her diploma studies at the Institute of Biological Sciences at the University of Rostock, Germany, on seasonal succession of microalgae in microbial mats in the wind flats of the Baltic Sea in 2004. Since 2005, she is working as Ph.D. student at the University of Rostock in a project relating to Arctic marine microphytobenthos and its primary production. Her research deals with the ecology of marine benthic microalgae, particularly diatoms, from extreme environments, and their ecophysiology, primary productivity and species composition.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 421–436
DOI 10.1007/978-94-007-1327-7_19, © Springer Science+Business Media B.V. 2011

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ECOPHYSIOLOGICAL PERFORMANCE OF BENTHIC DIATOMS FROM ARCTIC WATERS

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1. The Arctic Region as Habitat for Benthic Diatoms

The benthos of shallow water coastal zones in the Arctic region consists of a consortium of eukaryotic and prokaryotic microorganisms of high biodiversity. The dominant organisms are benthic diatoms, which form an assemblage referred to as microphytobenthos. This phototrophic community is generally known from temperate marine regions as being highly productive and providing a major food source for benthic suspension or deposit feeders (Cahoon, 1999), as filter for oxygen and other elemental fluxes at the sediment/water interface (Risgaard-Petersen et al., 1994) and as stabiliser of sediment surfaces by the excretion of extracellular polymeric substances (DeBrouwer et al., 2005). Consequently, microphytobenthos represents a key component in the functioning of trophic webs in many coastal regions. Some marine ecosystems, such as the German Wadden Sea, are mainly controlled by the production biology of benthic diatoms. However, structure and function of microphytobenthic communities are badly studied in the Arctic regions (Glud et al., 2009).

The Arctic is characterised by pronounced seasonal variations of the light regime, low temperatures as well as long periods of snow and ice cover. Shallow water communities are often strongly impacted by icebergs and sea ice, especially in areas with high wave action. Multi-year sea ice can reach depths of about 40 m in the Arctic (Gutt, 2001). In shallow waters, the seasons are characterised by short periods of photosynthetically favourable light conditions due to extended periods of darkness (polar night). The annual solar radiation is 30–50% less than in temperate to tropical regions at 80°N, which is the poleward distribution limit of phototrophic organisms (Lüning, 1990). The polar night lasts for about 4 months. This extreme light regime has strong implications for the primary production and seasonal growth of benthic diatoms. In addition, the long periods of darkness are further extended due to the formation of sea ice. If the ice is also covered by snow, irradiance can be diminished to less than 2% of the surface value. Consequently, phototrophic benthic communities may be exposed up to about 10 months of darkness or very low light conditions (Chapman and Lindley,

1980; Dunton, 1990). After sea ice break-up in spring, light penetrates deeply into the water. UV radiation (UVR) and blue light are, however, strongly attenuated in coastal waters because of the optical properties, which are influenced by particles and yellow substances. The 1% depth for UVB radiation, which represents more or less the threshold irradiance of UVB with the potential to affect primary plant productivity negatively, is located between 4 and 8 m on Spitsbergen (Hanelt et al., 2001). In summer, coastal water transparency in the Arctic regions decreases due to the development of phytoplankton blooms and the inflow of melt water carrying fine sediments. With increasing turbidity, light quality shifts from blue to green wavebands in deep waters (Jerlov, 1976). Consequently, microphytobenthic assemblages are exposed only to low irradiances even though the sun altitude is relatively high, i.e. about 34° elevation in July compared to only 14° in April at 79° North around noon. At a temperate location of 50° N, the sun elevation would be about 64° in summer around noon.

In contrast to the seasonally variable radiation conditions, water temperatures change only little between −1.8°C in winter and +5.0–6.0°C in summer in shallow Arctic waters, for example, in Kongsfjorden (Spitsbergen) (Hanelt et al., 2001). However, the inflow of melt water has considerable effects on the salinity and temperature regime in inshore waters in summer. During times of calm weather, stratified water bodies often occur with a layer of fresh water above a layer of denser sea water. However, due to vertical water mixing by wave action and wind, also deeper water zones may become affected and salinity decreases down to about 20-m depth (Hanelt et al., 2001).

High macronutrient concentrations are also present in the area of Spitsbergen, which obtains nutrient-rich water from the south during parts of the year (the so-called Spitsbergen current). This is one reason why the waters of the European Arctic belong to the most productive seas in the world (Orheim et al., 1995), but there is a considerable seasonal fluctuation of macronutrients. Nitrogen and phosphorus levels are relatively high during the winter months but both macronutrients are almost fully depleted in summer. However, sediment dwelling diatoms can exploit nutrients released by the underlying mineralising processes. They can also use dissolved nutrients from the water column, although they must compete here with phytoplankton. On the other hand, the pelagic community uses the down welling irradiance better compared to communities constrained on the sediment surface. Since the nutrient availability regulates the relative importance of phytoplanktonic versus microphytobenthic assemblages, oligotrophic settings generally favour the benthic production (Glud et al., 2009 and references therein). Since the coastal waters of the Arctic are mainly pristine and nutrient poor in summer, they can be expected to host a relatively important microphytobenthic production.

Due to many logistic constraints, basic research activities in the Arctic really begun few decades ago. Over the years, one particular fjord on Spitsbergen (Kongsfjorden) developed to a ‘high-latitude Arctic model ecosystem’ studied by scientists from different disciplines (Hop et al., 2002; Svendsen et al., 2002). Ny-Ålesund (78°55′ N, 11°56′ E) is the world’s northernmost permanent human settlement and situated at the Kongsfjorden on northwestern Spitsbergen.

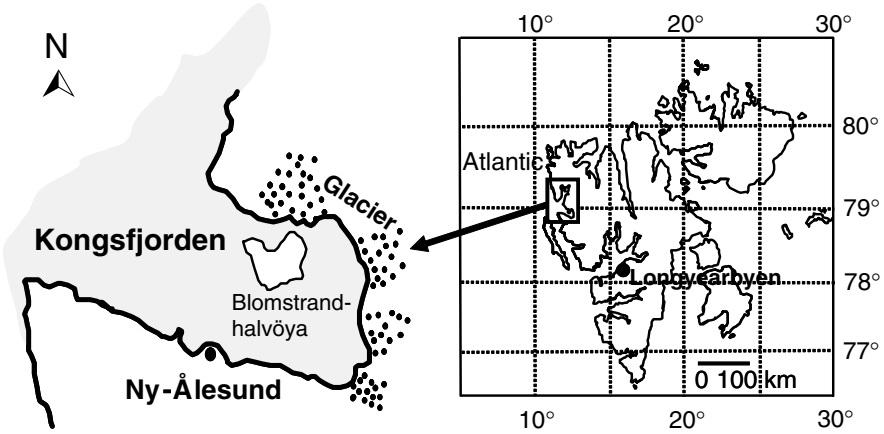

Figure 1. Map of Spitsbergen (*right*) with the Arctic model ecosystem Kongsfjorden near the international research platform Ny Ålesund at the northwestern coast (*left*).

Kongsfjorden extends 26 km from north–west to south–east, and the width ranges from 3 to 8 km. The fjord is influenced by the presence of four tidewater glaciers (Fig. 1). Maximum water depth is about 400 m and the tidal range is about 2 m (Svendsen et al., 2002). Since many decades, Ny-Ålesund has been used as an international research base for natural sciences in the Arctic (Wiencke, 2004), because it is easily accessible and has a well-developed infrastructure.

2. Primary Production of Benthic Diatoms in Arctic Waters

The Arctic shelf is characterised by high biomasses of infaunal and epifaunal organisms (Piepenburg et al., 1995; Sejr et al., 2000) as well as by high benthic mineralisation processes (Rysgaard et al., 1998), pointing to an important primary production in these high latitudes. Although pelagic and ice-related primary production can be high, this is of more local or regional significance, for example, at the ice edge. In addition, efficient microbial turnover rates for carbon and nutrients have been documented in the Arctic water column (Rysgaard et al., 1999), resulting in strongly reduced sedimentation events of particulate organic material. Consequently, heterotrophic benthic organisms do not benefit from the production of phytoplankton and ice algae and, hence, have to rely on benthic microalgae as the main food source (Glud et al., 2002). These authors were one of the first to document a high biomass and primary production of microphytobenthic assemblages in Young Sound, a fjord at the northeastern coast of Greenland. In their study, microphytobenthos accounted for about 40% of total benthic primary production (60% derived from macroalgae), indicating their ecologically important role for trophic relationships at these high latitudes.

While the ecological significance of diatoms in marine benthic habitats has long been recognised and studied extensively over decades in cold- to

warm-temperate and even tropical waters (Cahoon, 1999), the role of polar microphytobenthos has received much less attention. Moreover, the majority of studies on polar benthic microalgae have focussed on freshwater habitats and ice algae mainly from Antarctica (Wulff et al., 2009). This is surprising, because the Arctic Ocean is unlike other marine systems almost completely landlocked. A broad continental shelf of 5×10^6 km^2 in area is associated with the extensive land margin and accounts for 53% of the total Arctic Ocean (Pabi et al., 2008) and for approximately 22% of the global shelf area when related to depths <200 m (Menard and Smith, 1966). Since 1998, open water areas in the Arctic increased at a rate of 0.07×10^6 km^2 a^{-1}, particularly over the continental shelf (Pabi et al., 2008). As a consequence of these growing sea ice-free regions, pelagic production of the Arctic Ocean has increased by 5–6% annually in recent years as a result of increased light availability (Arrigo et al., 2008). In addition, Glud et al. (2009) speculated in their review that better light availability may also stimulate benthic primary production.

Primary production rates of benthic diatoms on sandy sediments of the Arctic Kongsfjorden (Spitsbergen) are similar to those in temperate waters despite lower water temperatures (Glud et al., 2009; Woelfel et al., 2010). Relatively high primary gross production rates of 2–62 mg O_2 m^{-2} h^{-1} seem to be associated with grain size, wave exposure and location in Kongsfjorden (Woelfel et al., 2010). In addition, increasing water depth down to 30 m in conjunction with decreasing light availability did not strongly inhibit production of the microphytobenthic assemblages. These data are well supported by the fact that benthic diatoms are capable to quickly and flexibly optimise their photosynthetic apparatus to the actual irradiance conditions (Glud et al., 2002; Karsten et al., 2006). Although the summer primary production rates of Arctic benthic microalgae may be high, the annual rates are low because of the long periods of light limitation. The ice-free summer of 90–120 days reflects undoubtedly the main period for benthic production. So far, not much is known to what extent Arctic benthic diatoms are able to cope with prolonged periods of low or even lacking light.

3. Ecophysiological Performance Under Different Light Conditions

As mentioned above, polar benthic diatoms generally live most of the time under low light conditions. However, these organisms have been shown to adjust very efficiently their photosynthetic activity to current radiation conditions (Kühl et al., 2001; Glud et al., 2002; Wulff et al., 2008, 2009). The shade acclimation of benthic diatoms in the Arctic was experimentally documented by the low light requirements for growth and photosynthesis in *Cylindrotheca closterium* (Reimann) Lewin isolated from sediment cores of Kongsfjorden (Figs. 2 and 3). This taxon is quite abundant in Kongsfjorden.

This species exhibited already at 15°C and under 0.5 µmol photons m^{-2} s^{-1} at continuous irradiation a relatively high growth rate of 0.38 day^{-1} (Fig. 2). A small rise to 3 µmol photons m^{-2} s^{-1} was accompanied by more than a twofold

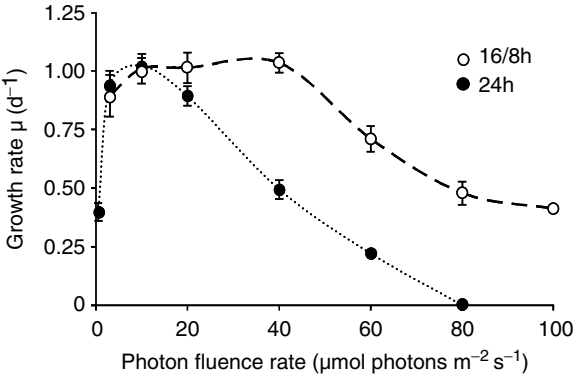

Figure 2. The effect of increasing photon fluence rates on the growth rate of the benthic diatom *Cylindrotheca closterium* isolated from a sediment core of Kongsfjorden, Spitsbergen. This species was kept at 15°C under continuous light or a 16:8 h light:dark cycle. Cells were grown in sterilised Baltic seawater enriched with sea salt (Sel marin hw professional, Wiegandt GmbH, Krefeld, Germany), vitamins and silicate resulting in a salinity of 33 PSU. Data shown represent mean values ± SD (n = 3).

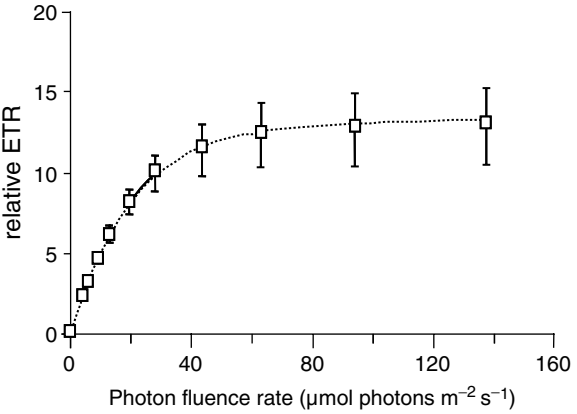

Figure 3. Relative electron transport rate under increasing photon fluence rates in *Cylindrotheca closterium* isolated from a sediment core of Kongsfjorden, Spitsbergen. Cells were acclimated for 72 h to 15°C and 3 μmol photons m^{-2} s^{-1} continuous irradiation. Algae were grown in sterilised Baltic seawater enriched with sea salt (Sel marin hw professional, Wiegandt GmbH, Krefeld, Germany), vitamins and silicate resulting in a salinity of 33 PSU. Data represent mean values ± SD (n = 3) and were fitted according to the photosynthesis model of Webb et al. (1974).

increase in growth (0.9 day^{-1}). Treatment with 10 μmol photons m^{-2} s^{-1} led only to a small additional stimulation of the growth rate up to the maximum value of about 1 day^{-1}. Further increases of the photon fluence rate to 20, 40, 60 and 80 μmol photons m^{-2} s^{-1} resulted in a linear decline of the growth response in *C. closterium* (Fig. 2). At 80 μmol photons m^{-2} s^{-1}, this species did not grow anymore. *Cylindrotheca closterium* exposed to the same photon fluence rates under a

photoperiod of 16 h light and 8 h darkness showed a slightly different picture. Although the growth response under the lowest photon fluence rates was unchanged, the optimum shifted from 10 to 20–40 µmol photons m^{-2} s^{-1} (Fig. 2). Further increases in photon fluence rate were also accompanied by a continuous decline of the growth in *C. closterium*, but the rates under all conditions were higher compared to continuous irradiation, and the cells still grew at 100 µmol photons m^{-2} s^{-1} with a rate of about 0.4 day^{-1} (Fig. 2).

These data are confirmed by a similar study on two *Fragilaria* species isolated as epiphytes from Arctic macroalgae. Both taxa grew also optimally, already at very low photon fluence rates of 10–20 µmol photons m^{-2} s^{-1}. Even at the lowest photon fluence rate tested (2 µmol photons m^{-2} s^{-1}), half of the maximum growth rate could be measured (Karsten et al., 2006). The ability of polar benthic diatoms to acclimate not only to extreme low light conditions but also to high light conditions has been shown in a number of studies mainly from Antarctica, emphasising that polar benthic diatoms are very well adapted to fluctuating radiation conditions (Wulff et al., 2008). In addition, vertical migration in the sediments contributes to light protective mechanisms.

The relative electron transport rate (rETR) as function of the photon fluence rate (PI-curve) was recorded for cells treated at 5°C for 72 h with low, but continuous irradiance (about 10 µmol photons m^{-2} s^{-1}) (Fig. 3). *Cylindrotheca closterium* exposed to these conditions exhibited an α-value of 0.6, a P_{max} of 12.8 and an I_k-value of 20.7. These PI-curve parameters clearly indicate adaptation to low photon fluence rates, and well confirm the low light requirements for growth (Fig. 2).

The low light requirements of polar benthic diatoms are also reflected in their dark survival potential as they can live at least up to 2 months in complete darkness (Wulff et al., 2008). From the literature, it seems that, particularly, polar diatoms have the capability to withstand long periods of darkness, which may be beneficial when considering the fluctuating and variable radiation conditions in Antarctica and the Arctic. The photosynthetic apparatus of dark-incubated temperate pelagic diatoms seems to be impaired already after few weeks as reflected by a very long recovery phase after re-irradiation. In contrast, light harvesting for photosynthesis and growth can fast resume in their Antarctic pendants after the polar night (Peters and Thomas, 1996). There are also reports that the survival of temperate taxa can be enhanced by lowering the dark incubation temperature (Antia, 1976). However, a systematic investigation of temperature effects on dark survival periods in benthic diatoms from the Arctic is lacking.

Experiments on the dark survival potential were performed also with the Arctic *Cylindrotheca closterium*. This species was kept for more than 5 months in darkness and subsamples were re-irradiated each month by continuous low photon fluence rates (10–15 µmol photons m^{-2} s^{-1}). After 1, 2, 3 and 5 months dark incubation, *C. closterium* exhibited high growth rates in the light (μ = 0.3–0.5 day^{-1}) within few days, indicating a high capability to withstand the polar night (Fig. 4). However, the longer the incubation time, the more the chloroplast size reduced. In the benthic diatom *Fragilaria striatula* from Spitsberghen, the length of the chloroplast

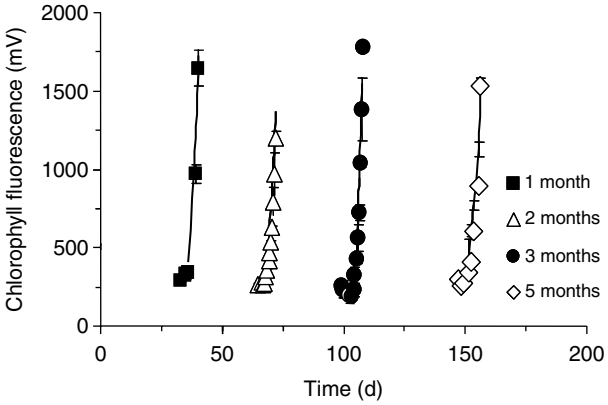

Figure 4. Dark survival potential of *Cylindrotheca closterium* isolated from a sediment core of Kongsfjorden, Spitsbergen. Cells were kept at 5°C for 1, 2, 3 and 5 months in darkness followed by re-irradiation with low photon fluence rates (10–15 µmol photons m^{-2} s^{-1}). Growth rates were measured as increase in chlorophyll fluorescence according to Gustavs et al. (2009). Cells were grown in sterilised Baltic seawater enriched with sea salt (Sel marin hw professional, Wiegandt GmbH, Krefeld, Germany), vitamins and silicate resulting in a salinity of 33 PSU. Data represent mean values ± SD (n = 3).

decreased from typical 9.8–13.3 to 5.9–9.6 µm after 3 months of dark incubation at 5°C, which equals about 30–40% (data not shown). However, the underlying mechanisms and physiological consequences are completely unstudied.

4. Ecophysiological Performance Under Different Temperature Conditions

Benthic diatoms in polar waters are preferentially growing in the subtidal on top of sediments and hard substrata at low, but relatively constant temperatures. As far as we know today, the temperature demand for the growth of Arctic benthic diatoms is somewhat higher than that of endemic Antarctic species. Two *Fragilaria* species from the Arctic Kongsfjorden (Spitsbergen) isolated as epiphytes on macroalgae with optimum growth rates at 12–14°C grew still well but with reduced rates at 0°C and did not survive 20°C (Karsten et al., 2006). Similarly, *Cylindrotheca closterium* from the Arctic grew between 0°C and 15°C and died at 20°C. At 0°C, already a relatively high growth rate of about one third of the maximum was measured (Fig. 5). The optimum growth temperature of *C. closterium* was between 8°C and 17°C, which clearly points to low to moderate temperature requirements.

Consequently, Arctic benthic diatoms can be characterised as eurythermal and psychrotolerant microalgae (organisms tolerant of low growth temperatures). This is in sharp contrast to a related Antarctic taxon, *Odontella litigiosa*, which typically exhibited maximum growth at 0°C and full inhibition of cell division already at 7–9°C (Longhi et al., 2003). Consequently, the studied Antarctic benthic diatoms were polar stenothermal and psychrophilic (organisms with a requirement

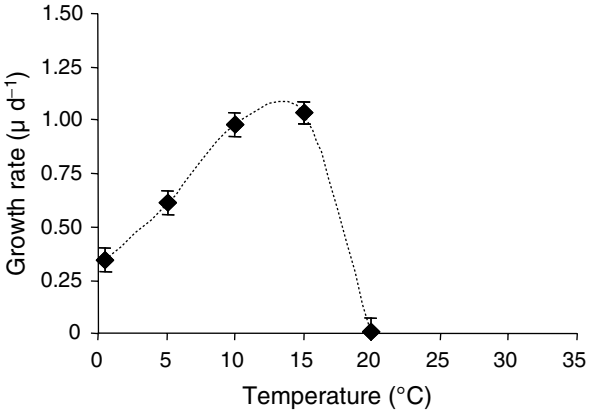

Figure 5. The effect of increasing temperatures on the growth rate of the benthic diatom *Cylindrotheca closterium* isolated from a sediment core of Kongsfjorden, Spitsbergen. Cells were grown in sterilised Baltic seawater enriched with sea salt (Sel marin hw professional, Wiegandt GmbH, Krefeld, Germany), vitamins and silicate resulting in a salinity of 33 PSU, and kept at 50 μmol photons m^{-2} s^{-1} under a 16:8 h light:dark cycle. Data represent mean values ± SD (n = 3) and were fitted according to Blanchard et al. (1996).

for low growth temperatures). These differences in the temperature requirements for growth in Arctic and Antarctic benthic diatoms can be related to the much longer cold water history of the Southern polar region (at least 14 million years) compared to the Northern high latitudes (approximately 3.5 million years) (Longhi et al., 2003), i.e. a longer exposure time and a higher degree of endemism in Antarctica. While the Antarctic benthic diatom taxon investigated are indeed characterised as endemic species, the respective information on strains from the Arctic is still missing. It is even still unclear, whether endemic Arctic benthic diatoms actually exist.

