Algal Chemical Ecology

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Prof. Dr. Charles D. Amsler
Department of Biology
University of Alabama at Birmingham
Birmingham, AL 35294-1170
USA
e-mail: amsler@uab.edu

Cover illustration: The brown alga *Fucus distichus* and associated grazing periwinkles *Littorina sitkana* and *L. scutulata*. (Photo Charles D. Amsler)

ISBN: 978-3-540-74180-0 e-ISBN: 978-3-540-74181-7

Library of Congress Control Number: 2007932727

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Dedicated to Rick Searles, Fritz Kapraun, and to the memory of Mike Neushul, three wonderful mentors and friends who taught me so much about the biology of algae

Preface

Studies of biotic interactions of algae that involve chemical defenses or signals are currently vibrant, active components of both marine ecological and phycological research. This field is rapidly growing, not only by delving deeper into relatively well-known aspects such as secondary metabolite defenses of tropical and temperate macroalgae to herbivores but also by broadening such work into new habitats and by expanding the field to include many other aspects of the chemical interactions of algae with other organisms or with their environments.

This book has attempted to span the breadth of algal chemical ecology from the perspective of basic ecology. To keep it of manageable length, its scope is restricted primarily to ecological aspects of the field and in doing so, it is unfortunate that a great deal of excellent work in applied areas of algal chemical ecology could not be included. Likewise, algal natural products chemistry is not emphasized in most chapters but the book begins with an introduction to the chemistry of algal defenses that is intended as a primer on natural products chemistry for algal ecologists. In addition, although intended to be broad, it could not be so comprehensively and consequently each author was asked to highlight new areas of research and simply refer a reader to previously published reviews where appropriate. Nevertheless, regardless of previous reviews, the authors were asked to go into depth in one or more areas that they felt would be particularly valuable to illustrate important concepts that could be incorporated into undergraduate- or graduate-level ecology or phycology courses.

As noted, Chap. 1 was intended to help algal ecologists understand the chemistry underlying chemical ecological studies. Chapters 2–5 focus on defenses of macroalgae, primarily though not exclusively on their defenses against herbivores. These chapters are divided by geography and habitat with the relatively well-studied tropical and temperate marine communities the focus of the first two and the relatively understudied freshwater and polar marine communities the focus of the latter two. Chapters 6 and 7 unify these by collectively examining new ways of looking at macroalgal chemical defenses as well as the utility of macroalgae across latitudes and habitats as models for testing and expanding broad ecological theories. Chapter 8 follows with a focused examination of a relatively new area of macroalgal chemical ecology, the multiple potential roles of dimethylsulfoniopropionate in algal ecology and physiology.

The chemical ecology of phytoplankton has been a major new focus of research over the past decade. Chapter 9 was originally intended as a detailed review of this field but unfortunately, for reasons both understandable and unforeseeable, the author was unable to complete it. Fortunately, most individual aspects of the field have been reviewed in a number of recent publications and I am very grateful to Professor Pohnert, himself the author of several of those reviews, for preparing this relatively short chapter that reviews these reviews and that can serve the reader as a unifying introduction into this literature.

Chapters 10–12 all deal with exciting and relatively new areas of study in both macroalgal and microalgal chemical ecology. Chapter 10 examines how herbivores "fight back" in adaptive response to the chemical defenses elaborated by their algal prey. Chapter 11 reviews the relatively few (and comparatively recent) studies of algal secondary metabolite defenses against biofoulers and pathogens that have been conducted with ecologically relevant methodology while Chap. 12 examines the relatively new field studying oxidative burst responses of algae as a defense against these same threats.

Finally, Chaps. 13 and 14 both review other areas of macroalgal and microalgal chemical ecology that have been studied to some extent for a number of years but which are both active areas of current research. Chapter 13 focuses on the multiple ways in which algae utilize defensive compounds to limit damage from ultraviolet radiation. Chapter 14 reviews studies of the behavioral sensory ecology of algae, which is very much understudied in comparison to such work on terrestrial and aquatic animals.