To successfully colonise low-temperature environments, polar benthic diatoms have developed adaptive, mainly physiological strategies. For example, strategies for cold adaptation are required for the maintenance of enzyme activity, for example, the photosynthetic key enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO). RUBISCO has a poor catalytic efficiency at low temperatures in isolated psychrophilic microalgae. Nevertheless, with decreasing temperatures, enzyme concentrations increased (Devos et al., 1998), a phenomenon known as quantitative strategy (Lesser and Kruse, 2004). The expression of many other genes encoding proteins involved in photosynthesis is upregulated under decreasing temperatures (Mock and Valentin, 2004). In contrast to temperature adaptation, acclimatory responses to transitory changes in the thermal environment are dependent upon sensor/signal pathways and involve, for example, modulations in rates of transcription or translation of enzymes (Morgan-Kiss et al., 2006).

Other adaptive mechanisms to low temperatures include the evolution of cold shock and antifreeze proteins, the modulation of the kinetics of key enzymes as well as the development of more fluid biological membranes by the accumulation of polyunsaturated fatty acyl chains (Morgan-Kiss et al., 2006). In association

with some sea-ice diatoms, such as *Navicula glaciei,* extracellular, secreted proteins are described to have an affinity for ice crystals (Janech et al., 2006; Raymond and Fritsen, 2001). Ice-binding proteins do not lower the freezing point. They rather seem to prevent membrane damages by inhibiting the recrystallisation of ice and, hence, may act as effective structural cryoprotectants (Janech et al., 2006). Various benthic diatoms from polar waters are known to contain high concentrations of low-molecular-weight solutes acting as organic antifreezing substances such as the amino acid proline and the tertiary sulfonium compound dimethylsulfoniumpropionate (DMSP) (Thomas and Dieckmann, 2002; Kasamatsu et al., 2004).

5. Ecophysiological Performance Under Different Salinity Conditions

The large discharge of melting water into nearshore waters during the summer months can temporarily decrease the seawater salinity. The effect of salinity on benthic diatoms and macroalgae from Arctic and Antarctic waters is generally badly studied in strong contrast to temperate regions (e.g. Kirst, 1990; Kirst and Wiencke, 1995). While temperate algae from the intertidal zone are generally euryhaline, subtidal organisms are more stenohaline.

Cylindrotheca closterium from the Arctic grew between 15 and 50 PSU (Fig. 6). While the growth rates were maximal between 15 and 40 PSU, growth was strongly inhibited at 50 PSU (Fig. 6). This species exhibited a growth pattern under the different salinities, which can be characterised as moderate euryhaline, because mild hyposaline conditions (<15 PSU) were accompanied by complete growth inhibition. The underlying mechanisms, such as osmotic acclimation, have not been studied so far in Arctic benthic diatoms.

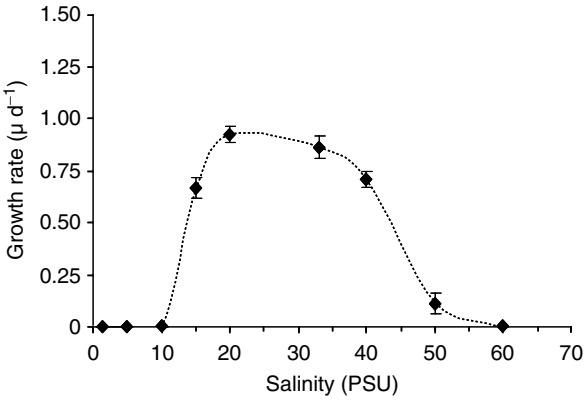

Figure 6. The effect of increasing salinities on the growth rate of the benthic diatom *Cylindrotheca closterium* isolated from a sediment core of Kongsfjorden, Spitsbergen. Cultures were incubated at continuous 50 μmol photons m^{-2} s^{-1} and 15°C. Cells were grown in sterilised Baltic seawater enriched with sea salt (Sel marin hw professional, Wiegandt GmbH, Krefeld, Germany), vitamins and silicate or diluted with distilled water resulting in different saline media. Data shown represent mean values ± SD (n = 5).

6. Conclusions

The microphytobenthos of Arctic shallow water zones is dominated by diatoms, which are highly productive during summer months. From the few data available on their ecophysiology, it can be concluded that these microalgae photosynthesise and grow at very low photon fluence rates. There are indications of low light requirements and adaptation, which well explain survival and performance under the often light-limiting and light-fluctuating conditions in the Arctic. On the other hand, sudden exposure to high irradiances can also be compensated by photoprotective and behavioural (vertical migration) mechanisms pointing to a high physiological plasticity. In addition, Arctic benthic diatoms seem to be capable to survive the polar night. However, the underlying mechanisms are not well understood. If environmental changes, such as the observed Arctic warming, are negatively affecting the dark survival potential of benthic diatoms during the polar night, their ecological function as important primary producers in polar regions may be strongly reduced with consequences for all higher trophic levels.

In contrast to Antarctic benthic diatoms, which can be characterised as polar stenothermal species, their Arctic pendants typically exhibit optimum growth temperatures between 10°C and 15°C and, hence, can be characterised as polar eurythermal organisms. These typical differences in the temperature requirements of benthic diatoms from Arctic and Antarctic waters can be explained by the much longer cold water history of the Southern polar region in conjunction with a high degree of endemism compared to the Northern high latitudes.

7. Acknowledgements

We thank Mandy Rickler for providing some of the ecophysiological data. The field work has been performed at the Ny-Ålesund International Arctic environmental Research and Monitoring Facility and under the agreement on scientific cooperation between the Alfred Wegener Institute and the University of Rostock. The authors thank the crew and divers at the AWIPEV-base in Ny Ålesund for technical support. We greatly appreciate financial support by the Deutsche Forschungsgemeinschaft (Projects Ka 899/12-1/2/3 and Ka 899/15-1) and by ARCFAC V (project nr. 026129–70).

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He first became interested in diatoms through studies of salt marsh aufwuchs food webs. These expanded into studies of niche partitioning and the heterogeneous distribution of diatoms, other algae and their consumers. Gnotobiotic studies with micro- and meiofauna led to his concept of food quality in lower food webs as an expression of informational energy flow. Practical application of the isolation and culturing skills acquired in studying benthic diatom ecology and physiology led to his long-time association with the National Center of Mariculture in Eilat, Israel. As part of a team, they discovered the particular species of diatoms that attracted abalone larvae to settle and metamorphose.

He had a long professional association with the late Dr. Charles Reimer, Philadelphia Academy of Sciences. Together they named many of the new species that were discovered in various studies.

He was one of the founders of the International Society of Symbiosis and served as its first President. He also has served as President of the Society of Protozoologists and was the Editor-in-Chief of the first and second editions of *The Illustrated Guide to the Protozoa.* He was named an honorary member of the Society of Protozoologists in 2001 and was given the Joseph A Cushman Award by the Society for Foraminiferal Research in 1999.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 437–464
DOI 10.1007/978-94-007-1327-7_20, © Springer Science+Business Media B.V. 2011

DIATOMS AS ENDOSYMBIONTS

DIATOMES AS ENDOSYMBIONTS**JOHN J. LEE**
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1. Introduction

No contemporary book on diatoms would be complete without a mention of diatoms as endosymbionts. It has been 20 years since the last review on the subject was presented at the 11th Symposium on Living and Fossil Diatoms in San Francisco (1990) to an audience primarily interested in diatoms (Lee, 1994). This review is an update for diatomists.

There are many examples of horizontally transmitted symbioses that had their origin in food organisms, that over time, evolved mechanisms to escape digestive processes. Thus, it is somewhat of a paradox, that although so many marine invertebrates and protists are major consumers of diatoms, only one group, the larger foraminifera, has become major hosts of them (Lee, 2006). Foraminifera seem especially well adapted to be the hosts for a broad spectrum of algae; some families host diatoms, dinoflagellates, chlorophytes, or unicellular rhodophytes, in addition to other minor endosymbionts (Lee and Hallock, 2000). Rationale for the preadaption of foraminifera for endosymbiosis has been recently summarized (Lee et al., 2010).

1.1. FORAMINIFERAN HOSTS FOR DIATOM SYMBIONTS

The four families of foraminifera that host diatoms are morphologically and taxonomically quite diverse. One family, Alveolinidae, belongs to the subclass Miliolina and three families, Amphistiginidae, Numulitidae, and Calcarinidae, belong to the subclass Rotalina. There is strong evidence that symbiosis with diatoms underlies the gross evolutionary morphological adaptations that each of the families has uniquely undergone in response to their symbiosis with diatoms. Each family, in its own way, has become larger (known by the general term "larger foraminifera" or lf for short), flatter, more compartmentalized, and has developed some system that facilitates nutrient exchange between the symbiotic partners and the external seawater environment outside the holobiont (Hallock, 1985; Hottinger, 1978, 2000; Leutenegger, 1984).

1.2. THE NUMMULITIDAE

The nummulitid foraminifera are well known because their fossil remains formed the limestone that was quarried to build the Egyptian pyramids. All of the living genera of the family are hosts for diatoms and we presume that symbiosis with diatoms drove the evolutionary morphological adaptations of these giant cells (Lee et al., 2010). To give you some idea of size, last summer Dr. George Kissil (National Center for Mariculture, Israel) discovered the largest nummulites ever reported lying beside the road between the Bahariya oasis and the Siwa oasis in the western desert of Egypt (28°46′32″N, 27°05′27″E). They were in excess of 4 cm in diameter (Fig. 1a, b)! Mountains of them remain in Giza, Egypt, and Wadi Taba Sinai (Fig. 1c–e). Nummulitids, as the name implies, are coin like in shape. One might think that with a shape that maximizes surface area and exposure of symbionts to light, there might be few other, internal or external, adaptations for symbiosis. But, in fact, they have very complex internal architecture. Externally, the tests of some of them reflect the depth of their ranges. For example, the test of *Assilina ammonoides* becomes thinner with depth (Fig. 1f). Interseptal blister-like pustules, which may act as light-condensing lenses, gradually inflate in size as the water depth increases. All nummulitids have a marginal cord and canal system. They have complex intraseptal canal systems that interconnect with each other with tubes and stolons and spiral canals in the umbilicus from which the pseudopods emerge (Hottinger and Dreher, 1974). There are pores in the surface of the test that have tubes that pierce the shell and reach the cell membrane of the chamberlets.

1.3. THE ALVEOLINIDAE

The alveolinids are fusiform in shape (Fig. 2a). *Alveolinella* has been observed to burrow in the upper layer of sediment and it has been suggested that this shape aids this behavior (Lipps and Severin, 1986). A reasonable explanation is that these forams hide under the shallow layers of coral sand as a means to regulate their irradiance (Hottinger, 1984); this is a hypothesis that remains experimentally untested. Alveolinid tests have a streptospiral involute coiling architecture. Tubiform chambers about half a whorl are flattened to become broad sheet-like spaces. These later become subdivided (Reichel, 1936, 1937). The apertures line up in a broad equatorial face and there are many additional polar apertures. Presumably, most feeding activity is by pseudopodia emerging from the polar apertures. Internally, there is a winding narrow coil of protoplasm called a columella that is formed by the chamber septa that meet at the shell pores. Interestingly, fusiform lineages have arisen twice in the history of lf: the fusulinids in the Pennsylvanian (they became extinct at the end of the Paleozoic) and the Cretaceous–Cenozoic aveolinids. There is no evidence for, or against, the possibility that diatoms, or their ancestors, were the endosymbionts of the fusilinids; however, since certified fossil formations are known and putative symbionts have been preserved and observed (Lee and Hallock, 1987), there is the possibility that molecular methods could answer this question.

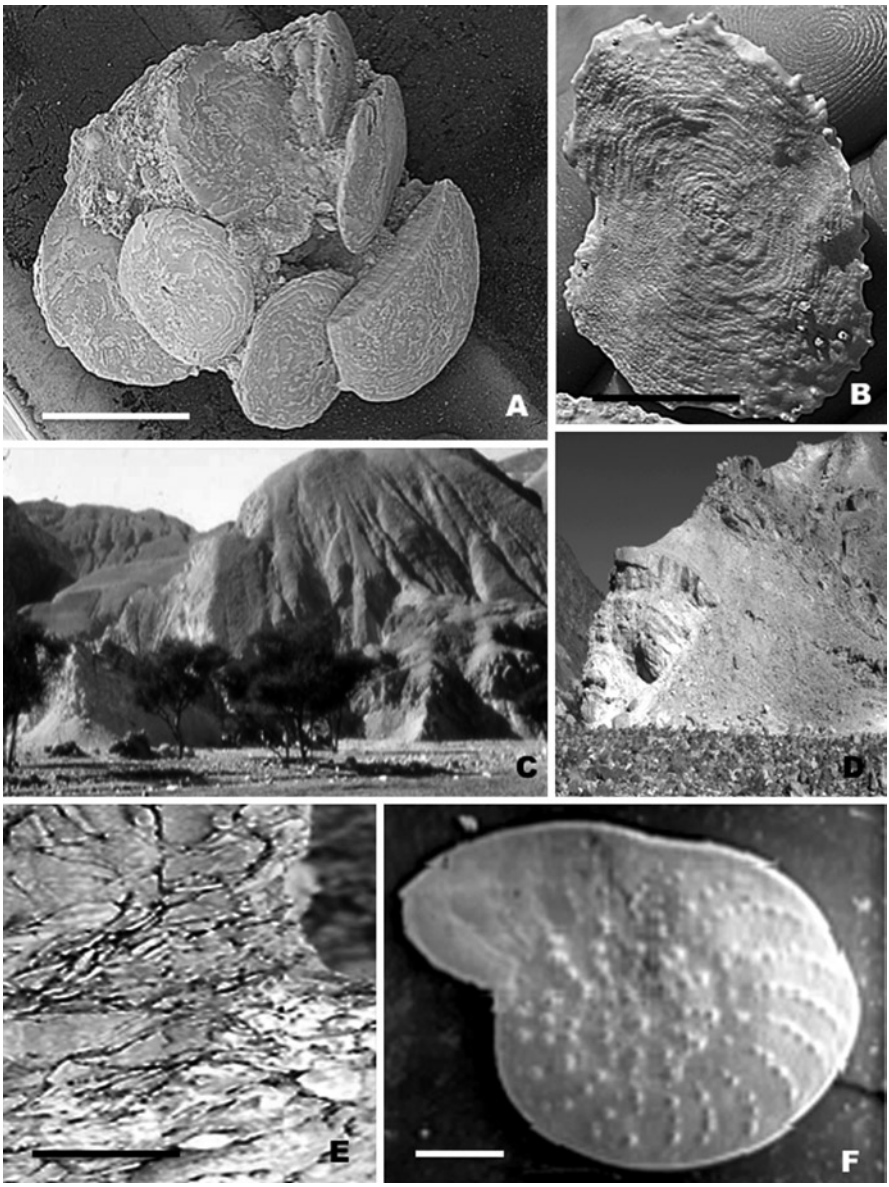


Figure 1. (**a**) Numulites in the soil of the great western desert of Egypt (scale = 4 cm). (**b**) Individual numulite in the palm of a hand. (Scale = 2 cm). (**c**) View of a mountain of nummulitic limestone in Wadi Taba, Egypt. (**d**) A closer look at the weathering edge of the mountain. (**e**) An even closer look showing that the limestone is a brecia of numulites; scale = 5 cm. (**f**) *Assilina ammonoides*; scale = 250 μm.

1.4. THE AMPHISTEGINIDAE

This distinct lineage of diatom-bearing foraminifera are cone-shaped foragers. They have evolved a cone within a cone shape. The single aperture to the test (Fig. 2b) is in the directrix of the cone so that the pseudopodia that emerge bind the organism to the substrate and pull the test along as foraminifera search for

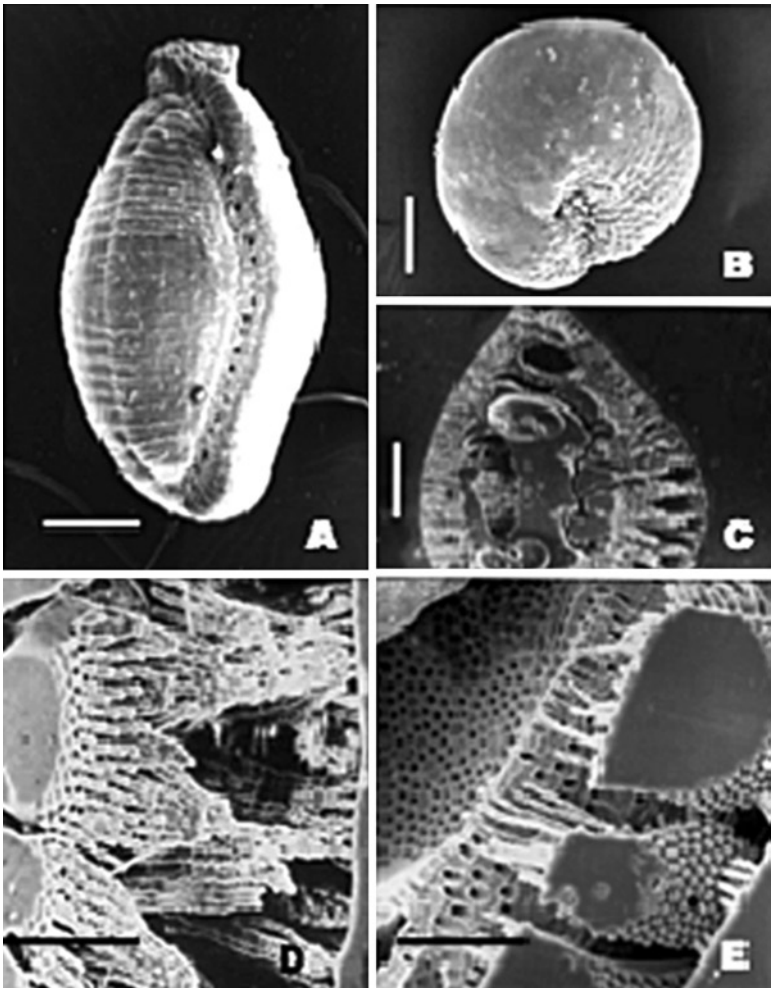


Figure 2. All figures are SEMs. (**a**) An alveoline foraminifera. Scale = 0.25 mm. (**b**) Umbilical view of *Amphistegina lobifera.* Surface is covered with fine pores. Scale = 0.5 mm. (**c**) Hottinger cast of *amphistegina lobifera*. White peripheral thread-like strands are pore liners. The pores bring seawater from the exterior of the shell to the cell membrane near each individual symbiont. Scale = 0.6 mm. (**d**) A higher magnification of (**c**). Scale = 150 μm. (**e**) Hottinger cast of a shell showing on the left the pore rims that house individual diatom symbionts, connecting pore-lining tubes and their connections to the next overlaying whorl of the shell. Scale = 100 μm.

food. The symbiotic diatoms are cortical on the vertex surface of the cone. In between the last whorl of chambers, there are stellar blind supplementary chamberlets. The blind diverticula of *Amphistegina lobifera* (see Hottinger, 2000; his text figure 11) remind one of the gut diverticula in sea slugs. The cell membrane surface of each chamber and chamberlet is bubbly (Fig. 2d, e). Each bubble (Fig. 2d, e) contains an individual symbiotic alga. A cup for each surface bubble is formed as an internal rim around a pore tube that runs through the shell wall (Fig. 2c) and presumably brings fresh seawater near to each symbiont (Leutenegger, 1979).

1.5. THE CALCARINIDAE

These unusual diatom-bearing hosts are well known as "star sands" to the peoples of the tropical pacific (Fig. 3a). In one place, the Ryukyu kingdom, their unusual characteristics gained them cultural and religious significance. Even today, one can buy small bottles of "star sands" in souvenir stores in Okinawa, Japan. The complexity of the canal systems in the calcarinids (e.g., Fig. 3c) is almost unbelievable and has attracted research almost from the beginning of micropaleontology (e.g., Carpenter, 1862). Hofker (1927) and Hottinger and Leutenegger (1980) have detailed the comparative anatomy of and taxonomy this group. Streams of pseudopods emerge from the spines of the stars which themselves are caniculate (Fig. 2b). Internally, the spinal canals are fed from enveloping canals at the distal dorsal end of the chambers. The chambers are connected with each other by multiple parallel stolons in alternating rows formed when previous structures are resorbed as new chambers are added. There are pores and canals through the shell (Fig. 2d), which probably allow nutrient exchange with the interior, but this remains to be tested experimentally. A recent cytological study of *Baculogypsina sphaerulata* by Hyams-Kaphzan and Lee (2009) found that the diatom symbionts are widely distributed in most of the chamberlets, especially in the umbilical and central parts of the test. In the proloculus and inner chamberlets, the symbionts were somewhat less abundant. The canal liners and the expanded internal pore domes were empty of individual symbionts. This is in contrast with *Amphistegina* where there is a special relationship between the symbionts and the pores. There is a strong inverse relationship in *Amphistegina* spp. between test thickness and habitat depth. Thicker tested forms tend to live in shallower, more turbulent environments, and thinner tested ones live in low energy and/or deeper habitats (Hallock et al., 1986).

1.6. THE ELPHIDIIDAE

This family has members that sequester photosynthetically active plastids only from diatoms (Correia and Lee, 2000, 2002a, b) (Fig. 4d). This phenomenon will be taken up separately in this chapter (Sect. 4), but it should be noted here that this family has no main aperture (Fig. 4a) and has evolved a unique and very complex canal system that is connected to openings (fossae) guarded by denticals (Fig. 2b, c)

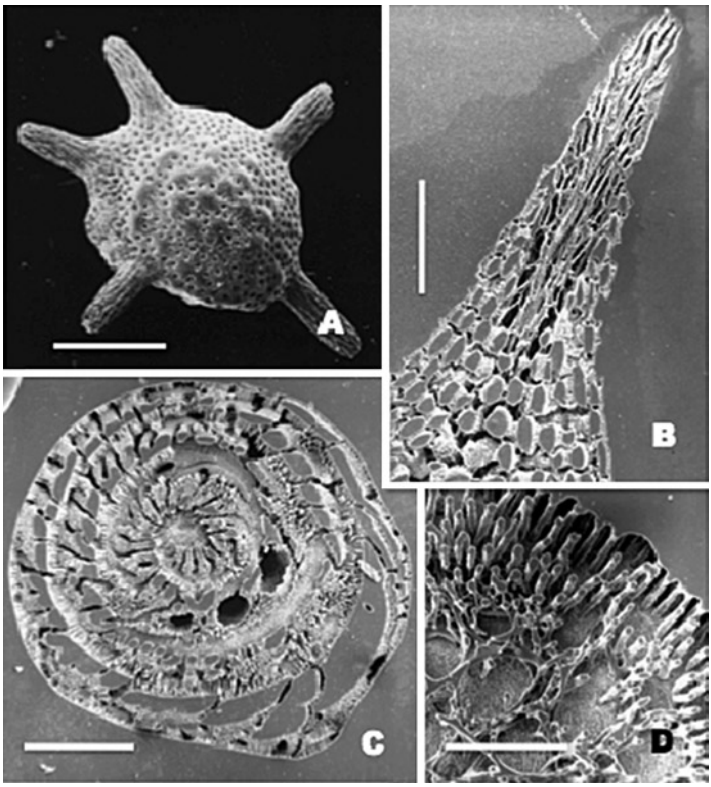


Figure 3. All figures are SEMs of *Baculogypsina sphaerulata*. (**b**–**d**) are Hottinger casts. (**a**) Exterior of test showing blisters and coarse pores. Scale = 750 μm. (**b**) Section through a spine showing canal system. Scale = 100 μm. Section through the central region showing coiled and overlapping growth with its interconnecting stolons. Scale = 100 μm. (**d**) Higher magnification showing stolon network. Scale = 25 μm.

that are somehow related to the kleptoplastid process. However, it should also be noted here that it has been reported that a totally unrelated microforaminifer also sequesters diatom plastids (E. Lanners, 1980; mentioned in Lee, 1983). In this latter case, there seems to be no special morphological adaptation to support the sequestration phenomenon. This is another intriguing facet of diatom symbiosis that remains to be experimentally probed.

2. Symbiotic Diatoms

2.1. METHODS OF SAMPLING OF POPULATIONS

From the outset, it must be clear that our knowledge of the diatoms involved in symbiotic relationships with foraminifera is very fragmentary and based on

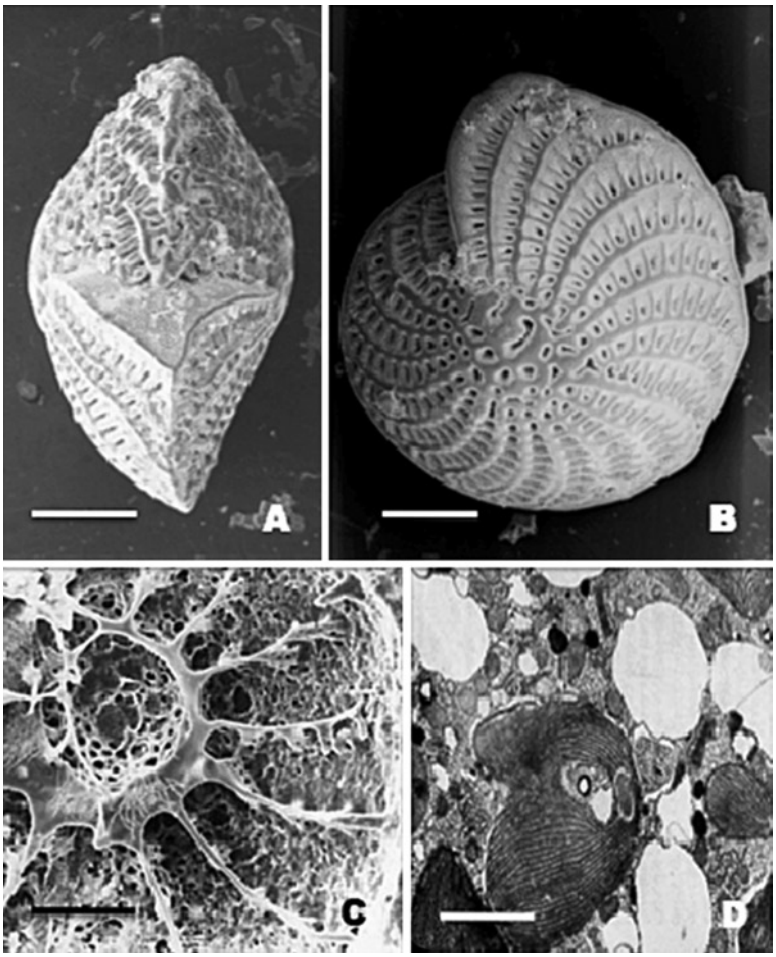


Figure 4. All figures are of *Elphidium*. (**a**–**c**) are SEMs. (**c**) is a Hottinger Cast. (**d**) is a TEM. (**a**) Edge view showing that there is no main aperture. Scale = 250 μm. (**b**) Lateral view showing fossae from which the pseudopods emerge. (**c**) The canal system. (**d**) Section showing Kleptoplastids..

opportunistic sampling. To place the methodology in context, it must be remembered that identifying the diatom species within foraminifera was begun in the late 1970s, long before molecular methods were available. It also must be acknowledged that, in general, the cell envelopes of endosymbiotic algae *in hospitae* are reduced, so that when the first modern studies of endosymbioses in foraminifera were begun in the early and mid-1970s, guesses were being made on which division of algae the symbionts might belong to (Leutenegger, 1977; Hansen and Burchardt, 1977; Dietz-Elbrächter, 1971; Schmaljohann and Röttger, 1978). It was common practice at the time to isolate the endosymbiotic algae and identify them by light and

electron microscopy (McLaughlin and Zahl, 1959; Freudenthal, 1962; Provasoli et al., 1957). With respect to the first diatom endosymbionts identified, it was a relief to know that the frustule-less diatoms, when released from their hosts, had not lost their abilities to form diagnostic frustules in culture (Lee et al., 1979). Although the methods for isolation of the diatoms have been modified over the years (e.g., Lee et al., 1980b, c, 1986, 1995; Lee and Correia 2005), the following has been the basic methodology:

1. The forams are brushed from their substrate (sea grasses, benthic rubble) with the aid of a #0 sable artist paintbrush into a petri dish containing a thin layer of sterile seawater.
2. Individually, they are picked up with the aid of fine forceps (Dumont #7) and transferred to a sterile petri dish with sterilized seawater.
3. They are then individually vigorously brushed with treated #000 sable brushes. (The treatment for the brushes is dipping them in 70% alcohol and letting them air-dry overnight.)
4. With the aid of alcohol sterilized fine forceps, the foraminifera are transferred to the wells of sterilized Pyrex® 9-hole spot plates containing 1 ml of sterile seawater. Once there, the plate is placed on the stage of a good-quality binocular dissecting microscope and the foram's surfaces are examined for any residual epiphytes. The turning and manipulating being aided with ethyl alcohol treated and dried #000 sable brushes. If need be, the foraminifera are brushed again and transferred to fresh sterile wells with sterilized seawater.
5. When satisfied that there are no surface contaminants, the foraminifera are transferred with the aid of sterilized forceps to fresh wells containing 1 mM HCl in sea water. This concentration is sufficient to gently decalcify the forams. There is usually gentle bubbling as the process of decalcification is observed under the microscope. If the bubbling stops, the decalcifying solution is replaced. Decalcification is complete when the test turns from opaque to transparent. (EDTA has also been used to effect the same change.)
6. At this point, the organic matrix of the test is easily torn apart with sterilized glass needles or fine forceps and the symbionts liberated.
7. There are a number of options that can be taken at this step. The symbionts can be transferred to liquid media or streaked on agar-solidified media. The solidified media have the advantage that it is usually easy to isolate axenic clone cultures and by examining many colonies, one can get a guess about the relative proportion of symbiont species that were in the host. The latter point may be spurious because the medium might be selective. In recent years, liquid media have been chosen. When the symbionts are isolated in liquid media, it is easier to detect multiple species. Following the traditional methods used at the time by Provasoli and the Haskins Laboratory group (Provasoli et al., 1957), the first diatom symbiont isolations were made in Erdschrieber and ASP_7 media. Common laboratory practice changed in the 1980s because many people found it difficult, or inconvenient, to make the soil extract needed to make Erdschrieber medium. Today it is standard practice to isolate symbiotic

diatoms using F/2, an "off the shelf" medium devised by Guillard (1975), purchasable from Sigma chemicals (Catalog #G9903), supplemented with Provasoli's antibiotic–antimycotic mix (Sigma Catalog #P8029). Incubation in the light at 23°C for 10–14 days is standard procedure.

2.2. PROFILE OF THE ISOLATES

After isolating the diatom symbionts from more than 3,500 hosts, we can safely conclude that the relationships between the partners are not finical. The same species of larger foraminifera has been found to host more than one species of diatom. In many cases, the foraminifera may host several species at the same time. The variety of diatoms hosted is not endless but is limited to a small number (≈25) of species. The most commonly isolated diatom symbiont from all species of hosts is one of the subvarieties or strains of *Nitzschia frustulum* var. *symbiotica* Lee and Reimer emend 2001 (e.g. Fig. 5a). While the variance in the length, width, fibulae, and striae stretch conventional boundaries for a diatom taxon, principal component analysis suggested that it was more reasonable to lump the isolates within a broad taxon rather than split them up (Lee et al., 2001). While the relative number of *N. f. symbiotica* varied in collections as widely separated as the Florida Keys, several stations in the Great Barrier Reef, Mombassa harbor Kenya, a number of stations on the west coast of the Gulf of Eilat, Israel/Egypt, Palu, and Sifnos Greece, overall it was found in ≈30–40% of the isolations (Lee and Correia, 2005). This species along with two other species of *Nitzschia*, *N. laevis* Hustedt and *N. panduriformis* var. *continua* Grunow in Cleve and Grunow, *Nanofrustulum shiloi* Lee, Reimer, and McEnery, *Amphora roettgerii* Lee and Reimer, and *Amphora erezii* Reimer and Lee, at one point (Lee et al., 2000) were isolated from over 75% of the associations. Less commonly isolated have been *Nitzschia valdestriata* Aleem and Husted, *Navicula hanseniana* Lee and Reimer, *Nav. muscatini*, *Nav. reissi* Lee, Reimer and McEnery, *Amphora tenerrima*, *Cocconeis andersonii*, *Protokeelia hottingerii* Reimer and Lee, *Achnanthes maceneryae*, and a few other yet to be described species (e.g., Lee and Correia, 2005; Mayama et al., 2000).

2.3. SIZE IS NOT A PROBLEM: NEW LIFE CYCLE OPTIONS

In their original description of *Nitzschia frustulum* var. *symbiotica*, Lee and Reimer (1982) noted that frustuleless diatoms studied to that date seem to be exceptions to the conclusions reached by Geitler (1932) with regard to the relationship of small size triggering the end of the vegetative stage. While it is true that many abnormal spheroid cells appear in cultures of *N. frustulum* var. *symbiotica* (illustrated in Lee et al., 2000), auxospore formation has yet to be observed in any culture. This, again, is a facet of symbiotic diatom biology that has not been addressed in any study.

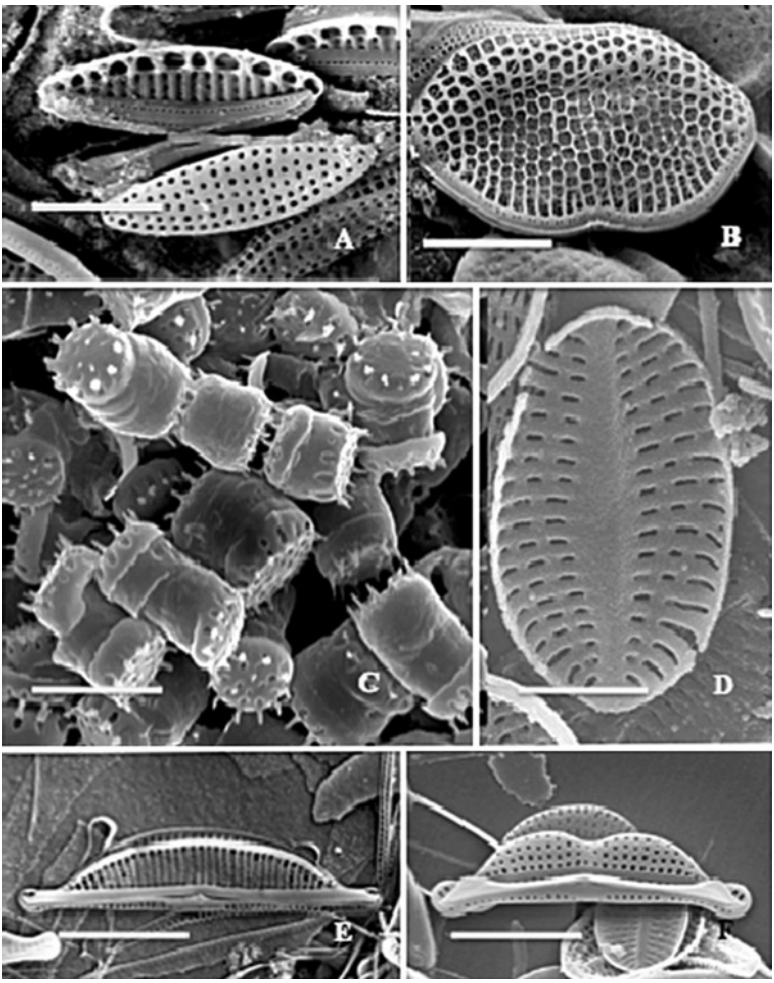


Figure 5. All figures are SEMs. Scale bars are 2.5 μm. (**a**) *Nitzschia frustulum* var. *symbiotica.* (**b**) *Nitzschia panduriformis*. (**c**) *Nanofrustulum shiloi*. (**d**) *Cocconeis andersoni*, (**e**) *Amphora tenerrima*. (**f**) *Amphora bigibba.*

On the other hand, it was noted that a high frequency of funnel-shaped (infundibuliform) frustules were found in a third (23/72) of the primary isolation cultures of *Nanofrustulum shiloi* made from hosts collected in Palau and Kudaka Jima. The frequency was high; ≈20 cells/1,000. Infundibuliform frustrules are formed of three distinct elements: (1) a normal valve, (2) a perforated cylindrical internal valve–girdle-like structure, and (3) a second internal valve that is thimble-shaped and flares out to produce a funnel-shaped structure. Flaring is also observed in copulae of the girdle (Fig. 6).

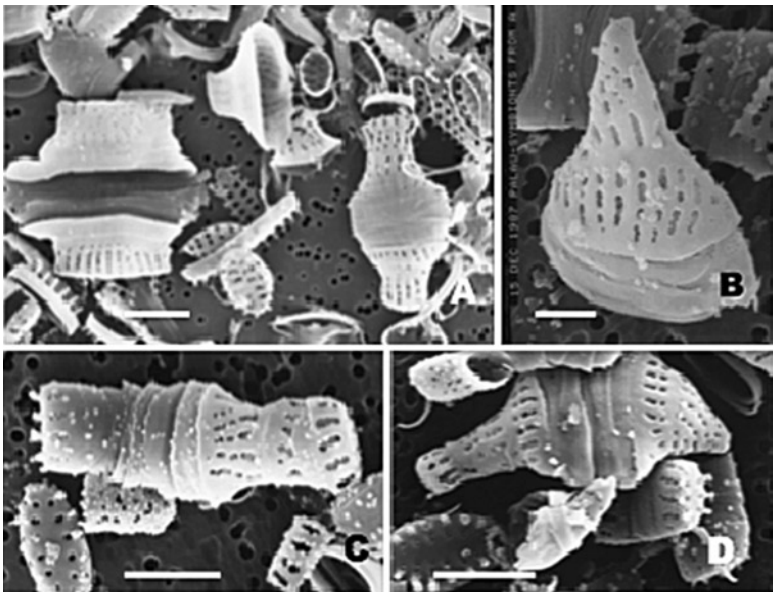


Figure 6. Infundibuliform frustrules of *Nanofrustulum shiloi* showing the wide variation in size regeneration even in the same culture. Scale bar = 3 μm.

Expansion in length (apical axis) (7.3 μm ± SD 1.78 μm, N = 100) varied from two to six times in the growth and cell division in a single generation (extremes 2.93 → 15.3 μm). Thus, these interesting endosymbiotic diatoms have retained a motif of size regeneration not normally expressed except when released from their hosts (Lee, 1995). While *Navicula muscatineii* is naked when it is in its host and it usually forms perfectly normal frustules in culture. However, in culture, it forms a small number of cells that do not fit the classic concept of an auxospore (e.g., Geitler 1932, 1969, 1979; Fritch 1935). It forms large spherical or dumbbell-shaped cells that could be considered autosporangia (Fig. 7a–d) (Lee and Xenophontos, 1989). The large cell walls are composed of fused ribbed scales. Some of the large cells are multinucleate.

2.4. CELL SIGNALING, ESTABLISHMENT OF SYMBIOSIS, AND MAINTENANCE OF THE SYMBIOTIC PHENOMENON

Since diatoms are a major component in the diets of many shallow water foraminifera, how do they recognize the difference between diatom as friends (symbionts) and diatoms as food? Fortunately, we have an answer to this question. Symbiotic diatoms have a recognition epitope on their surface. The protein profiles of diatom frustrules from 11 endosymbiotic isolates and 5 non-symbiotic isolates were

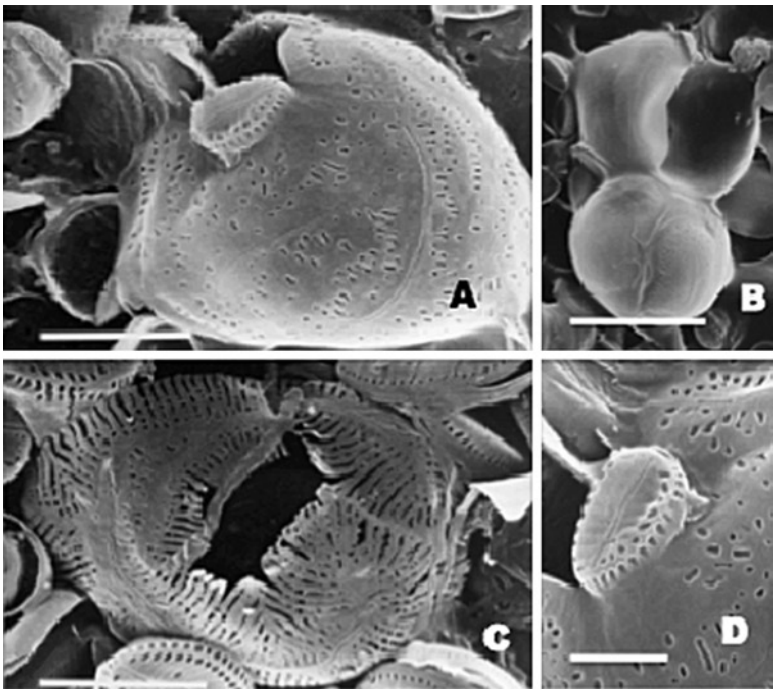


Figure 7. All are SEMs from cultures of *Navicula muscatineii*. (**a** and **d**) Autosporangium of *N. muscatinei.* Note normal valve fused to sporangium wall and missing scale next to it. Scale in (**a**) = 5 μm. (**b**) a low power view showing the hollow nature of an autosporangium once autospores are released. Scale = 14 μm. (**c**) A more oxidized autosporangium showing better its scale-like composition. Scale = 4 μm. (**d**) Enlargement of "(**a**)" more clearly showing the fusion of the valve on the sporangium. An autosporangium with several large missing scales. Scale 12 μm.

compared by immunoblotting them with polyvalent sera developed in rabbits against crushed and washed frustule fractions of symbiotic isolates of either *Nanofrustulum shiloi*, *Nitzschia frustulum* var. *symbiotica*, *Nitzschia panduriformis*, or *Amphora tenerrima*. A 104 kDa glycoprotein (CSSA, common symbiont surface antigen) was found on the surfaces of all the symbiotic species tested and was absent from the non-symbiotic species tested (Chai and Lee, 1999a, 2000). Blocking this antigen with antibody caused a loss of the ability of the diatom to bypass digestion and be drawn into the test to become an endosymbiont within the foraminifera. Using immunocytochemical and fine structural techniques, they found that receptors for the CSSA were abundant on the pseudopodia making initial contact with the diatoms and on the primary organic lining of the test. Thus, it is clear that the initial recognition between the host foraminifer and the potential symbiotic diatoms is mediated by a cell signaling system linking molecules on the surfaces of diatoms and the pseudopods of the foraminifera. This process takes place in the reticulopodial network outside the foraminifera. Soon after contact, the symbiotic diatom can be taken up by phagocytosis and subsequently brought into the interior of the

foraminifer away from the active digestive processes (Chai and Lee, 1999b, 2000). The CSSA is produced by the diatom even after it has lost its normal cell envelope, and it seems necessary to maintain the association even after the association is established (Chai and Lee, 2000). It seemed logical to see if the epitope on the surface of symbiotic diatoms was also found on other types of symbiotic algae found in LF. Lee and Reyes (2006) tested polyvalent antisera containing antibodies (including the CSSA) raised against the frustule fractions of three taxonomically diverse species of diatoms (*Nanofrustulum shiloi*, *Nitzschia panduriformis*, *Amphora tenerimma*) against *Symbiodinium* spp. isolated from dinoflagellate-bearing (soritine) foraminifera and from *Casseopea frondosa*. There was no reaction to the antisera, suggesting that a different surface antigen(s) or some other system must be involved in signaling between these dinoflagellates and their foraminiferan hosts.

2.5. DO THE SYMBIONTS REFLECT THE COMMUNITY IN WHICH THEY ARE FOUND?

Since the hosts have a loose fit with regard to their symbiotic diatoms, it was reasonable to hypothesize that the species they hosted were a reflection of the abundance of those species in their habitat; an easily tested hypothesis. Leaflets of *Halophila stipulacea* with abundant populations of *Amphistegina* spp., diatom-bearing hosts, were easily identified when diving with SCUBA. They were gently collected with minimal vibration. On shore, they were gently fixed with glutaraldehyde. Any epiphytes dislodged into the collection water were captured by filtration through a cellulose nitrate filter (Poretics, 0.45 μm). The leaves were critical-point dried and sputter-coated with 10-nm Pd, before they and the filtrate were examined in a scanning electron microscope (SEM). As part of the analysis, the endosymbionts isolated from the *Amphistegina* were morphologically compared to populations of diatoms living as epiphytes on the leaves and those ephiphytes washed from the leaves and (Fig. 8a–d). Of ≈10,000 diatoms recognized in the search, endosymbiotic species were rare (<<1%) or absent from the habitat. This suggests that the diatoms isolated as symbionts do not thrive in the native habitat of the foraminifera and the symbionts in their hosts are not a reflection of the most abundant diatom species available to the foraminifera as they feed.