With the exception of Chap. 9, all of the chapters were peer-reviewed and the thoughtful assistance of all the peer-reviewers is deeply appreciated. In almost every case there were at least two anonymous reviews of each chapter and I am grateful to Drs. Katrin Iken and Maureen Callow for coordinating the anonymous reviews of Chaps. 4 and 14, respectively. I am also grateful to Dr. James McClintock, Craig Aumack, and in particular to Margaret Amsler for help in editing several of the chapters. In addition, I thank Dr. Christina Eckey for inviting me to take on this project and Dr. Andrea Schlitzberger for a myriad of assistance in seeing it through to completion. In closing, I am sincerely grateful for the continuing financial support of my own work in algal chemical ecology from the Office of Polar Programs at the United States National Science Foundation and also past support from the Mississippi–Alabama Sea Grant Consortium.

August 2007 Charles D. Amsler

Contents

Contributors

Charles D. Amsler
Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA, amsler@uab.edu

Bill J. Baker
Department of Chemistry, University of South Florida, 4202 E. Fowler Ave CHE205, Tampa, FL 33620, USA, bjbaker@cas.usf.edu

Frank A. Camacho
Marine Laboratory, University of Guam, UOG Station, Mangilao, GU 96923, USA, frank.camacho@guamcc.edu

BAP da Gama
Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, C.P. 100.644, CEP 24001-970, Niterói, RJ, Brazil, bapgama@pg.cnpq.br

Tuija Honkanen
Section of Ecology, 20014 University of Turku, Finland, tuija.honkanen@utu.fi

Veijo Jormalainen
Section of Ecology, 20014 University of Turku, Finland, veijo.jormalainen@utu.fi

Ulf Karsten
Institute of Biological Sciences, University of Rostock, Albert-Einstein-Str. 3, 18059 Rostock, Germany, ulf.karsten@uni-rostock.de

Julia Kubanek
School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230, USA, julia.kubanek@biology.gatech.edu

Amy L. Lane
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA

J. Alan Maschek
Department of Chemistry, University of South Florida, 4202 E. Fowler Ave CHE205, Tampa, FL 33620, USA

James B. McClintock
Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA

Henrik Pavia
Tjärnö Marine Biological Laboratory, Department of Marine Ecology, Göteborg University, 452 96 Strömstad, Sweden, henrik.pavia@marecol.gu.se

Karen N. Pelletreau
College of Marine and Earth Studies, University of Delaware, Lewes, DE 19958, USA, kpellet@udel.edu

Renato Crespo Pereira
Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, C.P. 100.644, CEP 24001-970, Niterói, RJ, Brazil, renato.pereira@pesquisador.cnpq.br

Georg Pohnert
Department of Bioorganic Analytics, Institute for Inorganic and Analytical Chemistry, Friedrich-Schiller-Universität, Lessing Str. 8, 07743 Jena, Germany, Georg.Pohnert@uni-jena.de

Philippe Potin
Station Biologique, Marine Plants and Biomolecules, Unité Mixte de Recherche 7139, Centre National de la Recherche Scientifique-Université Pierre et Marie Curie and LIA DIAMS, Place Georges Teissier, BP 74, 29682 Roscoff, France, potin@sb-roscoff.fr

Erik E. Sotka
Grice Marine Laboratory and the Department of Biology, College of Charleston, 205 Fort Johnson Road, Charleston, SC 29412, USA, SotkaE@cofc.edu

Nancy M. Targett
College of Marine and Earth Studies, University of Delaware, Lewes, DE 19958, USA, ntargett@udel.edu

Gunilla B. Toth
Tjärnö Marine Biological Laboratory, Department of Marine Ecology, Göteborg University, 452 96 Strömstad, Sweden, gunilla.toth@marecol.gu.se

Kathryn L. Van Alstyne
Shannon Point Marine Center, Western Washington University, 1900 Shannon Point Road, Anacortes, WA 98221, USA, kathy.vanalstyne@wwu.edu

Kristen E. Whalen
Biology Department, Woods Hole Oceanographic Institution, MS-32, 45 Water St., Woods Hole, MA 02543, USA, kwhalen@whoi.edu

1
The Chemistry of Algal Secondary Metabolism

J.A. Maschek and B.J. Baker(✉)