2.6. IS IT POSSIBLE FOR SYMBIONT SPECIES TO BE REPLACED?

Because of amplified interest in global warming and worldwide reports of loss of coral habitats, there has been increased interest in endosymbiotic algae and their adaptive value in helping their hosts acclimatize to changing environmental parameters. While there has been great interest by coral researchers, there have been only two studies showing that it might be feasible that symbionts could be replaced in diatom-bearing larger foraminifera (Lee et al., 1983, 1986).

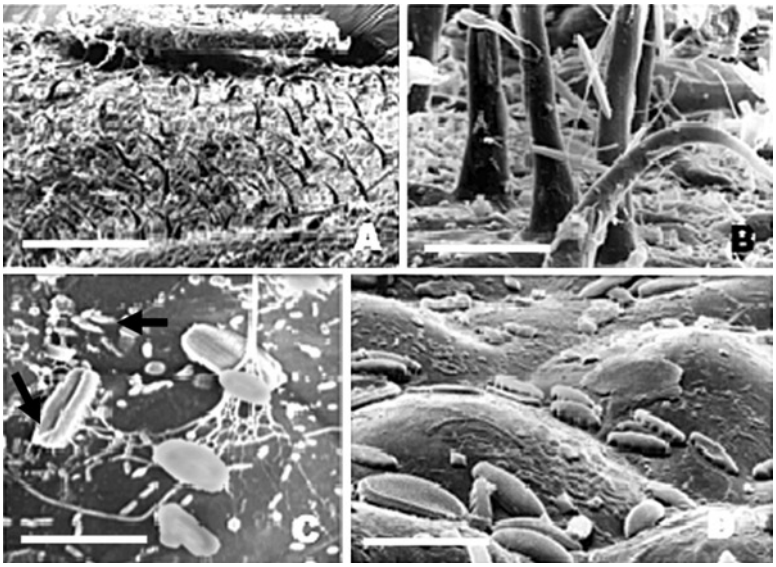


Figure 8. All figures are SEMs of the surfaces of Halophila leaves which supported abundant populations of symbiont-bearing foraminifera at the time of harvest from the Red Sea. (**a**) Low magnification (×30) showing an *Amphisauris hemprichii* feeding on the microflora on a leaf surface. (**b**) The "jungle" of the leaf surface showing many attached pennate diatoms. (**c**) A foraminiferan reticulopod (*arrow*) feeding on epiphytic bacteria and diatoms. (**d**) A view of the epiphytic community.

These experiments were carried out *in situ*. Plastic tissue culture flasks were modified by cutting circular windows (1.5 cm in diameter) in them and adhering nylon® filters (Millipore Cat. #SSWP14250) over the windows to allow free passage of seawater, but not the algae used in the trials. The flasks were anchored near the benthos seaward of the Steinitz Laboratory in Eilat Israel in the Red Sea (Gulf of Eilat) in custom-built translucent Plexiglas® racks. First the hosts, *Amphistegina lessonii*, a diatom-bearing host, were rendered nearly aposymbiotic by incubating them in seawater with DCMU (1×10^{-5} M (3–3,4-dichlorophenyl) -1,-dimethyl urea) in regular (unmodified) flasks at 10 m . They were then transferred to the windowed flasks and given randomized mixtures of diatoms (some isolated as symbionts, others isolated from the habitat) and 2 chlorophyte endosymbionts (*Chlamydomonas provasolii* Lee et al. and *C. hedleyi* Lee et al.) which had been isolated from *Cyclorbiculina compressa* d'Orbigny and *Archais anglatus* (Fichtel and Moll). One group of controls were treated with DCMU, but not exposed to any diatom mixture during the "re-browning" incubation. Another group of controls were untreated. After 4 weeks of incubation, the experiment was terminated and the hosts with their reestablished symbionts were examined. None of the free-living diatoms or the green symbionts was recovered from the "re-browned" hosts (Lee et al., 1983, 1986). Tritiated thymidine was used as a tracer to demonstrate that during the re-browning process diatoms were repro-

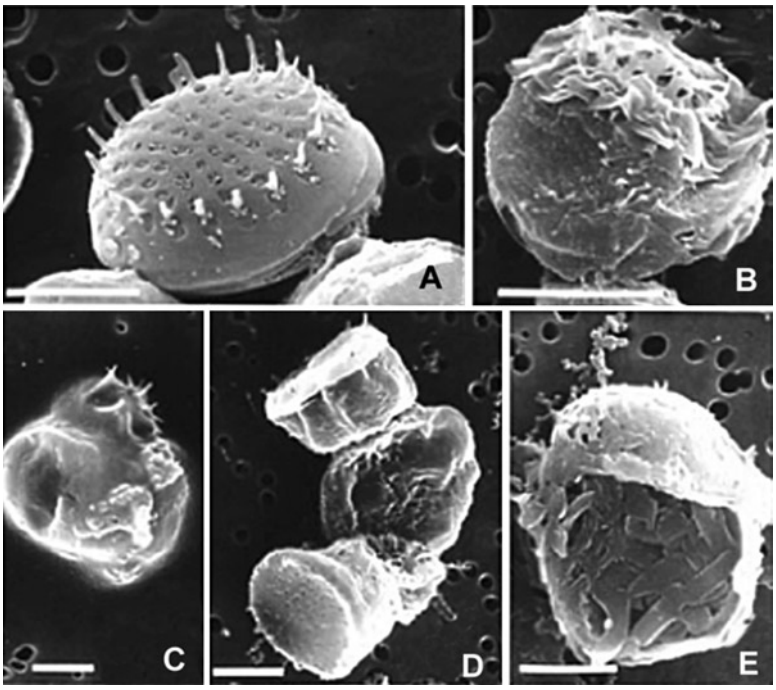


Figure 9. All are SEMs of *Nanofrustulum shiloi* after sterilized host homogenate was added to log phase axenic cultures. Scale bars 2 μm. (**a**) Control–normal cell. (**b**) Cell after five divisions with some frustule elements on part of surface. (**c**) Cell from culture examined after a week. No frustule elements. (**d**) Cell that divided shortly after the introduction of homogenate. (**e**) Cell in culture after a week with strange skeletal elements.

ducing within their hosts. Some endosymbiotic diatoms were recovered from the "re-browned" hosts more frequently than others, suggesting a "pecking order" of symbionts. When the aposymbiotic hosts were anchored at 10-m depth, *Nitzschia valdestriata* Aleem et Hust and *N. laevis* were the most successful species and *Nanofrustulum shiloi* the least. When the flasks were incubated at 20 m, *Nitzschia valdestriata* was more successful than *N. laevis*. While these experiments suggest the possibility of change, they are far from demonstrating change under more natural environmental incremental changes (e.g., seasonal) or dramatic stresses (e.g., storms). This is an issue begging for investigation.

2.7. NUTRITIONAL STUDIES

Only one study has concerned itself on the nutritional needs of symbiotic diatoms (Lee et al., 1980a). Isolates of *Nitz. frustulum* var.*symbiotica*, *Nitz. panduriformis*, *Nitz. laevis*, *Nitz valdestriata*, *A. tennerima*, *Navicula reissii*, and *Nanofrustulum*

shiloi required thiamin for continuous growth in axenic cultures. The *Nitzschia frustulum* var. *symbiotica* isolate also required vitamin B_{12}. Biotin stimulated the growth of six of the isolates tested. It seems reasonable to speculate that these vitamins would be acquired by the symbiotic diatoms from their hosts, who, in turn, obtain them in excess from the food they consume. Optimal concentrations of NO_3^{-1} varied among the isolates tested (0.2 μM–2 mM), but they exceeded the level found in the waters of the Gulf of Eilat by several orders of magnitude where the hosts were harvested. Again, this suggests that the host passes this nutrient to its symbiotic diatoms and that the host is a more favorable habitat for them than are open sea habitats.

2.8. PHOTOBIOLOGY

The patterns of the depth distribution of *Amphistegina* spp. in the Gulf of Eilat and the Red Sea suggest that light intensity and quality are the underlying factors for distribution of these larger foraminifera in the photic zone (Reiss and Hottinger, 1984; Hottinger et al., 1993; Hansen and Burchardt, 1977). Some support for this concept was obtained in manometric field experiments (Lee et al., 1980). They found that the photosynthetic rates of the holobionts were inhibited at natural surface light intensities, but not at reduced light levels. Also supporting this concept were behavioral observations on the phototactic behavior of three host species from the Gulf of Eilat (Zmiri et al., 1974; Lee et al., 1980a).

In one experiment, *Amphisorus hemprichii*, a dinoflagellate-bearing form, were placed in large tissue culture flasks that were wrapped in black plastic except for the end opposite the neck (Lee et al., 1980a). Light illuminated the flasks from the uncovered end. Neutral density filters were interposed between the light sources so that the intensity could be varied in different trial runs. Twenty-five specimens were placed in each flask along with adequate algal food. In nature, or in culture, when light is not so strongly unidirectional, individuals of *A. hemprichii* establish "feeding territories." They move away from each other when their reticulopodia come in contact. Eventually, they settle down and attach themselves to the substrate. In uncovered control flasks with moderate light, the organisms were randomly and widely distributed along the bottom and sides of the flasks. When the light was unfiltered (>30klx), *Amphisorus hemprichii* was negatively phototactic. At intensities between 10 and 20 klx, it was positively phototactic (Lee et al., 1980a). In the same experimental set up, *Amphistigina lobifera* were negatively phototactic at incident levels >10 klx and positively phototactic at intensities between 0.1 and 1 klx.

The photoadaptive responses of axenic cultures of four species of endosymbiotic diatoms isolated from the two species of larger foraminifera, *A. lobifera* and *Heterostegina depressa* were in consonance with distributional data and the phototaxic studies mentioned above (Lee et al., 1982). *Nanofrustulum shiloi* and *Nitz. laevis*, two diatoms isolated from *A. lobifera* grew fastest at 312 μW/cm^2. *Nitzschia*

laevis and *Nitz. valdestriata*, isolated from *H. depressa* grew best at much lower light levels (19 μW/cm^2). In nature, *A. lobifera* tends to be found at shallow depths than *H. depressa* (Hansen and Burchardt, 1977; Hottinger, 1977). The photosynthetic rates (measured by respirometry) of all four diatom species were depressed in high light 625 μW/cm^2 and all did well in moderate light (175 μW/cm^2). The photocompensation points of all four species were ~2% of the light level found at 1 m below the surface of the sea in Eilat in the summer (Lee et al., 1982).

3. Is the Holobiont System More Than the Sum of Its Partners? Do the Interactions of the Partners Bring New Characteristics to the Entire Holobiont System?

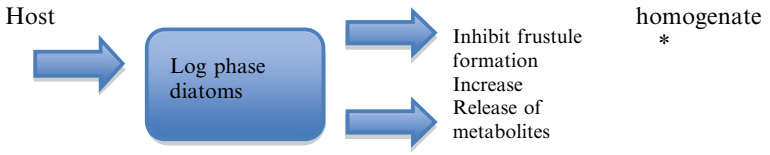
To some extent, these questions have been partially addressed. We have already touched upon the morphological changes noted in the host (Sect. 1), nutritional needs of the endosymbiotic algae (Sect. 2.7), and photobehavior of the holobiont (Sect. 2.8). In this section, several other aspects of holobiont interactions will be addressed.

3.1. ARE THERE MECHANISMS TO INCREASE METABOLIC EXCHANGES BETWEEN THE DIATOMS AND THEIR HOSTS?

Although we do not know any details of the mechanisms, one experiment has demonstrated that some factors present in the host cytoplasm inhibit both normal frustule formation and the release of metabolites synthesized after photosynthesis by the diatoms. In their experiment, Lee et al. (1984) carefully brushed and washed *Amphestigina lessonii* and *A. lobifera* and crushed them before squeezing the resulting homogenate through a sterile Swinney filter (0.45 μm pore). The filter-sterilized homogenate was then inoculated into axenic log phase cultures of symbiotic diatoms.

The homogenate affected the formation of new frustules (Fig. 9). After the first division, the cells were half naked and the next generation cells were spherical. The affect was variable among the species tested; *Nanofrustrulum shiloi* was the most sensitive under the test conditions with no vestiges of a frustule. Other species had disorganized strips of silicon on their surfaces. The researchers concluded that "host substances" are probably responsible for the maintenance of the frustule-less state *in hospite* and that if potential symbionts escape digestion, they lose their frustules as they grow and divide to fill host niches.

In a second experiment using $H^{14}CO_3^{-1}$ as a tracer, host homogenate increased the levels of ^{14}C labeled photosynthate released by the diatoms (Lee et al., 1984). The increase ranged from 190 to 9,000%. A similar host homogenate effect was noted in studies of zooxanthellae of cnidarians (Muscatine, 1967; Sutton and Hoegh-Guldberg, 1990).



3.2. GROWTH OF THE HOLOBIONT, FEEDING, AND CARBON BUDGETS: JUNK FOOD? WHERE DOES THE CARBON IN THE SHELL $CA(CO_3)_2$ COME FROM?

Which is more important in the carbon budget of holobiont: feeding by the host or photosynthesis by its diatom symbionts? Though it would seem that it would be simple to measure feeding rates, several factors make it difficult to make accurate assessments. First, feeding in foraminifera is episodic. Second, everything that is captured is not ingested, digested, or assimilated. Third, there is a great deal of recycling of nutrients between host and its symbiotic algae, a factor that needs to be carefully considered when using radionuclide tracer methodology.

One of the simplest first questions to ask is: can you prove that the diatoms that are being gathered by the foraminifera are being digested? Faber and Lee (1991a) addressed this in their study. *Assilina ammonoides* (Gronovius) Reiss and Hottinger and Heterostegina were collected in a *Halophila stipulacea* (Forssk.) Asch. sea grass meadow seaward of Taba. They were assayed using Napthol AS-BL phosphate for the presence of acid phosphatase, an indicator of digestive activity. Acid phosphatase was found in the food mats around the specimens, in the youngest chambers, along the marginal canal system and in the oblique plexis. The enzyme was not detected in the secondary or lateral canals or near the endosymbionts (Faber and Lee, 1991a). The scope of the answer to the question mentioned above was expanded to include more organisms (six species of larger foraminifera which were hosts for endosymbiotic diatoms, five species that sequester diatom plastids, and three species not known to host any symbionts) (Lee et al., 1991). Acid phosphatase was detected in all the foraminifera near their apertures and around the periphery of the shell and in the reticulapodial net. In many, it was detected in the last few chambers. It was never found near the location of the endosymbionts. The researchers reasoned that since algal endosymbiosis has arisen many times in the evolution of foraminifera, and involves a wide variety of algal types, it is possible that the extracameral initial digestion steps, coupled with intracameral partitioning, could be a fundamental property preadapting foraminifera for symbiosis.

Because of their abundance near the Inter-University H. Steinitz Biological Laboratory on the Gulf of Eilat, Red Sea, some of the most detailed studies on feeding, carbon budgets, and calcification have used *A. lobifera*, *Amphisorus hemprichii* (dinoflagellate-bearing), and *Peneroplis planatus* (Fichtel et Moll) (rhodophyte-bearing) as experimental organisms. Selective feeding was found in *P. planatus*. It ingested five times more ^{14}C labeled *Cocconeis placentula* Ehrenberg

and *Amphora* sp. than other algal species tested (Faber and Lee, 1991b). *Peneroplis planatus* did not grow if starved. It grew slowly when fed, but incubated in the dark. This organism was unusual in that its assimilation rates for some algal species was very high (~100%) for the first 24 h. The data suggested that even though light is necessary for the growth of *P. planatus*, it acquires most of its carbon and energy for growth from food and cannot grow solely on carbon compounds fixed, transformed, and released by its endosymbiotic algae (Faber and Lee, 1991b).

The photobiological effect on foraminiferal growth and calcification has been demonstrated many times (Lee and Zucker, 1969; Duguay and Taylor, 1978; Muller, 1978; Hallock, 1981; Röttger et al., 1980; Duguay, 1983; Kuile and Erez, 1987). Kuile and coworkers (1987) starved their experimental organisms, *Amphistegina lobifera* and *Amphisorus hemprichii*, before beginning their feeding experiments. Under these experimental conditions, which were attempting to model the episodic feeding behavior observed in the microscope, feeding was initially voracious and then slowed down after 8–24 h. Less than 5% of the carbon taken up as food ended up being incorporated into the test (shell). *Amphistegina lobifera* was used in an experiment to test whether dissolved inorganic phosphorous or nitrate in the medium could be a substitute pathway for these nutrients gained by feeding. Both enhanced growth for at least 2 weeks. Growth was five times greater in fed, or medium-enriched organisms, than it was in starved ones. Fed organisms grew slightly faster than medium-enriched ones. The growth of *Amphisorus hemprichii* was stimulated twofold in a parallel experiment. The researchers concluded that their observations indicated that *A lobifera* uses feeding mainly as a source of nitrogen and phosphorus, while *A. hemprichii* relies on food to satisfy its energy and carbon requirements, as well as nitrogen and phosphorus (Kuile et al., 1987).

Calcification rates of *A. lobifera* in the Gulf of Eilat ranged from 938 to 3,481 μg Ca/g $CaCO_3$ h (Erez, 1978) This rate was five times higher than the average calcification of the coral *Stylophora pistillata* Esper on the same reef. The enhancement of the calcification of *A. lobifera* in the light over that of controls was estimated to be ~×50 compared to an average of ×11 for corals. Stable isotope disequilibrium studies also provide evidence that symbiotic algae produce a light-dependent vital effect on shells of their hosts. (Buchardt and Hansen, 1977; Erez, 1978). The isotopic composition of the shell ($^{13}C/^{12}C$, $^{18}O/^{16}O$) becomes an average 1.5% lighter $\delta\ ^{18}O$ for symbiont-bearing species than the expected equilibrium value (Buchardt and Hansen, 1977; Erez, 1978). Controls, mollusc shells, and non-symbiont-bearing foraminifera were in isotopic equilibrium with the surrounding seawater. Kuile and coworkers (1988) did a very interesting experiment with *Nanofrustulum shiloi*. They asked the question whether this endosymbiotic diatom is uniquely adapted to be a symbiont with respect to its ability to accumulate inorganic carbon (Ci) at the photosynthesis carboxylating site. Internal accumulation of Ci was not demonstrated, suggesting *N. shiloi* does not possess the postulated active uptake mechanism for Ci accumulation. Calculations suggested that the generation of CO_2 from HCO^{3-} was sufficient under all experimental conditions to support the photosynthetic rates measured.

In other experiments, Kuile (1991) and Kuile et al. (1989a, b) concluded that *A. lobifera* and *Amphisorus hemprichii* differ also in their calcification mechanisms.

Experiments using DCMU and carbonic anhydrase suggested that there is a competition for inorganic carbon between photosynthesis and calcification in *A. lobifera*, while he found none in *A. hemprichii.* Observations led him to conclude that the symbionts in *A. lobifera* take up inorganic C in the form of CO_2 from the seawater and the CO_2 deposited in the test comes from an internal pool destined for this purpose. *Amphisorus hemprichii* does not have an internal pool. The CO_2 uptake is not energy dependent and is more easily modeled by diffusion.

4. The Kleptochloroplast Story

As mentioned earlier in this chapter (Sect. 1.5), there is a family of foraminifera, Elphididae, with complicated canal systems and modified apertures and fossae, which is highly evolved to retain diatom plastids (~$1 \times 10^{2-4}$ host cell^{-1}). While this aspect of foram–diatom interaction has barely explored, some aspects of the phenomenon are known: (1) the plastids are functionally undergoing photosynthesis; (2) diatom plastids are the only plastids retained; and (3) the plastids have a predetermined life, but they persist for a long time.

4.1. ABUNDANCE

Studies of this symbiotic phenomenon by Correia and Lee (2000) showed that in culture, each *Elphidium excavatum* retained ≈ 3.7×10^4 plastids. This number is slightly higher than the number that Lopez (1979) found for fresh specimens of *Elphidium williamsonii* (9.7×10^3), *E. excavatum* (1.2×10^3), and *Haynesina germanica* (5.2×10^3) collected from the shallow waters of Limfjorden, Denmark. Lopez estimated that under normal conditions at Limfjorden, individuals of the former host species needed to capture at least 65 plastids h^{-1} and while individuals of the latter host needed to capture 20 plastids h^{-1} (Lopez, 1979). Lee and Lee (1990) noted that lower numbers of plastids were sequestered by *Elphidium crispum* collected near Drake's Island in Plymouth Harbor, England.

4.2. PRIMARY PRODUCTION OF THE HOLOBIONTS

Diatom plastids sequestered by species of Elphidiidae are as photosynthetically active as they are in their natural state (Lee et al., 1988; Lopez, 1979). Using tracer labeled $H^{14}CO_3^{-1}$ to measure primary production in the holobiont, *Elphidium williamsoni* fixed carbon at a rate of 2.3 μg C ash-free dry weight^{-1} h^{-1} and *Haynesina germanica* fixed at a rate of 0.5 μg C ash-free dry weight^{-1} h^{-1} (Lopez, 1979). Specimens of *Elphidium* from the Gulf of Eilat, Red Sea fixed 1.5 μg C ash-free dry weight^{-1} 48 h^{-1} (Lee et al., 1988).

4.3. ARE ALL CHLOROPLASTS AND PLASTIDS EQUALLY VIABLE IN THE FORAMINIFERA?

In an experiment, Correia and Lee (2000) isolated axenic cultures of algae from the natural habitat of *Elphidium excavatum* collected in Lake Tashmoo, Martha's Vineyard, Massachusetts. The test algae included four diatom species, a dinoflagellate, and four unicellular chlorophytes. Each experimental group of *Elphidium* was fed one of the algal cultures or a mixture of algae and incubated in a 12 h light/dark cycle or in complete darkness. Starved foraminifera were the baseline controls. The number of plastids retained by each host was determined by viewing their autofluorescence in a laser scanning confocal microscope. Diatom plastids ($\sim 3.7 \times 10^4$) were the only plastids retained.

4.4. HOW LONG DO THE PLASTIDS LAST AFTER THEY HAVE BEEN CAPTURED?

Using HPLC to study pigments extracted from *H. germanica* from a UK salt marsh, Knight and Mantoura (1985) found very little phaeophytin (degradation products of chlorophyl). They concluded from their results that proportionately little digestion was taking place at one time. Only diatom plastids were retained by *E. excavatum* (Correia and Lee, 2000). Experiments with starved *E. excavatum* in the laboratory indicated that plastids decreased over time. The number of plastids remaining in the cytoplasm of the foraminifer was higher for foraminifera that were incubated in a 12 h day/night cycle than it was for those incubated in complete darkness. The half-life of the plastids of *Amphora coffeaeformis* was estimated to be ≈9 weeks (Correia and Lee, 2002a, b).

5. Conclusions and Future Directions

More questions are raised by the state of our knowledge of the interaction of diatoms and foraminifera than have been answered in the past 40 years when the renaissance in research on algal symbiosis in foraminifera began. From the perspective of the diatomist, only the superficial question of why are foraminifera hosts for diatom symbiosis has been partially answered. Why isn't the phenomenon wider spread among other protists and marine invertebrates? Why is the CSSA restricted to so few species of diatoms? Why are the host–symbiont relationships less than specific? Are there any host–symbiont relationships that are more restrictive? Are there particular species of diatoms that are favored as symbionts at different depths? Do the hosts change symbionts with the seasons? Although we can glibly conclude that symbiosis with diatoms has driven the morphological

evolution of 4 lines of ordinary sized foraminifera to become giants, the physiological and genetic mechanisms which drove these changes challenge intellectual curiosity. What is so special about diatoms belonging to the *Nitzschia frustulum* var. *symbiotica* complex that makes them the most frequent cosmopolitan symbiont? How does the host inhibit diatom frustule formation? Since the diatom frustule has no survival value in the host, the symbionts can be passed vertically from one generation to the next, and since each host is an isolated island, why haven't some symbiotic diatoms lost the ability to form frustules? With respect to the kleptoplasty phenomenon, why are just diatom plastids retained by their hosts? What are the mechanisms allowing only the partial digestion of the diatoms whose plastids are retained? The answers to these questions, and many more, whet the intellectual appetite for the results of future research.

6. Acknowledgments

Research over the years was supported by grants from the NSF, NIH, BSF, and PSC-CUNY. Many people have organized students or have themselves collected specimens for research including: Amatzia Gennin, Pamela Hallock, Arik Diamont, Angelo Colorni, Jonathan Erez, Beverly Goodman, and Beno ter Kuile. Among the many students and colleagues who have been involved in various phases of the work, particular mention should be made of the major contributions of Drs. Charles W. Reimer, Walter Faber, Pamela Hallock, Maria Correia, Jianyuan Chai, Zeev Reiss, Moshe Shilo, Megan Cervasco, and Jorge Morales.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 465–490
DOI 10.1007/978-94-007-1327-7_21, © Springer Science+Business Media B.V. 2011

MARINE PLANKTONIC DIATOMS, INCLUDING POTENTIALLY TOXIC SPECIES

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1. Introduction

Marine pelagos is the largest habitat on our planet; it covers ~70% of the earth's surface area, and the average 200-m-illuminated upper layer encompasses an enormous body of water that extends from the Arctic through the tropics to the Antarctic Ocean. The microscopic inhabitants of this vast environment are comprised by organisms adapted to life in suspension, the (phyto)plankton, which collectively constitutes the base of the food webs involving nearly all life at sea. Among these, diatoms alone are thought to contribute, depending on the area, up to 35% or even 75% of local primary productivity (Nelson et al., 1995) which bears on the well-being of the great majority of inhabitants on the planet that also require the by-product of photosynthesis, oxygen. Diatoms in high productivity areas are the base of short food chains that sustain fisheries, e.g., the upwelling region off the Peruvian coast (Cushing, 1975). In contrast, the ability of some species to produce the toxin domoic acid which cascades through the food web and may affect the neural system of higher vertebrates, including humans, adds economic and social significance to marine diatoms (Villac et al., 2010). These three contexts alone validate the merit of research in marine planktonic diatoms.

Diatoms are tremendously successful for a relatively recent evolutionary form of eukaryotic life that quickly and effectively colonized nearly all the planet's niches where some moisture and light exists. What makes diatoms so successful ecologically has been a matter of long-standing debate. Most current genomic research affords new insight into diatom competitive capacity by showing that they possess a number of unorthodox metabolic pathways most likely acquired by lateral gene transfer from bacteria (Armbrust et al., 2004; Bowler et al., 2008). This places diatom association with other biota, e.g., N_2-fixing cyanobacteria, in a new context.

In this chapter, we review selected aspects of the biology and ecology of extant marine planktonic diatoms. We first present diatom diversity and their adaptations to the pelagic environment which will steer the reader through the subsequent discussion about distributional patterns that reveal the astonishing ability of planktonic diatoms to exploit a myriad of niches at overlapping scales in space and time. We will close by providing an updated account about an intensively studied genus, *Pseudo-nitzschia,* which has several potentially toxic species.

2. The Diversity of Planktonic Diatoms

2.1. EVOLUTION

Planktonic life-forms are thought to have derived from ancestral benthic diatoms. Extrapolating from present-day distribution patterns of the species known to produce resting spores, we may speculate that spore-like fossilized remains of diatoms found in Lower Cretaceous sediments, the oldest and well-preserved diverse assemblage of diatoms (Harwood and Gersonde, 1990; Gersonde and Harwood, 1990), may represent meroplanktonic species because spore-forming diatoms are predominantly found in coastal plankton today, although other hypothesis are also considered (Kooistra and Medlin, 1996; Harwood et al., 2007). The pelagic habitat might have been first colonized by diatoms with radial centric valves such as solenoids (Sinninghe-Damsté et al., 2004), but since then repeatedly diversified further by radiations of evolutionary younger lineages (Kooistra et al., 2007, 2009). Consequently, a wide range of morphological and phylogenetic diversity and examples of convergent and parallel evolution among extant members of planktonic diatoms should be anticipated, for example, colonies of *Pseudo-nitzschia* are reminiscent of those of *Rhizosolenia*, colony-linking apparatus of *Aulacoseira* is similar to that of *Detonula* or fragilarioid species.

Presence of silica in planktonic cell walls seems to be an ill-adopted evolutionary legacy in diatoms that live in suspension; mean seawater density is 1,027 kg/m^3 while opaline silica alone is 2,400 kg/m^3. Mean diatom cell densities vary from 1,020 to 1,250 kg/m^3 for the few freshwater species examined, leaving most cells considerably heavier than seawater (Reynolds, 1984). Adaptive "disadvantage" of siliceous "ballast" is reflected in global distribution of diatoms in higher production in turbulent and cool waters (upwelling regions, polar seas) rather than in stratified seas.

Silica is absolutely required for normal functioning of a diatom cell. Silica metabolism and its role in diatom cell cycle is summarized by Simpson and Volcani (1981) who speculate on possible advantages of using silica in constructing cell walls, for example, energetically lower cost of silica compared to cellulose or carbonates, protection from pathogens, grazing, among other suggested benefits. More recently, Medlin (2002, 2007a) suggested that silica conferred adaptive advantage on the earlier (UR-diatoms) by expanding longevity and/or affecting resting stages, but such advantages would apply to all diatoms, not only planktonic forms.

2.2. SIZE RANGE

The planktonic habitat hosts some of the most extreme cell sizes. They range from the smallest to the largest cells: single pico-sized cells of *Minidiscus variabilis* are only 2.0 μm (0.0018 mm, Kaczmarska et al., 2009) while the largest cells of

Ethmodiscus reach 2 mm in diameter (Hasle and Syvertsen, 1997) with cell volume of 10^9 μm^3 (Villareal, 1992a). The large cell-size end of the range may be, interestingly, apparently inherent to marine environment (Litchman et al., 2009). This represents 10^9 times difference in cell volume and 10^6 in cell surface area, yet both sustain themselves in seawater medium with physicochemical properties that do not differ in similar order of magnitude. Clearly, these two species evolved quite different means to maintain their cell populations in the ocean's photic zone. Small cells benefit from frictional drag of their large surface area relative to cell volume. The large cells may contain large vacuoles that could become positively buoyant, for instance, *Ethmodiscus* (Villareal, 1992a; Villareal et al., 1999) and some *Rhizosolenia* (Moore and Villareal, 1996). Although both *Minidiscus* and *Ethmodiscus* cells are solitary and drum shaped, the circular valve face outline is not the only or even the most common form among planktonic species. Single-cell diatoms in marine plankton are in the minority; a wide range of colonial forms dominate coastal plankton and individual cells in a colony are of intermediate size relative to the two extremes discussed above.

2.3. FORM AND FUNCTION

Diatoms demonstrate many adaptations to planktonic lifestyle that are also known among other phytoplankton (Reynolds, 1984), all basically aiming to reduce cell excess weight relative to density of seawater, and thus slow the sinking. Diatoms specifically covet the ability to overcome cell wall weight-ballast that all diatoms possess. This may be achieved by modification of cell size and shape.

At a basic level, three types of cells may be found in the plankton. These are, following the classification of Medlin and Kaczmarska (2004), although alternative schemes do exist (Round et al. 1990, and references therein): (1) cells with circular valve face outline, mostly members of the Class Coscinodiscophyceae, containing some of the most ancient of planktonic diatom lineages (e.g., Coscinodiscales, Ethmodiscales, Rhizosoleniales); (2) cells with polar outline in valve face that compose most of the Class Mediophyceae, a younger lineage (e.g., Chaetocerotales, Hemiaulales, Thalassiosirales); and (3) cells with elongated linear-fusiform valve face outline (some with raphe) that compose the Class Bacillariophyceae, the youngest of the three. Marine planktonic species are found predominantly among the radial and bi(multi)polar cells. Each of the three lineages evolved many overall similar cell forms, though these similarities arise via expansion of different cell axes following class-specific auxospore ontogenetic pathways (Kaczmarska et al., 2002; Medlin and Kaczmarska, 2004).

Filamentous assembly of cells closely abutting by their valve faces occurs mostly among coscinodiscophycean plankton (*Leptocylindrus*, *Rhizosolenia*). Here, cell size increase is achieved by the expansion of pervalvar axis, often many times longer than is the valve diameter. Such colonies form a relatively rigid bar suspended in seawater.

Polar species, in addition to the previously mentioned colony type, evolved colonies with loosely aggregated cells, often with considerable space between the sibling cells, thus spreading the heavy cell "load" over longer linear distance. Such colonies will less likely be subject to a normal stress, in contrast to the earlier case. Here, the individual cells may possess additional, permanent or temporary, extensions enlarging surface area relative to cell volume (*Chaetoceros* or *Thalassiosira*, respectively), but in general, all three cell axes are relatively similar in linear dimension while it is the colony that elongates.

Among pennate species, some colonies are more similar in design to those of radial centrics with cell colony members in close contact (*Fragillariopsis*); in others, cells use exudates to connect members of a colony (*Asterionellopsis, Thalassionema*), combining the approaches of the two previous classes to maintain colony integrity. Planktonic pennate species more often than other classes use "long thin needle" cell design, with some species reaching record length of single cell in plankton. Two araphid genera, *Lioloma* and *Thalassiothrix*, contain very long and thin species with apical axes reaching 2–6 mm and transapical axes no wider than 5–8 μm (Hasle and Syvertsen, 1997). These are good examples of form adaptation of pennates to the planktonic environment because this surface area-to-volume ratio (SA/V) is comparable to that of relatively small centrics, such as *Cyclotella* or *Thalassiosira* (Reynolds, 1984).

While single-celled planktonic diatoms are mostly drum or cylinder shaped, cells of colonial species present a seemingly unlimited range of variations in outline and valve face protrusions; most, however, conserve specific SA/V ratios (Reynolds, 1984). The fact that different diatom lineages evolved similar cell shapes, conserving SA/V ratios of ~1–3, presents examples of functional/shape convergence. Physical properties of planktonic environment and diatom cells (if considered as inanimate, suspended particles) do not fully explain all seemingly limitless variations in diatom frustule shape and size. Other factors operating within these physical constraints are, for example, competition for resources (Litchman et al., 2009) and grazing deterrence (Smetacek, 2001), discussed further below.

2.4. TAXON RICHNESS

Planktonic marine diatoms are perhaps one of the most studied functional groups of diatoms, far exceeding other marine habitats (e.g., salt mashes, mudflats, benthos) in scientific attention received. Nonetheless, it is very likely that diatom plankton represent a minority of marine genera richness. A perusal of Round et al. (1990) reveals 61 marine planktonic genera which constitutes only ~30% of marine and ~20% of all genera presented in detail allowing such determination. Focused on plankton alone, Ricard (1987) and Hasle and Syvertsen (1997) list, respectively, ~190 and 140 often found in plankton, including those genera that contain at least a few planktonic species.

The introduction of electron microscopy to diatom research greatly accelerated the discovery of new species (synthesis in Round et al., 1990), and systematic application of molecular methods since the 1990s clearly shows that diatom biodiversity is far from being unraveled, even among plankton. For example, five new species were found hidden within the "well-known, cosmopolitan" diatom "*Skeletonema costatum*" (Sarno et al., 2005, 2007). This phenomenon, now recognized as (semi-)cryptic diversity has been found to be widespread among diatoms examined using a combination of microscopical, molecular, and breeding approaches (e.g., Amato et al., 2007; Kaczmarska et al., 2008). One important consequence of this research is emerging understanding of the geographic patterns of distribution. In contrast to postulated intrinsic lack of biogeography among microbes (Finlay, 2002), it becomes clear that planktonic diatoms, like more complex biota, contain some species (or even genotypes) with widespread distribution and others with geographically restricted occurrence (Godhe et al., 2006; Medlin, 2007b; Kooistra et al., 2008; Casteleyn et al., 2008).

3. Time and Space Distributions

3.1. GENERAL FRAMEWORK

The major parameters that determine the abundance and distribution of phytoplankton in nature are the availability of light and nutrients (central to growth rates) in association with physical forcing that promote accumulation or dispersal (e.g., water column stability, frontal zones, lateral transport) and intrinsic losses due to biological controls (predation, competitive exclusion, cell lysis) and/or sinking out of the photic layer. Certainly, the interactions among these parameters vary widely from place to place and it is beyond the scope of this chapter to detail the multitude of strategies adopted by such ubiquitous organisms as diatoms. Although we will present data referring to different regions, for sake of simplicity, let's keep in mind the division of the plankton realm into two ecological categories: communities that comprise recycling systems as opposed to those that make up new production systems (as summarized in Smetacek, 1999). The first one consists of small cells ranging from submicron cyanobacteria to flagellates and diatoms ca. 5–10 μm in size whose biomass is kept low by predation; this is the steady-state community found in the nutrient-limited waters of subtropical gyres, at high latitudes during winter and in the "high-nutrient, low-chlorophyll (HNLC)" conditions found in areas of the equatorial and subarctic Pacific and in the Southern Ocean. In contrast, along the continental shelves, especially in upwelling areas and during springtime at higher latitudes, the input of allochthonous nutrients in a shallower mixed layer promotes the growth of larger diatoms (>10 μm), often chain-forming species.

Another way to provide a framework for the vast data on diatom distributional patterns is to use Margalef's (1978) approach of "life forms" as the

expression of adaptation to recurrent patterns. His mandala links nutrient availability to degree of turbulence and thus defines stages in the "succession" of phytoplankton communities: from small diatoms in nutrient-rich, turbulent environments to large dinoflagellates with mobility and widespread heterotrophy in a nutrient-deprived and less mixed water column. Actually, there is a gradation between these two extremes, in which distinct niches could be explored by a host of sizes and forms, thus SA/V ratios (see Reynolds, 1988 for concept; see Alves-de-Souza et al., 2008 for application). Another contention for size-dependent distribution of phytoplankton, not fully developed when the original mandala was proposed, is that cells from the smallest spectrum (picoplankton, ≤2 μm) thrive and dominate in oligotrophic oceans where nutrient uptake by larger cells (ca. ≥5 μm in radius) can be limited by molecular diffusion at very low nutrient levels (Chisholm, 1992).

Although it is true that diatom-dominated communities are more characteristic of non-stratified coastal waters and upwelling regions, which complies with both claims presented above, there are exceptions. Diatoms can also succeed in offshore waters and/or downwelling seasons due to survival strategies involving variations in size, shape, and life cycles, as well as the incidence of symbiosis or buoyancy regulation, as discussed below. We will not detail the ecology of diatoms in polar systems, which would call for the description of the intricacies of these environments that impose constraints such as life close to freezing point, 6-month light:dark cycle and salinity gradients associated with seasonality in ice cover. Despite these apparently harsh conditions, diatoms thrive on/in the ice and in the water column of polar regions, and the reader is referred to Medlin and Priddle (1990) as a first, basic appraisal.

3.2. SUCCESSION AND SEASONALITY

It is not possible to explore phytoplankton succession without acknowledging Margalef's generalization of three stages in which the sequence of community organizations is a result of the influence of external perturbations and the interactions between species (Margalef, 1958, 1978). As we describe these stages, which are here regarded as the sequence of species shifts at an area after a disturbance, the reader should be aware that: (1) there is a heated debate on theoretical and operational issues involving this concept since its original proposition in the 1920s in the context of land plants (see Benedetti-Cecchi, 2003); (2) the interplay of the underlying processes that may drive the course of phytoplankton succession will vary from place to place, so that both equilibrium and nonequilibrium situations may be found (see Harris, 1986); and (3) this is an idealized scenario applicable more closely to upwelling systems and with deviations particular to any other given coastal area. The overlap of space and time scales comes into play as well. Succession can be recognized as a shorter term (local upwelling) or a longer term (regional seasonality) process, at the same time that a synoptic depiction of coastal

and oceanic communities along the same latitude may correspond to distinct successional stages. In other words, species that bloom in the early stages are typically abundant in eutrophic coastal waters whereas those that develop later are often found in oligotrophic offshore environments.

The first stage begins with the enrichment of the euphotic layer by upwelling, wind mixing, or land runoff that triggers the intense growth (>1 division/day) of diatoms with high SA/V ratios (~1+) within colonial genera such as *Skeletonema*, *Leptocylindrus*, and *Thalassiosira*; communities can reach 10^6 cells/l and population density is often regulated by cell dispersal around patches of high productivity. During the second stage (at times split into two shorter stages), one can observe a larger number of medium-size species whose SA/V ratios are ~0.5, excluding appendages, commonly referred to as *Chaetoceros*-dominated assemblage. Population densities of 10^4–10^5 cells/l can be detected during the second stage which is often followed by the rapid sinking of various members of the community, mostly those that make up aggregates or form resting spores once nutrients are exhausted in the surface mixed layer. The third stage is usually associated with the development of thermal stratification, cell abundances of 10^4 cells/l or less, and the occurrence of species with a very low SA/V ratio, some of them already detected in the second phase. Various diatoms can thrive during this more advanced stage of succession, for instance, large species within Rhizosoleniaceae (*sensu lato* according to Hasle and Syvertsen, 1997), *Coscinodiscus*, *Ditylum*, *Pseudo-nitzschia*, among others (e.g., Guillard and Kilham, 1977; Margalef, 1978; Valentin et al., 1985).

Following succession on a seasonal scale requires sampling strategies that can adequately assess that the rate of succession can actually vary with latitude and from year to year. A diatom spring bloom (and often a less conspicuous autumn bloom) is very characteristic in temperate waters as the response to the disruption of the seasonal thermocline that makes nutrients again available in the photic layer, although exact timing and intensity can be highly variable; such seasonality, however, is much less pronounced in tropical/subtropical regions, if at all present (Guillard and Kilham, 1977; Smayda, 1980; Harris, 1986, and references therein for all). This is an adaptable process in which variations in grazing pressure associated with frequent (days, weeks) readjustments in water column stability can push succession back-and-forth between the first stages in shallower waters, whereas more advanced stages in successions could be prolonged in offshore, more stable water columns that relies on regenerated production.

The fate of the biomass generated by a diatom bloom, frequently referred to as the loss term in trophic dynamics modeling, can be attributed to biological controls such as cell lysis and grazing in association with aggregation/flocculation of senescing populations and subsequent sinking out of the photic layer. Cell lysis may be due to natural (programmed) cell death (Franklin et al., 2006), as well as to pathogen-mediated mortality (Brussard, 2004), all still in a nascent state of understanding. Grazing has been studied in more detail in relation to copepod feeding habits and the production of compounds affecting fertility and

fecundity of some grazer species, as reviewed in Paffenhöfer et al. (2005): diatoms are not, necessarily, their major diet and feeding behavior may change in response to patchy as opposed to heterogeneous food availability. One interesting finding was grazing-induced increase in cell wall silicification of *Thalassiosira weissflogii*, thus improved protection for the diatom, suggesting that plant–herbivore interactions may drive ecosystem structure beyond grazing itself (Pondaven et al., 2007). Grazing by microzooplankton (ciliates and heterotrophic dinoflagellates) is another top-down control to consider (Buck and Newton, 1995; Strom et al., 2007). However, grazing does not seem to be the major player in mediating diatom export from surface layers. Fecal pellets remineralize in the upper layers and mass diatom downward flux in some areas can take place within days to weeks of surface blooms, a time scale not always compatible with zooplankton dynamics (Smetacek, 1985). Although more significant than the sedimentation of fecal pellets, the extent (and ecological role) of mass phytoplankton flux can vary from place to place depending on interacting factors such as composition, sinking rates, local depth, microbial colonization, and ingestibility by consumers (Turner, 2002).

Rapid diatom vertical flux can be attained by the formation of aggregates (reviewed in Thornton, 2002) and by spore formation of, most often, chaetoceroid species (reviewed in McQuoid and Hobson, 1996). Diatoms are one of the major contributors to the visible aggregates known as marine snow, responsible for sinking rates that may exceed 100 m/day. Spore formation, that is, change in valve thickness and shape that precedes a period of slower physiological rates, can happen fairly quickly (1–5 days) and is most often triggered by nitrogen depletion. However, according to Smetacek (1985), sinking does not necessarily equate to mortality and, although bloom sedimentation does promote a true "pelagic spring cleaning," there is evidence to evoke sinking as a survival strategy for diatoms still in the water column, before the actual contribution to benthos ecology and/or to the formation of diatomaceous oozes. Indeed, resting spores do not function solely as benthic resting stages; their elevated sinking rates may prevent wind-driven surface advection away from upwelling centers which, added to pelagic retention in circulation cells, can reseed intermittent blooms as observed in some upwelling systems (Garrison, 1981; Pitcher, 1990).

Marine aggregates of diatoms may serve other functions, for example, as a platform where sex-compatible cells can maintain position (Fryxell et al., 1991), thus facilitating gametangial copulation. Most diatom species examined *in vitro*, to date, sexualize when in exponential stage of population growth (Amato et al., 2007; Kaczmarska et al., 2008), with light and temperature affecting onset of sexualization (Hiltz et al., 2000) more often than does nutrient depletion (Jewson, 1992). This likely scenario can be suggested for *Corethron criophilum* in nature, as observed at the Antarctic polar frontal zone during a spring bloom when massive sexual reproduction was associated with rapid downward flux of half frustules and auxospores (Crawford, 1995).