1.1 Introduction

Natural product chemistry is a branch of organic chemistry that touches upon many other fields of science, especially applied sciences such as medicine, agriculture, and engineering. It is fundamentally a basic science, involved in the discovery, characterization and cataloging of new chemical substances found in nature. Among other basic scientists, biologists and ecologists recognized some time ago that natural products might help explain species composition, distribution, and diversity in some settings, establishing the field of chemical ecology (Harborne 1989). The field has developed largely around collaborations of chemists and biologists, each bringing their own knowledge base to chemical ecological questions. This chapter is intended as an overview of natural product chemistry with an emphasis on the chemical aspects of algal defensive metabolites. It is our hope that algal ecologists will gain insight into the chemistry in the same manner that chemists will acquire a deeper understanding of the ecology from the remaining chapters of this book.

1.2 Conceptual Framework

1.2.1 Natural Products

Similar to those of other sessile organisms, successful life history strategies employed by algae must include adaptations to their biological communities. Such survival strategies may include behavioral, physical, or chemical means (Hay and Fenical 1988; Lobban and Harrison 1994; Dawes 1998; Stachowicz 2001). Chemical strategies, whereby an organism may produce a toxic defensive compound or an

J.A. Maschek
Department of Chemistry, University of South Florida, 4202 E. Fowler Ave CHE205, Tampa, FL 33620, USA

B.J. Baker
Department of Chemistry, University of South Florida, 4202 E. Fowler Ave CHE205, Tampa, FL 33620, USA. e-mail: bjbaker@cas.usf.edu

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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antifouling metabolite, utilize what chemists refer to as "natural products," literally alluding to products made by living organisms. Defining what a natural product is can be a slippery slope; generally, we know one when we see it, but describing the identifying characteristics inevitably invites divisiveness. It is not the intent of our discussion here to devolve into the philosophy behind the concept, but rather to present typical characteristics observed among natural products.

The term natural product is used synonymously with "secondary metabolite ," the latter of which may have more obvious characteristics. In particular, "secondary" is used here in the context of functional significance, so that something that is secondary is less important than something that is primary. Indeed, primary metabolites are the nuts and bolts of a living system: amino acids, cofactors, and lipids, for example. Cellular function is not possible without primary metabolites. Similarly, organismal function is dependant on primary metabolism, extending "primacy" to large molecules, such as proteins, but perhaps enveloping other small molecules such as visual pigments and neurotransmitters. Primary metabolites tend to be ubiquitous; proteins and enzymes from all organisms are composed from a selection of 20-odd amino acids, and genes are built from combinations of four purine and/or pyrimidine bases. Thus primary metabolites are largely limited to several dozen small molecules, and polymers thereof, and drive all living systems, providing energy, structure, and reproductive capacity.

One of the ways to identify a secondary metabolite, then, is to establish that it is not a primary metabolite. For example, only a small group of red algae make C_{15} halogenated acetogenins (Sect. 1.3.1). These fatty-acid-derived compounds are not nuts and bolts, nor are they ubiquitous, and so they are clearly secondary. Consider, however, steroid derivatives produced by algae and plants known as phytosterols (Parish and Nes 1997). These sterols (cholesterol-like compounds) bear alkylation at C-24, differentiating them from animal sterols. They have roles in membrane structure, a primary characteristic, but they are often species-specific and usually fall under the guise of secondary metabolites. Most often, secondary metabolites are found to be associated with an organism's interaction with its environment. Phlorotannins (Sect. 1.4.2), for example, can impart distastefulness to potential predators, which is a secondary characteristic even though phlorotannins can have primary roles in brown algal cell wall biosynthesis (Ragan and Glombitzka 1986; Amsler and Fairhead 2006). Thus, in the modern use of the term, when we speak of a secondary metabolite or a natural product, we are referring to compounds that are not involved in the development or maintenance of an organism, limited in their biological distribution, often species-specific, and most often produced by an organism for intervention in ecological interactions (Williams et al. 1989). Note that the lack of a demonstrated ecological role for a natural product does not mean one does not exist, and even the failure to identify a particular role is not sufficient to argue against ecological relevance. Careful and thorough investigations of the ecological roles for many natural products remain to be accomplished.