3.3. LARGE-SCALE SPATIAL PATTERNS

To illustrate large-scale distributional patterns, that is, coastal as opposed to oceanic, we rely on the extensive survey of the literature carried out by Guillard and Kilham (1977). They assessed the differences and similarities between major bioregions within the Atlantic, Pacific, and Indian Oceans, based on a comparison between diatom biocoenoses and the underlying thanatocoenoses. Although there are copious examples at the species rank, their approach was to discuss trends at the community level, operationally defined as "recurrent association of species, with the proviso that the association is flexibly defined and need only to include a certain percentage of the species in some definite set of species in order to qualify." Patterns drawn from this 30-year appraisal still hold true at present. For additional examples of the global biogeography of planktonic marine diatoms, the reader is referred to Hasle (1976), Semina (1997), Semina (2003), and references therein.

Some general patterns described in Guillard and Kilham (1977) are:

- Thanatocoenoses reflect a distinction between neritic and oceanic populations but, as expected, the zonation pattern for the overlying biocoenoses may be more complex than the thanatocoenoses, and the boundaries do not match exactly.
- Species can be (a) restricted to oligotrophic regions or (b) ubiquitous, but more abundant in eutrophic waters; examples of tropical, oceanic forms are: *Asterolampra marylandica*, *Chaetoceros tetrastichon*, *Gossleriella tropica*, *Hemidiscus cuneiformis*, *Mastogloia rostrata*, *Pseudosolenia calcar-avis*, and *Rhizosolenia hebetata*.
- Indian and Pacific assemblages are more similar and set apart from the Atlantic.
- In the North Pacific, abundance and number of species decrease dramatically westward from the North American coast into progressively less fertile waters until it reaches the influence of the nutrient-enriched Oyashio and Kuroshio current systems.
- Phytoplankton biomass in the Indo-Pacific is mostly dominated by dinoflagellates, except in nutrient-rich neritic and upwelling waters where diatoms thrive.
- In the North Atlantic, populations are horizontally exchanged by relatively small-scale lateral eddies so that the transition between coast and offshore is less abrupt than that observed in the North Pacific; for example, *Hemiaulus hauckii* is perceived as neritic in the first and oceanic in the latter; indeed, neritic species can be found at all times from New England to Bermuda, albeit in very low numbers offshore; resemblance is also evident in seasonal succession.
- There are orders of magnitude difference in diatom cell abundances between impoverished Central South Atlantic (10^1 cells/l, 2–5% of total phytoplankton) to Antarctic and African upwelling regions (10^5 cells/l, 40–80% of total phytoplankton).

Estimates of biogenic silica production and export confirm the patterns described above. Nelson et al. (1995) performed such an exercise in which the upper limit estimate of 280 Tmol Si $year^{-1}$ was based on empirical data available for global ocean primary productivity (60 Gt C $year^{-1}$), the relative contribution attributable to diatoms (~26 Gt C $year^{-1}$), and diatom elemental composition (Si/C = 0.13). Since at least 50% of this diatom-produced silica dissolves in the upper 100 m, the resulting export to the deep ocean becomes a maximum of 140 $\times 10^{12}$ mol Si $year^{-1}$. The authors further estimated the contrast between coastal and offshore systems which are, respectively an upper limit of 75% and 35% for the contribution of diatoms to total primary productivity.

Although there is a sharp overall difference between coastal and oceanic diatom communities at their extremes, our perception and interpretation of some deviations from such a clear-cut pattern can be revealed by our ability to sample at appropriate scales and to apply suitable techniques. Venrick (1992), for example, demonstrated the importance of long-term studies to understand the phytoplankton species structure within the central North Pacific gyre. In her study, spatial differences on a scale of 3,200 km became negligible over temporal intervals of 4–5 years for the shallower assemblages and 12 years for the deeper ones. Through remote sensing, global SeaWiFS chlorophyll data from 1997 to 2007 detected summer blooms in areas of oligotrophic gyres that did not coincide with areas of nitrogen fixation nor high eddy kinetic energy that could promote such high productivity (Wilson and Qiu, 2008). Going for an offshore oceanographic cruise in a very unlikely time of the year, during late winter storms in the Sargasso Sea, allowed Krause et al. (2009) to observe the effect of nutrient entrained into the euphotic zone. Diatoms comprised <5% of total chlorophyll, but accounted for an estimated 25–50% of the nitrate uptake in the upper 140 m and 35–97% of the particulate organic nitrogen export from the upper 200 m.

3.4. VERTICAL DISTRIBUTION

3.4.1. Diatoms in "Deep Chlorophyll Maxima" and "Thin Layers"

Phytoplankton distribution in stratified water columns varies with depth resulting from the interplay between availability of light and nutrients. Productivity is generally limited by nutrients in the upper layer whereas light plays a role either by excess at the very surface through photoinhibition or by restrictive low levels at the base of the photic zone (Lalli and Parsons, 1993). This feature in which phytoplankton may concentrate at distinct water depths that do not necessarily correspond to a maximum in either irradiance or nutrient concentration was revealed as such in the pioneer work of Venrick et al. (1973) and is nowadays widely referred to as the "deep chlorophyll maximum (DCM)." The dynamics of DCMs have been studied in many regions of the world; it may range from 90 to 130 m in the open ocean to 15–30 m in more coastal areas, which can be more related to the depth of light penetration rather than local depth alone; and its contribution to total areal

production can vary from place to place, and seasonally within the same system, from 20% to 75% (Scharek et al., 1999, and references therein). The formation of a DCM may be solely the physiological increase of chlorophyll per cell without an increment in cell numbers, but other processes may be involved such as the physical accumulation of sinking cells at the pycnocline or behavioral aggregation of motile cells (especially dinoflagellates) as defense against grazing (Cullen, 1982).

A common feature of most DCMs is the presence of diatoms, especially small-sized (10–60 μm) pennates (e.g., Kimor et al., 1987; Estrada and Salat, 1989; Scharek et al., 1999; Hanson et al., 2007). One can speculate that their needle shape increases SA/V ratio that enhances nutrient absorption once in contact with the nutricline. However, species larger than >50 μm (*Rhizosolenia* and *Thalassiothrix*), including some with a drum/sphere morpho-type (*Stephanopyxis palmeriana* and *Coscinodiscus*) are also often found at the base of the euphotic zone. Evidence from laminated sediments and sediment traps indicate that there is substantial population growth of this "shade flora" over the entire period of stratification (from late spring to autumn) so that its rapid downward flux, once stratification breaks, is intense enough to be referred to as "fall dump" (Kemp et al., 2000, and references therein). Indeed, the specific growth rates of several of these larger diatoms are great enough to meet the requirements for growth at ca. 1–2% light levels, provided that some mechanism such as episodic eddies lift nutrients up to the lower portion of the euphotic zone (Goldman and McGillicuddy, 2003; Brown et al., 2008). Smetacek (2000) ponders whether possible advantages of large, robust diatoms in this environmental setting might result from their relative low abundance; that is, added to the reduced need to invest in mechanical defense, a lower rate of encounter with pathogens and predators could translate into low mortality rates. Alternatively, Litchman et al. (2009) point out that nutrient pulses at certain frequencies – in otherwise oligotrophic conditions – may select for large-sized diatoms considering their capability of nitrogen storage in large vacuoles.

Diatoms are present in another bio-oceanographic feature known as subsurface "thin layers" which is distinct from DCMs. The first is described as accumulations that are vertically compressed, from centimeters to only a few meters (<5 m), that may extend horizontally for kilometers, and can persist for days, as opposed to DCMs which are typically a less intense increase in chlorophyll, expressed over a depth range of tens of meters (Cowles et al., 1998). According to McManus et al. (2008, and references therein), "thin layers" have been identified as a common, recurrent feature in a variety of coastal systems and that it is imperative to implement the use of high-resolution vertical sampling for their detection. Some potentially harmful species can comprise the bulk of "thin layers," as will be the case for *Pseudo-nitzschia* discussed below.

3.4.2. Beach Surf Zone Diatoms

The surf zones of exposed oceanic beaches seem inhospitable, but the following diatoms thrive in these moderate- to high-energy, unstable environments: *Anaulus australis* (so far restricted to the southern hemisphere), *Asterionella socialis* (endemic

to the west coast of North America), *Aulacodiscus africanus* (apparently a tropical species), *Attheya armatus*, *Aulacodiscus kittonii*, and *Asterionellopsis glacialis*, the first four recorded only in beach surf zones (Campbell, 1996). They form semi-permanent accumulations that can reach 10^7–10^9 cells/l and appear as brown foam on the surface of dissipative beaches all over the world (e.g., Lewin et al., 1989; Talbot et al., 1990; Odebrecht et al., 2010). Such large biomass may fuel different pelagic, benthic, and shore food webs (Brown and McLachlan, 1990).

Surf diatoms are well equipped to develop high abundances in a turbulent, very shallow water column. An important adaptation of these species is the production of mucus that helps cells to adhere to bubbles, floating to the surface between wave bores, which initiates a mechanism responsible for the maintenance of a rather self-contained environment, as follows: with time, the mucus gets stickier, collects particles that increase overall cell density thus enhancing sedimentation; settling cells caught by rip currents are transported to the sediment behind the surf zone where they stay until the next perturbation allows for the reseeding of the population to the actual surf zone (Talbot and Bate, 1987; Talbot et al., 1990). The photophysiology of these diatoms seems to be adjusted to exposure to a fluctuating light environment that oscillates, in a matter of seconds, from near aphotic conditions close to the bottom to inhibitory, full sunlight at the surface (Du Preez and Campbell, 1996).

3.5. THE ROLE OF DIATOMS IN RECYCLING SYSTEMS

3.5.1. High Nutrient, Low Chlorophyll Areas

Low phytoplankton biomass and excess nutrients coexist in the well-lit surface waters of three large open ocean regions in the subarctic Pacific, the equatorial Pacific, and the Southern Ocean. These are known as HNLC areas. Although it is widely recognized that phytoplankton dynamics are governed by a complex interdependent suite of factors, research about persistence of unused nutrients (or lower than expected levels of chlorophyll and primary productivity) has operationally focused on two main alternate hypotheses: failure of phytoplankton to exploit major nutrients or effective control by herbivores (Cullen, 1991). The deficiency of iron (Fe), which can limit photosynthetic and respiratory processes (Geide and La Roche, 1994), is the best studied example of bottom-up control of phytoplankton in HNLC areas initiated by Martin and Fitzwater (1988) and leading to a series of *in situ*, ecosystem-scale experiments of Fe fertilization.

As predicted, enhanced Fe availability in seawater resulted in greater phytoplankton biomass and changes in community composition in which, according to de Baar et al. (2005, and references therein), "Large diatoms always benefit the most from Fe addition, where a remarkably small group of thriving diatom species is dominated by universal response of *Pseudo-nitzschia* spp." The proliferation of pennate diatoms was a common feature of on-deck Fe-enrichment experiments carried out in HNLC areas under coastal upwelling regimes as well

(Hutchins et al., 1998; Hutchins et al., 2002). Although exceptions to this trend do exist, such as the dominance of the more delicate, hyalochaetan *Chaetoceros* species (Tsuda et al., 2003). Interestingly, cell size reduction has been shown to occur to *Pseudo-nitzschia* under Fe-stressed conditions (Hutchins et al., 1998; Marchetti and Harrison, 2007), a strategy also known as microbial miniaturization that leads to higher SA/V ratios.

Although proposals of large-scale Fe fertilization were initially viewed as an ecosystem engineering option to reduce atmospheric build up of CO_2 by activating the biological pump, export carbon into deep waters is difficult to assess and, when proven, seems to be quite modest (de Baar et al., 2005). In the long term, a myriad of group-specific responses of autotrophs to the artificial addition of Fe may lead to changes in the phytoplankton community structure with unforeseen and untested consequences to food webs. In one, controlled experiment, variations in the abundance and cellular physiology of different populations within the same community indicated that cells can be Fe-starved (large eukaryotes), Fe-stressed (cyanobacteria), and Fe-replete (picoeukaryotes) (Eldridge et al., 2004). There is some indication that the first, immediate growth response of a given population is due to their presence as a local, more abundant seed stock (Smetacek et al., 2004), added to their corresponding growth rate under an iron-replete condition (Tsuda et al., 2005), provided that grazing pressure is low enough to allow diatom biomass built up (Tsuda et al., 2007).

3.5.2. Diatom–Cyanobacteria Symbiosis and Other Associations

The occurrence of diatoms hosting cyanobacteria endosymbionts, taxonomic and biological aspects particular to such consortia, and the role of some of these as a nitrogen-fixing system in oligotrophic waters were reviewed in Villareal (1992b). At that time, the following associations were known and documented: coccoid cyanobacteria as endosymbionts in *Neostreptotheca subindica* and filamentous, heterocystous cyanobacteria in *Rhizosolenia* spp. (10+ species), *Guinardia cylindrus*, *Proboscia alata*, *Hemiaulus hauckii*, and *H. membranaceus*. Cocoid cyanobacteria were later found in *Neostreptotheca torta* (Zehr et al., 2000) and *Climacodium frauenfeldianum* (Carpenter and Janson, 2000). *Richelia intracellularis* is frequently cited as the filamentous endosymbiont, despite some confusion in nomenclature and conflicting field reports (reviewed in Gómez et al., 2005). Molecular genetic studies of natural populations of *Richelia* associated with *H. hauckii* (North Atlantic) and *R. clevei* (North Pacific), as well as from a culture isolate of *Calothrix* associated with *Chaetoceros* (North Pacific) indicated that the symbionts in those different hosts are distinct species or strains (Foster and Zehr, 2006).

Rhizosolenia-*Richelia* associations are easily observed at the light microscope, the symbiont is usually located at the apex of the host valve and, at least for some species, in the periplasmic space between the plasmalemma and the frustules, as mentioned by Villareal (1992b), who suggests that this may facilitate excretion of recently fixed nitrogen into the surrounding medium. The author also points out that it is unknown what factor other than host size limits the

number of symbionts per cell, a thought exemplified by *R. clevei*, whose diameter ranges from 7 to 250 μm and each cell may contain 1–31+ symbionts. The *Hemiaulus-Richelia* association, however, is said to be cryptic because the cyanobacteria are obscured by the dense cytoplasm of the host so that epifluorescence is required to effectively detect the symbiont (Heinbokel, 1986).

N-fixation has been clearly shown for *R. intracellularis* present in *Rhizosolenia* and *Hemiaulus* (Villareal, 1992b), and there is indirect evidence for nitrogen fixation for the symbiont found in *C. frauenfeldianum* (Carpenter and Janson, 2000). Extensive blooms of *Hemiaulus* or *Rhizosolenia* bearing the cyanobacteria have been reported in oligotrophic waters, either in the Pacific or the Atlantic, and indicate that N_2 fixation by such association may be very relevant not only locally but also at basin-scale nitrogen budgets (Zehr et al., 2000, and references therein). These diatoms, however, can also be found symbiont-free in coastal, nutrient-rich waters.

Richelia intracellularis as epiphytes on *Chaetoceros* and *Bacteriastrum* are more rare (less documented?) and, in the case of the former, it often involves another epiphytic pennate diatom tentatively identified as belonging to the *Pseudo-nitzschia americana* species complex (Gómez et al., 2005). Either as epiphytes or endosymbionts, diatom–cyanobacteria associations seem to be more common in warm, nutrient-poor waters. Indeed, the following diatom associations with other organisms are also detected in subtropical/tropical waters and/or during summer/fall of temperate regions: *Chaetoceros tetrastichon* or *Chaetoceros dadayi* attached onto the lorica of tintinnid ciliates (Cupp, 1943), a consortia with apparently coevolutionary significance (Gomez, 2007); 20+ diatom species have been found to host a parasite, whether a fungi or an amoeboid/flagellated protozoan (Hoppenrath et al., 2009).

3.5.3. Vertical Migration

Some of the largest diatoms known, *Ethmodiscus* and *Rhizosolenia*, are positively buoyant with ascent rates of up to 7–8 m/h as demonstrated by Moore and Villareal (1996). The authors also point out that there is a critical minimum size for diatoms to achieve positive buoyancy, that maximum ascent rates decrease with decreasing cell size, and that overall morphology is also important, for example, *Rhizosolenia* chains ascend with the long axis parallel to the direction of motion. A large vacuole comprises the majority of the volume of these cells whose buoyancy is determined by variations in intracellular density which is, in turn, associated with internal ion and/or carbohydrate concentrations according to physiological status (Richardson et al., 1996; Villareal et al., 1999, and references therein both). In the case of *Rhizosolenia*, these are also found as macroscopic aggregates (1–30 cm) of multiple species, known as *Rhizosolenia* mats, that can achieve extensive vertical migrations in the Atlantic, Pacific, and Indian open oceans (e.g., Carpenter et al., 1977; Pilskaln et al., 2005).

The ability for vertical displacement allows these diatoms to apply a competitive strategy to thrive in the open oceans, otherwise an inhospitable

oligotrophic milieu for autototrophic cells with such high SA/V ratios. Solitary *Ethmodiscus* cells, as well as individual chains or mat-forming *Rhizosolenia*, descend to or below the nutricline (ca. 100–300 m) where nitrate is acquired for use during photosynthesis at the surface (e.g., Villareal and Lipschultz, 1995; Pilskaln et al., 2005). Both *Ethmodiscus* and *Rhizosolenia* that migrate to the photic layer have high $\delta^{15}N$ values, typical of deep nitrogen pools (Villareal et al., 1993; Villareal et al., 1999). The dominant form of nitrogen release at the upper mixing layer in one study at the central North Pacific was nitrate, with a contribution of at least 4–7% of the nitrate pool per day and, possibly, as much as 27% (Singler and Villareal, 2005).

4. Potentially Toxic Diatoms

In the mid-1960s, Grethe Hasle monographed a small group of species from the genus *Pseudo-nitzschia* (then ascribed to the genus *Nitzschia*) using light and electron microscopy (Hasle, 1965). This basic research played a key role 20 years later in tracing the cause of a serious shellfish poisoning event, the first time a diatom was ever shown to be the source of such phenomenon. In the fall of 1987, a toxin outbreak was observed in Prince Edward Island, Canada, which resulted in human deaths and disabilities. The syndrome was called amnesic shellfish poisoning (ASP), the toxin was determined to be domoic acid (DA), and the diatom was identified as *P. multiseries*. Although toxin transfer to humans has not been reported since this first DA outbreak, evidence has accumulated and testifies to the pervasiveness of DA entry and movement through marine food webs in regions subject to toxigenic *Pseudo-nitzschia* blooms with noticeable effects, even death, to marine birds and mammals. A great body of literature is available on the subject (see http://www.glf.dfo-mpo.gc.ca/os/aes-sae/dapr-radp/index-e.php; Villac et al., 2010) with unabridged evaluations of the biogeography of *Pseudo-nitzschia* (Hasle, 2002; Lundholm and Moestrup, 2006), their bloom dynamics and ecophysiology (Bates, 1998; Bates et al., 1998; Bates and Trainer, 2006), toxin transfer in food webs (Bates and Trainer, 2006; Bejarano et al., 2008), and the epidemiology of DA poisoning (Todd, 1993).

Other diatoms, *Amphora coffeaeformis* and *Nitzschia navis-varingica*, have been found to produce DA in culture, but their potential harmful effects have not yet been detected in nature (Moestrup et al., 2009). We will, therefore, focus on the ecology of the genus *Pseudo-nitzschia* and will follow the topics presented above for planktonic diatoms in general. The great morphological and ecological versatility of diatoms is mirrored within this single genus, which is of great socio-economic relevance as well.

- Biodiversity: There are 37 taxa of *Pseudo-nitzschia*, including two varieties, of which 14 have been described (or redefined) since 2002, mostly with the aid of electron microscopy and molecular characterization, illustrating (semi-)cryptic

diversity within the genus (Villac et al., 2010). Domoic acid production has been shown for 12 species: *P. australis*, *P. calliantha, P. cuspidata, P. delicatissima, P. fraudulenta, P. galaxiae, P. multiseries, P. multistriata, P. pseudodelicatissima, P. pungens, P. seriata,* and *P. turgidula* (Moestrup et al., 2009; Villac et al., 2010).

- Associations: *Pseudo-nitzschia* cells may occur within colonies of *Chaetoceros socialis* (Rines et al., 2002) and of *Phaeocystis globosa* (Sazhin et al., 2007).
- Size range, form, and function: The diagnostic staggered chains of needle-shaped frustules suggest an apparently simple overall morphology. In fact, specific sizes vary widely (e.g., from a 12-µm-long *P. brasiliana* to a 174-µm-long *P. pungens*), the ultrastructure is quite diverse (e.g., Hasle, 1965; Skov et al., 1999), and ecophysiological requirements *in vitro* are also species specific (Bates, 1998; Bates et al., 1998; Bates and Trainer, 2006). The needle shape of any size is a significant departure from sphericity that increases the SA/V ratio of the cell, affording those species that are considered a larger microphytoplankton (>60 µm), a broader niche than expected. The same species may show a wide range in length due to reduction in size typical of vegetative growth in diatoms. Size restoration through sexual reproduction is seldom observed in nature because sexualization seems to be constrained in time and to a limited fraction of the population (Holtermann et al., 2010; Sarno et al., 2010).
- Large-scale biogeography: This is a widely distributed marine genus, found from polar regions to warm waters. It is an important component of coastal floras, though also well represented in oceanic environments, including warm core rings (Kaczmarska et al., 1986). At the species level, however, *Pseudo-nitzschia* may show different distribution patterns (Hasle, 1972, 2002; Lundholm and Moestrup, 2006), and cosmopolitanism of individual species is still to be confirmed (Villac et al., 2010).
- Local and meso-scale blooms: Increasing abundances in some enclosed and semi-enclosed coastal systems can be associated with the growth of local seed stocks due to land runoff (Villac et al., 2004), they may result from advection onshore of blooms generated and retained in offshore cyclonic eddies (Trainer et al., 2002) or be the effect of accumulation in salt wedge zones of estuarine areas (Dortch et al., 1997).
- Succession and seasonality: The versatility afforded by a microphytoplankton cell with a high SA/V ratio has clear implications for succession. *Pseudo-nitzschia* spp. are frequently present year round. They are quick to respond to nutrient input from river discharge (e.g., Dortch et al., 1997; Trainer et al., 2007) or upwelling waters (e.g., Walz et al., 1994) and are abundant in the initial stages of the spring bloom of some warm temperate regions (e.g., Orsini et al., 2004; Schnetzer et al., 2007). They also contribute to the later stages of succession and during more stratified water columns of late summer/autumn periods (e.g., Valentin et al., 1985; Buck et al., 1992).
- Vertical distribution: Actively growing cells have been found as the major component of "thin layers" (Rines et al., 2002; McManus et al., 2008). As seen in culture, some species form clumps that sink to the bottom of the flask at senescence and return to forming long and spiraling planktonic chains at exponential

growth, after the addition of new media (e.g., Fryxell et al., 1990). In nature, such dynamics of sinking to the nutricline and returning to the photic zone has been suggested based on indirect evidence (Fryxell et al., 1997), but see physical displacement of thin layers as a likely mechanism (Rines et al., 2002). At any rate, despite relatively low sinking rates of individual chains (Smayda and Boleyn, 1965), frustules and particulate DA have been found in sediment traps placed at depths below the general occurrence of blooms (Buck et al., 1992; Dortch et al., 1997; Schnetzer et al., 2007). Moreover, layers of *Pseudo-nitzschia* have been found in cores of recent sediment (Parsons et al., 2002) and detectable levels of DA in benthic species, indicating a pelagic–benthic coupling in terms of pathways for toxin transfer (Kvitek et al., 2008). The report of apparently viable cells in zooplankton fecal pellets (Buck and Chavez, 1994) provides an additional means of *Pseudo-nitzschia* transport through the water column.