Chemical structure can often be used by itself to recognize a secondary metabolite because of their common biosynthetic origin. Terpenes and polyketides, for

example, account for most of the secondary metabolites (Buckingham 2002) and can be recognized as oligomers of the primary metabolites isoprene and acetate, respectively. An expert can discern arrangements of these oligomers and their associated source(s). Specific details of these chemical classes will be discussed in Sect. 1.3.

1.2.2 Natural Product Names

Like in other fields of science involved in characterizing and cataloging, natural product chemists name their discoveries. The International Union of Pure and Applied Chemistry (IUPAC) has rules of nomenclature, much to the chagrin of many a student of organic chemistry. However, systematic names of natural products, as IUPAC dictates, are inappropriate for common usage, bearing multiple nests of convoluted descriptions of functionalization (IUPAC 1976; Giles 1999; Favre et al. 2004). Common usage allows for nicknames, which are bestowed at the time a new compound is described in the literature, and are generally more succinct and easy to pronounce, and especially, more easily associated mentally with a chemical structure. As an example, most of us are aware of our personal cholesterol level, but few of us would fully comprehend the significance of our (3*S*,10*R*,13*R*,17*R*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-1H-cyclopenta[a]phenanthren-3-ol level. Thus, a common naming system is critical for natural product chemists. Nonetheless, many scientists outside the field of natural products can be baffled by the odd amalgamation of terms that comprise the common names assigned, and perhaps worse, may still find difficulty in pronouncing them. A brief explanation is presented here to assist the nonspecialist.

A newly described natural product will normally be assigned a common name based either on its species of origin or, alternately, on a geographic characteristic associated with its acquisition. **Laurin**terol (Fig. 1.5a), for example, is a common name assigned to a compound first isolated from a red alga of the genus ***Lauren****cia* (Irie et al. 1966), while **kahala**lide (Fig. 1.7e) describes a series of compounds isolated from a green alga (and its sacoglossan predator) from the **Kahala** district of O'ahu, Hawaii (Hamann and Scheuer 1993). Purists will argue the capriciousness of the latter, and certainly a natural product isolated from a Caribbean brown alga may one day turn up in another collected from Okinawan waters. However, as biological taxa undergo constant revision, genus and species names may become obsolete or their members reassigned, so that neither of these guidelines bestows durability or accuracy. Biologically based names can also suffer the discovery of a symbiotic or dietary source, such as the case for aplysiatoxin, which was first described from Hawaiian sea hares (Aplysiidae) (Watson and Rayner 1973; Kato and Scheuer 1974) but later proved to be of cyanobacterial origin. The ultimate aim in assigning a common name is to achieve uniqueness with some semblance of historical origin, be it biological or geographical.

The origin of the natural product occupies the part of the compound name that IUPAC refers to as the "parent" structure. Appended to the name of the parent is a suffix that indicates the primary chemical function (functional group) of the molecule. For instance, in the earlier-mentioned example, laurinterol has an alcohol functional group, which is denoted by IUPAC systematic nomenclature with the "-ol" ending. The "-ide" ending of kahalalide similarly identifies a lactone functionality. Caulerpenyne (Fig. 1.7a), isolated from the green algae ***Caulerp****a flexilix* (Amico et al. 1978), contains a carbon-carbon double bond, which is referred to as an alk**en**e, as well as a carbon-carbon triple bond, known as an alk**yne**. Alternately, the suffix may convey biological activity information, or may categorize the structural class (Sect. 1.2) to which the natural product belongs. Aplysiatoxin (Fig. 1.8g), for example, is broadly cytotoxic and has been implicated in "swimmers itch" in shallow water environs of the South Pacific (Fujiki et al. 1985). Table 1.1 illustrates several other ways the suffix of a compound may provide further information.

There are exceptions to these guidelines. Ilimaquinone, for example, is a compound isolated from a Hawaiian sponge (Luibrand et al. 1979). The suffix, as guidelines suggest, imparts information about the chemical function present in the natural product, the quinone function. Ilima, rather than deriving from the species binomial or a geographical region, is the Hawaiian word for yellow, the characteristic color of the natural product. Putricine and menthol have obvious odoriferous characteristics associated with their constitution. Honorific names are rare among natural products (Herb et al. 1990; Cooray et al.1988).