- HNLC: *Pseudo-nitzschia* species thrived in most ecosystem-scale Fe fertilization experiments carried out to date (de Baar et al., 2005). Miniaturization as a possible survival strategy to enhance nutrient uptake by increasing SA/V ratio was suggested for *Pseudo-nitzschia* acclimated to low Fe concentrations (Marchetti and Harrison, 2007). A low-Fe adaptive strategy was shown to be associated with toxicity: DA production can be stimulated by low-Fe availability, and the resulting release of DA to solution, in turn, increases rates of Fe uptake (Maldonado et al., 2002).
- Annual, interdecadal trends: Species shifts involving *Pseudo-nitzschia* have been associated with nutrient loading on coastal systems, El Niño-ENSO events, and global climate change (Avaria and Muñoz, 1987; Hasle et al., 1996; Parsons et al., 2002; Lundholm et al., 2010; Jester et al., 2009). The postulated tendency is that the decrease in the Si:N and Si:P atomic ratios due to cultural eutrophication would favor flagellates as opposed to diatoms. Possible effects of global warming are less intuitive because temperature tolerances can be species specific across all taxonomic groups, although many marine dinoflagellates and coccolithophorids are particularly known to thrive in warm waters (Steidinger and Tangen, 1997; Heimdal, 1997).

Our understanding about the nature of the selective pressure of environmental fluctuations on phytoplankton communities, members of the genus *Pseudo-nitzschia* included, is still too incomplete to accurately predict the outcome of long-term trends, despite the great contribution of long time-series studies (Smetacek and Cloern, 2008). Since the Late Cretaceous–Early Tertiary, diatoms have shown an ever-increasing diversity preserved in the fossil record and now dominate the modern marine phytoplankton (Kooistra et al., 2007). Extrapolating from this success, one ought to expect that diatoms are well equipped to face new challenges. After all, this is a photosynthetic microeukaryote of a broad size range, protected in a silica case, with acquired unorthodox metabolic pathways and other niche-broadening associations, and well adapted to floating and even extensive vertical migration despite the lack of flagella. Looking through a microscope, planktonic marine diatoms are simply as beautiful as they are interesting.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 491–512
DOI 10.1007/978-94-007-1327-7_22, © Springer Science+Business Media B.V. 2011

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TOXIC DIATOM *PSEUDO-NITZSCHIA* AND ITS PRIMARY CONSUMERS (VECTORS)

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1. Introduction

Algal species can be established in the water column via their natural presence or their introduction from currents, ballast water, and aquacultures. When favorable environmental conditions occur, periods of rapid cell reproduction, called blooms, can be triggered. Generally, during the bloom or at the end of the bloom, different types of harmful effects are observed ranging from discoloration of the water to production of toxins to contamination and death of higher organisms in the food chain. Harmful algal blooms (HABs) are, therefore, widely accepted to represent toxic species as well as species that alter habitat (anoxia, hypoxia, persistent turbidity), create physiological dysfunction, or disturb community relationships.

More than 5,000 species of microalgae exist worldwide, of which about 100 are toxic. Most of the well-known algal toxins are associated with dinoflagellates. However, toxin-producing species are found in other groups besides the dinoflagellates, including diatoms and cyanobacteria. The primary groupings of algal toxins are described based on the syndrome they caused in humans and the first identified vector, usually shellfish that transferred the toxin to humans. These groups include syndromes such as paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), *diarrhetic* shellfish poisoning (DSP), ciguatera fish poisoning (CFP), and cyanobacteria toxin poisoning (CTP). The cell concentrations at which harmful algae can become toxic are very species specific. With some toxic taxa, harmful levels can be reached when concentrations are only 500 cells/l, which may not be considered a bloom. In some cases, algae belonging to different genera can produce similar toxins and cause the same syndrome. As Smayda described in 1997, "Harmful algal blooms have regional, seasonal, and species-specific aspects; it is not simply a biomass issue" (Smayda, 1997).

HABs are increasingly noted around the coastlines of the world's ocean, with the growing numbers of reports resulting possibly from increased surveillance or possibly linked to anthropogenic influences (Smayda, 1990; Hallegraeff, 1993; Van Dolah, 2000; Anderson et al., 2002). It is argued in these reviews that human activities have been changing the nutrient ratios and these changes alter

the phytoplankton species composition of coastal marine ecosystems and favor toxic species. Alternatively, marine organisms can respond to natural processes such as changes in water temperature that are related to climate fluctuations (Balech, 1960; Jester et al., 2009). Among all the known toxic algal species, recently diatom genus *Pseudo-nitzschia* has been shown to be affected both by climate shifts (Jester et al., 2009) and nutrient enrichment (Dortch et al., 1997).

2. Diatom *Pseudo-nitzschia* and Its Toxin Domoic Acid

The diatom *Pseudo-nitzschia* (Fig. 1) is a common coastal and oceanic phytoplankton with worldwide distribution (Hasle, 1994, 1995; Hasle et al., 1996). Multiple species in the genus with similar appearance are capable of producing the toxin domoic acid (DA), which is a neurotoxic amino acid responsible for deaths of higher trophic-level organisms, including humans (Wright et al., 1989; Bates, 2000). Eleven species have been identified so far as being capable of producing DA from areas around the world (Moestrup and Lundholm, 2007). Most DA-producing *Pseudo-nitzschia* species are cosmopolitan and reports of their blooms, like those of toxic dinoflagellates, are increasing (Hasle, 2002; Mudie et al., 2002). There have been problems identifying these species and the genus and species names were revised when some species in the group were found to be toxic. Historical records of the DA-producing diatoms (usually designated as *Nitzschia*) are limited worldwide as well as on the US coasts. Limited data on the DA-producing species are the result of the relatively recent recognition of the toxin and the consequence of taxonomic problems among species that appeared similar in light microscopy studies. A number of species formerly identified as *Nitzschia* are now recognized to include one or more species of *Pseudo-nitzschia*, including both known toxin producers and nontoxic species. *Nitzschia seriata*, widely reported on the west coast since the 1920s, is known to include California's now most commonly observed toxic species, namely, *P. australis* (Fig. 1) (Fryxell et al.,

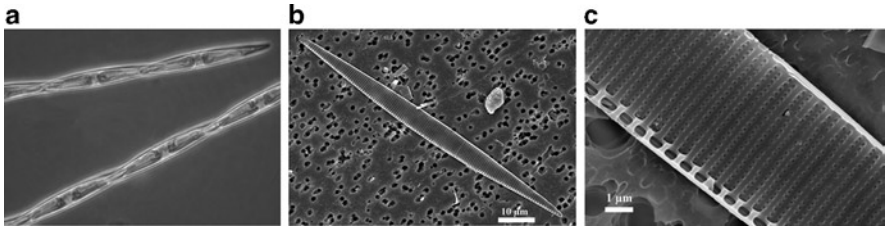

Figure 1. *Pseudo-nitzschia australis*. (**a**) Light microscopy image of *Pseudo-nitzschia australis* (Image courtesy to Dr. Rozalind Jester.) (**b**) Scanning electron micrograph of *P. australis*, whole valve with length = 89 µm, width = 6.1 µm, stria = 15 in 10 µm, fibulae = 15 in 10 µm. The poroids are arranged in two rows with four in 1 µm. A central nodulus is absent. (**c**) Closer look to (**b**) showing the pore division structure. Water samples are taken from Monterey Bay, CA, USA.

1997). However, no records of the patterns of the toxin producers are available on the west coast prior to the 1990s. With the developments of species-specific DNA probes, many other *Pseudo-nitzschia* species are now identified as toxin producers. While some toxic *Pseudo-nitzschia* species have been known to produce the toxin in every growth stage (e.g., *P. australis*), others have production limited to the stationary phase (e.g., *P. multiseries*) (Bates et al., 1991). However, Wells et al. (2005) stated that all *Pseudo-nitzschia* species studied to date, using the highly sensitive toxin assays, have been shown to produce DA under appropriate conditions.

DA was first isolated from the red alga *Chondria armata* by Japanese scientists and used as an anthelmintic in 1950s (Daigo, 1959a, b, c). It is water soluble, a rare tricarboxylic secondary amino acid and structural analog of glutamic acid, the major excitatory neurotransmitter in the brain (Kramer et al., 1991; Berman and Murray, 1997). It mimics glutamate and binds to glutamate receptors, which can result in damage to the central nervous system in vertebrates (Takemoto, 1978; Stewart et al., 1990; Hampson et al., 1992). The toxin damages the nervous system of vertebrates, including humans, causing permanent short-term memory loss, seizures, and sometimes death (Todd, 1993). This fairly new phycotoxin was discovered when over 100 people suffered from gastrointestinal disorders, and four people died after eating cultured blue mussels (*Mytilus edulis*) in Prince Edward Island, Canada in 1987 (Wright et al., 1989; Todd, 1993). Interestingly, the origin of the toxin was found to be the diatom *P. multiseries,* which occurred in high numbers during the event (Bates et al., 1989). The event itself was called "Amnesic Shellfish Poisoning (ASP)" after the effects on humans caused by the toxin. Ingested DA can cause serious neurological and gastrointestinal effects and, in humans, result in ASP with symptoms including abdominal cramps, vomiting, disorientation, extreme headaches, seizures, confusion, hallucinations, chronic memory loss, and death (uncommon and only in elderly) (Todd, 1993). In animals, symptoms included vomiting, diarrhea, confusion, disorientation, scratching, seizures, coma, and death, and the event was called "domoic acid poisoning (DAP)" (e.g., Gulland et al., 2002). The first report of a DA's neurotoxic effects on higher level animals was observed in brown pelicans and cormorants (Work et al., 1993). Later, these symptoms were observed in sea lions and male dolphins (Goldstein et al., 2008; Ramsdell and Zabka, 2008; Torres de la Riva et al., 2009).

In the USA, Monterey Bay, CA seems one of the hot spots for these domoic acid poisoning events. Toxic diatom species are found periodically in Monterey Bay throughout the year and often form blooms in spring and fall (Buck et al., 1992; Garrison et al., 1992; Walz et al., 1994; Fryxell et al., 1997; Scholin et al., 2000; Bargu et al., 2010). Over the last decade, California coastal waters have been shown to be impacted by DA, mostly associated with *P. australis*, with toxic events recognized not only by the presence of DA in shellfish but also by deaths of charismatic megafauna, especially marine birds and mammals. The toxin was first discovered on the US west coast in Monterey Bay, California, in 1991, when more than 200 brown pelican (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) were found dead on local beaches (Work et al., 1993). There were other mass bird kills before 1991 but these were never previously linked to algal toxins. Similarly, Monterey Bay was the site of other well-publicized DA

poisoning incidents, including ones in 1998 and 2000 when beached sea lions (*Zalophus californianus*) exhibited diagnostic neurological symptoms now associated with DA (Lefebvre et al., 1999; Scholin et al., 2000; Gulland et al., 2002). In all the California events, *P. australis* was the primary DA source, and filter-feeding anchovies (*Engraulis mordax*) were the apparent toxin vector (Fritz et al., 1992; Garrison et al., 1992; Work et al., 1993; Walz et al., 1994; Lefebvre et al., 1999; Scholin et al., 2000). Since the 1991 DAP event in central California, high toxic *Pseudo-nitzschia* cell numbers and DA contaminations have been also reported from southern California and Gulf of Mexico (Dortch et al., 1997; Parsons et al., 1999; Schnetzer et al., 2007).

3. Primary Consumption of DA

Toxins of photosynthetic organisms are common in terrestrial environments. On land, plant toxins are largely removed/reduced in food crops and mainly used as anti-herbivory (Rosenthal and Janzen, 1979; Fox, 1981). In aquatic environments, phycotoxins are uncommon having only a few dozen out of 5,000+ species, which are mainly known as coastal, shellfish poisons (known as human toxins). These toxic compounds in aquatic environments, as in terrestrial environments, are thought to be secondary compounds produced from primary metabolic activities but their function, if any, is often poorly understood (e.g., Hay and Fenical, 1988). While some of these compounds can be released by phytoplankton into the water to sequester nutrients and vitamins (Ladizinsky and Smith, 2000; Bates et al., 2001; Rue and Bruland, 2001), others can be held inside the cells and used as a feeding deterrent (e.g., Paul, 1987; Bargu et al., 2003), a well-known mechanism in terrestrial systems (e.g., reviewed in Rosenthal and Janzen, 1979; Duffey, 1980; Fox, 1981). Variations in herbivore consumption rates can be caused by differences in size, mobility, shape, and nutritional value of their food as well as by its concentration (e.g., Frost, 1972; Huntley et al., 1986; Price et al., 1988). Among the factors that influence how food is chosen and how much is consumed is the quality of available food (e.g., Huntley et al., 1986; Opalinski et al., 1997).

Production of phycotoxins that might reduce the feeding rates of herbivorous zooplankton are mainly known for dinoflagellates (e.g., Huntley et al., 1986; Ives, 1987; Turriff et al., 1995; Bagoien et al., 1996; Teegarden and Cembella, 1996; Shaw et al., 1997). Contamination of fishery products or death of higher organisms in the food chain is also known (e.g., Geraci et al., 1989; Anderson and White, 1992; Lefebvre et al., 1999; Scholin et al., 2000). The direct and rapid transfer of these toxins to the highest levels of the food chain requires herbivores that consume the toxic species. In the case of water-soluble toxins like DA, the direct and rapid transfer of these toxins to high levels of the food chain requires herbivores that consume large quantities of toxic algal species and have the cells (and the associated toxin) in their gut at the time they are consumed by their predator.

There are two possible routes by which herbivores acquire DA. First, they could ingest particles containing DA, possibly also consuming some additional dissolved DA in the water accompanying the particles. Second, herbivores could absorb dissolved DA directly from the surrounding water through their body surface, especially through lightly chitinized gills. If DA is outside or concentrated in the boundary layer around the cells, chemosensory systems on mouthparts could also provide info causing herbivores to reduce feeding. Once it is inside of the gut, however, chemosensory detectors appear to be minimal and glutamate receptors will be fairly nonspecific, binding both glutamic acid and domoic acid. In this second scenario, animals could use other mechanisms to protect themselves from toxin that has already entered the gut. Below, we present the types of feeding strategies of potential toxin vectors and list the expected outcomes for DA transfer by these organisms through the food webs:

1. The vectors consume toxic cells with no ill effect: they are now potent toxin vectors.
2. In a possible variant of scenario 1, above, the vector may have an evolutionary history of exposure to the algal toxins and therefore have developed chemical or physiological strategies to avoid assimilating or to mitigate the toxic effects of consumed toxins: the species are now potent toxin vectors.
3. The vector may reduce its feeding rate, with consequent reduction in growth or reproduction if the DA event lasts sufficiently long: the species is a potential toxin vector but less contaminated than other species that continue feeding at their normal rates.
4. The vector may starve or migrate from the bloom area: the vector does not transfer DA to its predators.
5. For omnivorous consumers, the species may change their diet and consumption of nontoxic prey, if such items are available: limitation of their role as toxin vectors.

It is clear that identification of different herbivorous vectors that can transfer phycotoxins to higher trophic levels and knowledge about their feeding behaviors (and hence, toxin acquisition) become essential for understanding the fate of domoic acid in marine ecosystems. Since DA is not expected to bioaccumulate in the food chain due to its water-soluble nature, a short food chain is required to transfer DA very efficiently, such as large immediate consumers, with its direct link between the algae and large predator. A wide variety of pelagic organisms and some benthic forms as well, have been shown to contain DA. After the first DA poisoning event in Monterey Bay, CA in 1991, with northern anchovies (*Engraulis mordax*) found to be the DA vector (Lefebvre et al., 1999; Scholin et al., 2000), researchers identified additional DA vectors on the US west coast: e.g., mussels (*Mytilus californianus*), razor clams (*Siliqua patula*), Dungeness crabs (*Cancer magister*), mole crabs (*Emerita analoga*), and krill (*Euphausica*

pacifica and *Thysanoessa spinifera* (Loscutoff, 1992; Wohlgeschaffen et al., 1992; Langlois et al., 1993; Horner and Postel, 1993; Lund et al., 1997; Lefebvre et al., 1999; Bargu et al., 2002, 2003; Bargu and Silver, 2003). An increasing number of fish have also found to contain DA, including northern anchovies, Pacific sardines, sand dabs, white croaker, sculpins, and menhaden (Lefebvre et al., 1999, 2002a, b; Fire and Silver, 2005; Del Rio et al., 2010). In some blooms, *Pseudo-nitzschia* is the overwhelmingly dominant phytoplankton. Under those conditions, *Pseudo-nitzschia* is essentially the only available food, so their primary consumers will ingest the cells and, presumably, the toxin. If they are not the victim of domoic acid, they can efficiently transfer the toxin through the food web and inflict severe damage on higher organisms. The main groups of primary consumers of the toxic diatom are detailed in the following sections.

3.1. SHELLFISH

Shellfish acquire toxins when filtering phytoplankton in shallow coastal waters, where humans can readily harvest them and, consequently, have encountered and then identified the toxins (Silver, 2006). DA has been found to contaminate a large variety of shellfish from around the world (e.g., Mos, 2001; Blanco et al., 2006; Amzil et al., 2001; Vale and Sampayo, 2001). Reported shellfish vectors range from shallow benthic, immobile organisms that filter feed, to a few mobile shellfish and cephalopods (e.g., Ferdin et al., 2002; Costa et al., 2005). The issues of how DA in shellfish are effectively transferred to humans and what is being done to monitor this toxin together with the concern of how the vector itself is affected by the toxin are of great interest to scientists.

As mentioned earlier, the first known occurrence of ASP was in 1987 in Prince Edward Island, Canada where more than 100 illnesses and three deaths were traced back to shellfish. Blue mussels (*Mytilus edulis*) were the first known shellfish that were contaminated and responsible for the ASP outbreak in Canada with levels of DA up to 790 μg/g tissue (Bates et al., 1998). Since that time, there have been several other areas around the world that contaminated shellfish have been reported (Fig. 2). For example, king scallop (*Pecten maximus*) has often been found to be contaminated by DA, with levels ranging from 53 to 534 μg/g tissue, in several European countries including Scotland (Gallacher et al., 2001), France (Amzil et al., 2001; Liu et al., 2007), Spain (Blanco et al., 2006; Bogan et al., 2007), and Ireland (Bogan et al., 2007). In Greece, mussels (*Mytilus galloprovincialis*) and venus clams (*Venus verucosa*) have also been monitored since a low first detection of 5.5 μg DA/g tissue in 2002 (Kaniou-Grigoriadow et al., 2005). Other shellfish markets in Denmark, (Lundholm et al., 2005) on the Mediterranean coasts of France and Italy (Kaniou-Grigoriadow et al., 2005), and in Portugal have been affected by levels of DA of 32–90 μg/g tissue as well. Specific shellfish from Portugal include the common cockle, *Cerastoderma edule,* and carpet shell, *Venerupis pullastra* (Vale and Sampayo, 2001). In the USA, shellfish species, specifically the Pacific oyster (*Crassostrea gigas*) (Jones et al., 1995), dungeness

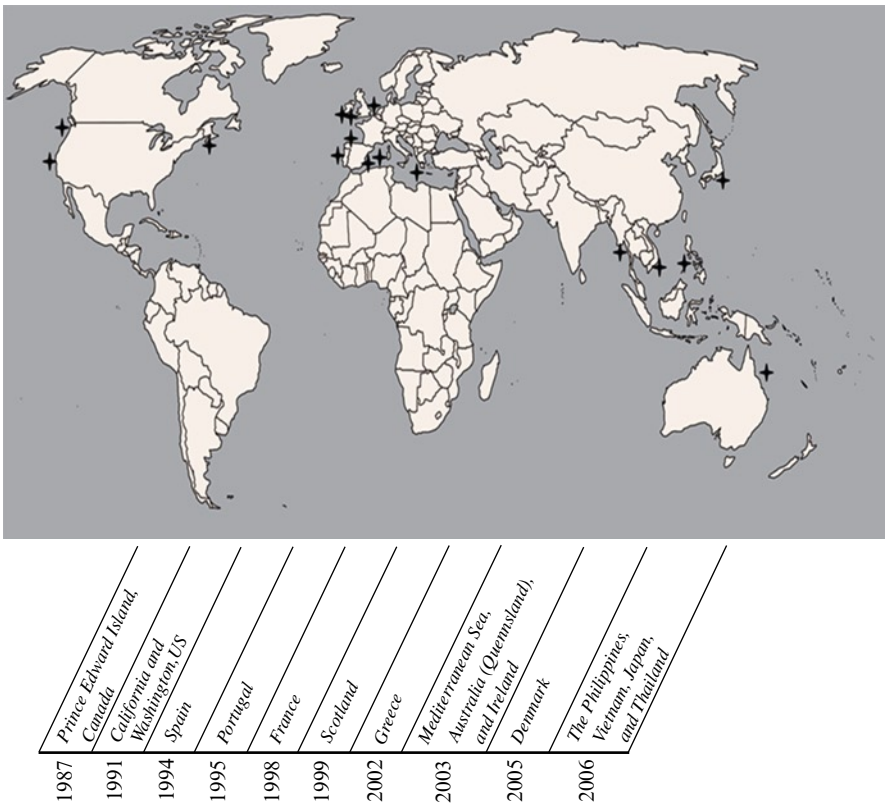

Figure 2. DA detection in shellfish around the world.

crab (*Cancer magister* Dana), and razor clam (*Siliqua patula* Dixon), have been reported to contain high DA levels up to 125 μg/g tissue (Lund et al., 1997). A recent study also reported that *Pseudo-nitzschia* blooms are affecting shellfish in tropical waters of the Philippines, Vietnam, Japan, and Thailand with levels of DA ranging from 0.003 to 42 μg/g tissue (Dao et al., 2009; Takata et al., 2009). In the Southern Hemisphere, there has been a study off of the coast of Australia in 2004, which showed levels of DA in shellfish up to 256 μg/g tissue (Takahashi et al., 2007). Another potential vector of DA to humans is the shellfish group of cephalopods. Even though cephalopods do not filter feed, but rather eat planktivorous fish are showing levels of DA that may need to be watched in the future. For example, squid (*Loligo opalescens*), in the USA, have shown very low levels of DA (0.1–0.37 μg/g tissue), but octopi (*Eledone cirrhosa* and *E. moschata*) in Portugal had DA levels up to 127 μg/g tissue (Costa et al., 2005; Bargu et al., 2008).

Levels of DA in shellfish have been reported from around the world and vary in all regions. The max DA level found in shellfish will even vary in countries that are geographically close together (Table 1). Most studies of DA accumulation show that the area with the highest concentration is in the hepatopancreas of

Table 1. Maximum amount of DA found in shellfish around the world.

Year	Country	Species	Max DA level (μg/g tissue)	Source
1987	Canada	*Mytilus edulis* (blue mussel)	790	Bates et al. (1998)
1999	Scotland	*Pecten maximus* (king scallop)	534	Gallacher et al. (2001)
2000	France	*Donax trunculus* (bean clam)	53	Amzil et al. (2001)
2000	Portugal	*Mytilus edulis* (blue mussel)	90	Vale and Sampayo (2001)
2002	Greece	*Venus verucosa* (Venus clam)	5.5	Kaniou-Grigoriadow et al. (2005)
2002	Spain	*Pecten maximus* (king scallop)	175 (Digestive gland)	Blanco et al. (2006)
2003	Australia	*Donax deltoides* (Goolwa cockle)	256	Takahashi et al. (2007)
2003	Ireland	*Pecten maximus* (king scallop)	154	Bogan et al. (2007)
2005	Vietnam	*Spondylus versicolor* (golden thorny oyster)	147	Dao et al. (2009)
2005	Denmark	*Mytilus edulis* (blue mussel)	32	Lundholm et al. (2005)
2006	The Philippines	*Spondylus squamosus* (ducal thorny oyster)	42	Takata et al. (2009)
2006	Japan	*Spondylus sinensis*	0.5	Takata et al. (2009)
2006	Thailand	*Spondylus versicolor* (golden thorny oyster)	1.8	Takata et al. (2009)

shellfish (Arévalo et al., 1998; Bogan et al., 2007). The Dungeness crab has only shown accumulation in the digestive system (Lund et al., 1997), unlike mussels that has accumulation in several different body segments (Novaczek et al., 1991). Another study showed that king scallop larvae have a possible DA-binding site that could be a role in transferring DA into the food web. This has possible serious implications, if the larvae are accumulating the DA, and it is not detrimental on their development, it could be lethal in high doses to their consumers (Liu et al., 2007). The shellfish that serve as the DA vector for humans can also be negatively impacted by this toxin. There have not been many studies on how DA directly affects shellfish reproduction; however, some studies on king scallop showed that concentrations of DA possibly present in the environment will have no significant effect on the larvae, but consistently affects the amount of DA in the gonadal tissue (Campbell et al., 2003; Liu et al., 2007). The overall role of sexual reproduction and how it is affected by DA is still under speculation (Mos, 2001).

The variety of methods that shellfish use to depurate DA is as varied as the animals themselves. Since DA is a water-soluble toxin, the kidneys are a very effective means to disperse DA, resulting in many organisms having a high level of the toxin in the kidneys compared to other body parts (Novaczek et al., 1991). The period over which depuration takes place also varies with the organism. There are several species of shellfish like mussels, oysters, and soft-shell clams that

can depurate DA in a very short period of hours or days. However, there are other bivalves like scallops and razor clams that retain high DA levels for up to a year (Trainer et al., 2007; Mafra et al., 2010). The king scallop showed a great decrease of DA in all of its body parts except the abductor muscle, but compared to most bivalves, it was still a very slow rate (Blanco et al., 2002). However, not all shellfish need to depurate the toxin through their body systems, some like oysters use feeding mechanisms to distinguish the toxin diatom while feeding and avoid even ingesting it (Mafra et al., 2009, 2010).

Reports of DA detection in shellfish around the world (Fig. 2) have been happening with a more frequent basis and may be attributable to more countries looking for this toxin. If a country has not previously had a problem with shellfish poisoning events, then there is usually no regulation in place to monitor for phycotoxins. After the 1987 outbreak of DA in Canada, Health Canada (Canada's health care regulatory body) set a regulatory limit of 20 μg DA/g tissue for shellfish in the late 1980s. The amount of toxin found in the 1987 human contamination case was 790 μg DA/g, showing how much higher the amount of toxin consumed by humans with obviously disastrous results. A few years later, there was discovery of DA in finfish off of the west coast of the USA. Since there was no reported human DA poisoning in the USA, the US Food and Drug Administration adopted the Canadian limit for DA amounts in shellfish (Wekell et al., 2004). Europe started regulations of the same maximum limit in 1997, which has caused several shellfish harvesting bans since, including one in Scotland in 1999 where toxin level reached 534 μg DA/g tissue in shellfish. There may have been outbreaks before this, but since there were not any official regulations in place, there is no data available (Gallacher et al., 2001). There are several concerns raised by researchers and agencies from different countries on how monitoring efforts should be conducted. The regulations are very firm in the 20 μg DA/g tissue limit; however, in several countries that have since adopted this limit, there has been debate over whether this should apply to the whole organism or just to the part that is consumed by humans. For example, in several scallop studies, the whole body limit was below regulation, but the gonadal tissue (the main part used for consumption) was above the regulatory limit (Blanco et al., 2002; Campbell et al., 2003). Also, the problem of inter-animal variability within each sample has been shown (Bogan et al., 2007; Mafra et al., 2010), so the replication is another essential step but can be very costly for government agencies. There is the additional issue of a few studies showing that onshore collections of shellfish and their toxin levels differ from the offshore collection levels (Bogan et al., 2007). This could potentially cause a problem with the current methods of collection since mostly inshore samples are taken for convenience. There have also been a couple of cases where DA levels were not in sync with *Pseudo-nitzschia* blooms, suggesting a time lag between the two occurrences. When samples of the water and shellfish are taken the same day, the phytoplankton levels may be very high at the surface, but the shellfish may have not had enough time to uptake the toxin (Takahashi et al., 2007), or the bloom can exhibit a very patchy distribution that

sessile shellfish species may not be exposed to the toxins directly. Scientists now are suggesting using mobile organisms as the sentinel species as opposed to the common practice of using shellfish species. Obviously, there is a need to develop region-specific monitoring protocols, but also to develop long-term monitoring and research efforts in order to increase our understanding on the ecology of these blooms and contamination levels of different shellfish organisms to be able to generate better warning outcomes for consumption, as well as to increase our prediction capabilities of such events.

3.2. ZOOPLANKTON

Zooplanktons are important consumers of diatoms as well as valuable food for a variety of higher organisms such as birds, fish, and baleen whales in the pelagic food web of the world oceans (e.g., Nemoto, 1971/1972; Holm-Hansen and Huntley, 1984; Price et al., 1988; Schoenherr, 1991; Turner and Tester, 1997; Doucette et al., 2006). The food preference of zooplankton depends on the availability and the quality of food, as well as the developmental stage of the animals. Toxin effects may vary due to variations in toxin concentrations but also due to different effects such as feeding inhibition, reduction in fecundity and mortality may have different concentration thresholds.

Studies on zooplankton response to the neurotoxic DA are very limited. Results from previous laboratory studies with zooplankton reveal various effects of exposure to both particulate and dissolved DA, depending upon the types of assays performed and the taxa involved. Microzooplankton grazing has been studied by Olson and Lessard (2010) and Olson et al. (2008) and concluded that DA did not act as a feeding deterrent nor did it suppress their growth. Different copepods species have been found to accumulate DA in several lab studies (Windust, 1992; Lincoln et al., 2001; Tester et al., 2001; Maneiro et al., 2005; Leandro et al., 2010). Similar to microzooplankton, researchers have not found any negative effect of particulate DA on copepod gut-filling rates, survival, or reproductive success. They concluded that DA did not act as a grazing deterrent for copepods and, therefore, copepods have great potential to transmit DA to higher trophic levels during toxic *Pseudo-nitzschia* blooms. On the other hand, dissolved DA seemed to act differently on copepods' responses. Lincoln et al. (2001) found no adverse effect on copepods exposed to dissolved DA at nanomolar levels, whereas Shaw et al. (1997) showed that dissolved DA caused death at micromolar levels.

Krill (Euphausiids) are dominant macro-zooplankton and are also the major consumers of phytoplankton, especially diatoms. They can feed on detritus, small zooplankton, and large phytoplankton. However, the shifts in their diet can be dependent on the abundance of food or structure of their mouth parts. It is shown that krill feed on toxic *Pseudo-nitzschia* and that the quantities of cells consumed and the feeding pattern over time are dependent on the species of euphausiid and the cellular DA content of the food (Bargu et al., 2003; Bargu and

Silver, 2003). Bargu et al. (2003) has demonstrated the temporal feeding pattern of krill on the toxic diatom species differed from that of the temporal feeding pattern of krill on nontoxic diatom species. The observed feeding patterns may have contributed to the maintenance of toxin body burdens below a certain threshold, where no immediate ill effects to the herbivore were apparent. Additionally, high levels of dissolved DA exposure have been reported to suppress krill grazing (Bargu et al., 2006). The DA content in krill collected from Monterey Bay, CA ranged from 0.1 to 44 μg DA/g tissue, depending on the concentration of toxic *Pseudo-nitzschia* in the water (Bargu et al., 2002). Toxin concentrations, similar to those found in krill in the laboratory study, have also been observed in field-collected specimens, suggesting that krill may have a DA threshold at a level above that which ingestion of toxic cells is inhibited. The highest detected concentration of 44 μg DA/g krill exceeded the upper limit allowed for human consumption, which is 20 μg DA/g of shellfish. Krill are important forage species for fish, squid, seabirds, and baleen whales (e.g., Morejohn et al., 1978; Schoenherr, 1991; Starr et al., 1998). Their large size gives them a potentially critical role in toxin transfer. Blue whales (*Balaenoptera musculus*), collected when they were foraging on krill swarms, have already been reported to contain high DA levels (max levels of 207 μg DA/g feces) during a bloom of toxic *Pseudo-nitzschia* in Monterey Bay, CA (Lefebvre et al., 2002b).

3.3. PLANKTIVOROUS FISH

After preliminary studies substantially included bivalves, planktivorous fish became the primary focus after the first *Pseudo-nitzschia* bloom occurred on the Pacific coast of the USA in 1991. Mortality of the brown pelican (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) was reported and attributed to their consumption of northern anchovies (*Engraulis mordax*) contaminated with DA (Fritz et al., 1992; Work et al., 1993). Previous reports indicated that DA levels detected in anchovy's viscera was up to 1,815 μg DA/g tissue (Lefebvre et al., 2002a) and in the gut was 2,300 μg DA/g tissue (Altwein et al., 1995), which is almost 100 times greater than the regulatory level for fish containing DA. After this event, filter-feeding fish were identified as major vectors that transferred the toxin to higher tropic levels. They are thought to be a more potent vector than bivalves since they are the direct link between a toxic algal bloom and large, higher-level consumers (Lefebvre et al., 2002a).

The high abundance of northern anchovies and Pacific sardines (*Sardinops sagax*) in Monterey Bay and other regions of the California current have made these regions quite attractive for extensive studies on planktivorous fish and DA intoxication. In addition to the 1991 incident involving pelicans, a second case of DA toxicity in sea lions (*Zalophus californianus*) was reported in California in 1998 as the result of their consumption of contaminated anchovies (Lefebvre et al., 1999; Scholin et al., 2000). In 1996, after a mass death of

pelicans in Cabo San Lucas, Mexico, mackerel were also identified as a vector of DA (Sierra-Beltran et al., 1997). Yet another incident of DA toxicity was reported in 1998 (Scholin et al., 2000) on the central California coast, during which more than 400 California sea lions died from consuming contaminated anchovies. Seizures were observed in some of the sea lions after consuming the toxic fish (Lefebvre et al., 1999; Scholin et al., 2000).

Despite the obvious connection between DA-contaminated fish and toxicity to their predators, the point at which DA levels in water reach toxicity has been unknown. Lefebvre et al. (2002a) clearly demonstrated the correlation between toxic cell densities in water during *Pseudo-nitzschia* bloom and DA levels that accumulated in fish. DA was only detected in fish viscera samples when the toxic cell densities reached levels of 10^3 cell/l in surface waters. When the toxic cell levels reached 10^4 cell/l, DA concentration was reported above the regulatory level for fish containing DA. Undetected DA in fish viscera pre- and post-bloom suggested that DA is a threat only during the bloom.

Studies clearly showed that higher-level consumers, affected by DA, experience neurologic symptoms. The observation of neurologic effects in fish, however, was not as obvious. After first failing to show any symptoms in rainbow trout that had been fed DA-contaminated fish (Hardy et al., 1995), experiments involving the intracoelomic (IC) injection of DA into anchovies resulted in serious neurologic symptoms such as head shaking, spinning, disorientation, inability to school, and death (Lefebvre et al., 2001). IC injections of DA confirmed that anchovies (Lefebvre et al., 2001), zebrafish (Tiedeken et al., 2005), and rainbow trout (Bakke et al., 2010) are neurologically susceptible to DA. It is believed that these neurotoxic symptoms allow contaminated fish to become an easier target for predators, thereby spreading the toxin to other populations.

DA is not only a threat for pelagic species but also shows a threat to benthic-feeding species such as rex sole (*Errex zachirus*), Dover sole (*Microstomus pacificus*), English sole (*Pleuronectes vetulus*), and curlfin turbot (*Pleuronectes decurrens*). There are also benthopelagic-feeding species such as Pacific sanddab (*Citharichthys sordidus*), slender sole (*Eopsetta exilis*), petrale sole (*Eopsetta jordani*), sand sole (*Psettichthys melanostictus*), and Pacific halibut (*Hippoglossus stenolepis*). The amount of DA that was measured in these benthopelagic-feeding species was up to 53 μg DA/g tissue (Vigilant and Silver, 2007). Once planktivorous fish die and sink to the seafloor, DA can also be transferred to benthic communities (Lefebvre et al., 2001). Other pathways suggested that fecal pellets of planktivorous fish and invertebrates containing DA and flocculated cells of *Pseudo-nitzschia* can reach the seabed and become part of the diet of benthic fish species (Vigilant and Silver, 2007).

There have been extensive studies between fish and DA to reveal the effect of DA on immediate and higher-level consumers over two decades. These studies are dominated around California, Mexico, and Portugal. As shown in Table 2, DA is found predominately in fish viscera and gut rather than in their edible parts. This may simply suggest that DA is a more potent biotoxin for higher tropic level organisms other than human consumers.

Table 2. Planktivorous fish species that were studied for DA analysis over two decades around the world.

Fish species	Trophic level	Region	Body part analyzed	Max. DA (µg/g tissue)	Source
Atherinopsis californiensis (*Jacksmelt*)	Omnivore	Monterey Bay, CA	Viscera	275	Lefebvre et al. (2002b) and Mazzillo et al. (2010)
Brevoortia patronus (*Gulf menhaden*)	Filter feeder	Terrebonne Bay, LA	Viscera	0.3	Del Rio et al. (2010)
Engraulis mordax (*Anchovy*)	Filter feeder	Monterey Bay, CA	Viscera	1,815	Lefebvre et al. (1999, 2001, 2002a), Fritz et al. (1992), Mazzillo et al. (2010), and Work et al. (1993)
Engraulis mordax (*Anchovy*)	Filter feeder	Monterey Bay, CA	Body tissue	39	Lefebvre et al. (1999, 2001)
Engraulis mordax (*Anchovy*)	Filter feeder	Monterey Bay, CA	Gut	2,300	Altwein et al. (1995) and Scholin et al. (2000)
Engraulis mordax (*Anchovy*)	Filter feeder	Monterey Bay, CA	Whole body	55	Lefebvre et al. (1999) and Langlois et al. (1993)
Engraulis mordax (*Anchovy*)	Filter feeder	Monterey Bay, CA	Caudal	4.7	Haywood (1995)
Engraulis mordax (*Anchovy*)	Filter feeder	Monterey Bay, CA	Muscle	38	Fritz et al. (1992)
Sardina pilchardus (*Sardine*)	Filter feeder	Portugal	Gut	492	Vale and Sampayo (2001) and Costa and Garrido (2004)
Sardina pilchardus (*Sardine*)	Filter feeder	Portugal	Muscle	3.5	Vale and Sampayo (2001)
Sardina pilchardus (*Sardine*)	Filter feeder	Portugal	Brain	1.7	Vale and Sampayo (2001)
Sardinops sagax (*Pacific Sardine*)	Filter feeder	Monterey Bay, CA	Viscera	588	Lefebvre et al. (2002a) and Mazzillo et al. (2010)
Sardinops sagax (*Pacific Sardine*)	Filter feeder	Monterey Bay, CA	Body tissue	2.2	Lefebvre et al. (2002a)
Scomber japonicus (*Mackerel*)	Omnivore	San Diego, CA	Body tissue	7.3	Busse et al. (2006)
Scomber japonicus (*Mackerel*)	Omnivore	Monterey Bay, CA	Viscera	1.4	Lefebvre et al. (2002b)
Scomber japonicus (*Mackerel*)	Omnivore	Monterey Bay, CA	Viscera	1.5–2.3	Mazzillo et al. (2010)
Scomber japonicus (*Mackerel*)	Omnivore	BCS, Mexico	Viscera	142.9	Sierra-Beltran et al. (1997)
Trachurus symmetricus (*Jack Mackerel*)	Omnivore	San Diego, CA	Body tissue	5.5	Busse et al. (2006)

4. Conclusion

Toxic algae have long been part of coastal ecosystems and toxin contamination is widespread in pelagic and benthic consumers. Humans may be changing the algal species by changing their growth environment, introducing new algal species, or by altering predator communities. To be able to predict the consequences of DA in marine systems, a better understanding of feeding behaviors of their immediate consumers is essential. Understanding why the diatom does not consistently produce the toxin is an area that needs to be explored to better explain the variability in contaminations of primary consumers. Awareness of this potential toxin needs to be raised especially in coastal communities. If the primary consumers of toxic *Pseudo-nitzschia* are mobile, then resulting sickness in higher trophic organisms may be more geographically spread. If the trends over the years can tell us anything, then more occurrences of DA in shellfish and fish can be expected.

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PART 5:
SUMMARY

Seckbach
Kociolek

Biodata of **Joseph Seckbach,** editor of this volume and author of opening chapter with **J. Patrick Kociolek,** editor and coauthor of two chapters in this volume.

Professor Joseph Seckbach is the founder and chief editor of *Cellular Origins, Life in Extreme Habitats and Astrobiology* ("COLE") book series. He has coedited other volumes, such as the *Proceeding of Endocytobiology VII Conference* (Freiburg, Germany) and the *Proceedings of Algae and Extreme Environments meeting* (Trebon, Czech Republic). See http://www.schweizerbart.de/pubs/books/bo/novahedwig-051012300-desc.ht). He coedited the recent volume (with Richard Gordon) entitled *Divine Action and Natural Selection: Science, Faith, and Evolution* published by World Scientific Publishing Company.

Dr. Seckbach earned his Ph.D. from the University of Chicago (1965) and did a postdoctorate in the Division of Biology at Caltech, in Pasadena, CA. He was appointed to the faculty of the Hebrew University (Jerusalem, Israel). He spent sabbaticals at UCLA and Harvard University. and DAAD-sponsored periods in Tübingen, Germany, and at LMU, Munich. Dr. Seckbach served at Louisiana State University, Baton Rouge, as the first selected occupant of the Endowed Chair for the Louisiana Sea Grant and Technology transfer.

Beyond editing academic volumes, he has published scientific articles on plant ferritin–phytoferritin, cellular evolution, acidothermophilic algae, and life in extreme environments. He also edited and translated several popular books. Dr. Seckbach is the coauthor, with R. Ikan, of the Hebrew-language *Chemistry Lexicon* (Deveer publisher, Tel-Aviv). His recent interest is in the field of enigmatic microorganisms and life in extreme environments.

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J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19, 515–517
DOI 10.1007/978-94-007-1327-7_23, © Springer Science+Business Media B.V. 2011

Professor J. Patrick Kociolek is the **coeditor** of this book (COLE volume 19). He received his M.S. (1983) from Bowling Green State University (Ohio, USA), working with Dr. Rex Lowe on the diatom flora of the Great Smoky Mountains (in the Southeastern portion of the USA), and obtained his Ph.D. (1988) from the University of Michigan (Ann Arbor) in the laboratory of Dr. Eugene F. Stoermer. He spent nearly 20 years as the G Dallas Hanna Chair in Diatom Studies at the California Academy of Sciences, before joining the faculty at the University of Colorado, Boulder. His interests include the taxonomy and systematics of diatoms, as well as developing an understanding of their evolutionary histories and patterns of distribution. He is also interested in developing tools to further research on diatom taxonomy, nomenclature, and ecology. Dr. J. Patrick Kociolek, is the author of over 150 peer-reviewed articles and has edited or coedited eight books.

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SUMMARY

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The remarkable contributions in the 23 articles by the 49 authors from a dozen different countries, presented in *The Diatom World*, offer new data on diatoms. This volume celebrates the diatoms' diversity, emerging areas of research on them, and their fascinating ecology. The chapters relate to age-old questions and topics, such as the classification of diatoms and the habitats they occupy, as well as address new areas of research and interest, such as diatom viruses, symbioses, nanotechnology, and toxicity. In this combination of persistent questions and new horizons, it is clear that the diatoms remain as alluring and intriguing today as they were when Von Leeuwenhoek first spied them over 300 years ago. We suspect interest in this group will grow in the years to come as many new fields emerge around their biology, geological record, genomics, incredible morphologies, and ecological breadth.

ORGANISM INDEX

J. Seckbach and J.P. Kociolek (eds.), *The Diatom World*,
Cellular Origin, Life in Extreme Habitats and Astrobiology 19,
DOI 10.1007/978-94-007-1327-7, © Springer Science+Business Media B.V. 2011

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