Nomenclature based on a parent term with an appended suffix accounts for most natural product names. However, as related compounds are identified, or even when a suffix has to denote multiple functional groups, a variety of modifying terms can be employed. For example, the common prefix "nor-" denotes the removal of a skeletal atom from the parent structure; the loss of two or more skeletal atoms is indicated by combining an appropriate numerical prefix with "nor-", e.g., "dinor-", "trinor-" (Giles 1999). Table 1.2 lists additional examples of commonly encountered modifying terms.

Table 1.1 Examples of natural product nomenclature suffix usage

Functional group	Structural class	Bioactivity	Biological origin
-al (aldehyde)	-sterol (steroid)	-toxin (toxin)	-mycin (Actinomycete)
-ol (alcohol)	-oside (sugar)	-statin (inhibitor)	-gorgin (gorgonian)
-one (ketone)	-ceramide (ceramide)	-lysin (lytic)	-spongin (sponge)

Table 1.2 Nomenclature modifying terms

Atom replacement	Bond rearrangements	Skeletal modifications
Aza (C → N)	Abeo (general rearrangement)	Apo (side chain loss)
Oxa (C → O)	Cyclo (cyclic rearrangement)	De (functional group loss)
	Iso (isomeric form)	Homo (add skeletal atom)
	Seco (bond removal)	Nor (skeletal atom loss)

The pronunciation of natural product names stresses the individual contributions. Thus, biological or geographical names incorporated into parent names of natural products are generally pronounced the way they are as biological or geographical names. Caulerpenyne, with its alkene and alkyne functionality described earlier, is pronounced to emphasize each of those three units: Caulerp en yne (kôl erp ĕn īn). Isocyanopupukeanane (Burreson et al. 1975) looks like a mouthful, but when dissected into its functional group, the isocyano (ī sō sī an ōh) group, its geographical origin, Pupukea, Hawaii (pu pu kay a), and finally its structural class, an alk**ane** (-ān), it is much more manageable: īsō sīanōh pu pu kay ən ān. Structural classes can often combine some of the same terms, so that a diterpene (dī tûr pēn) that has been metabolized to remove two carbon atoms from its skeleton becomes a "dinor" (dī nôr) diterpene or a dinorditerpene (dī nôr dī tûr pēn). Select common names and terms used in this chapter will include phonetic pronunciation.

1.2.3 Bioactivity of Natural Products

Natural products are inherently bioactive. Bioactivity is a physiological response to a molecule or ion binding to a ligand, with downstream cascading consequences. Natural products are themselves products of enzymatic processes, demonstrating their ability to interact with receptors. Whether they bind other, nonsynthetic receptors likely depends on whether they have evolved in response to environmental pressures or whether they accumulate as a result of diverted primary metabolic pathways (Williams et al. 1989; Clardy 1995).

Receptor binding requires exquisite molecular organization, as generalized in Fig. 1.1. Proteins and enzymes present a plethora of functional groups on their surface, providing opportunity for molecular interactions. It is only when a potential binding molecule has its own array of functionality that complements those presented on the surface, or more likely in a pocket of an enzyme, that binding can take place. Binding, as measured by the ratio of bound to unbound natural product, is dependent on the strength of the molecular interactions depicted in Fig. 1.1 in order of decreasing strength: ionic bonds (**A**, structure II), hydrogen bonds (**B**, structure II), π-stacking interactions (**C**, structure II), which involve noncovalent aromatic interaction in which p-orbitals of flat aromatic molecules overlap and align parallel to each other, much like stacked coins, and, finally, van der Waals forces (**D**, structure II), also referred to as London dispersion forces.

The receptors of ecological interactions are still not well understood. In fact, many ecological studies have failed to demonstrate well-defined roles for natural products (Pawlik 1993). Most experimental evidence for natural product receptors derives from biomedical applications. Kahalalide F, for example, is a potent cytotoxic depsipeptide (see Sect. 1.3.2.3) initially found in the sacoglossan mollusc *Elysia rufescens* and later in the green alga it feeds upon (Hamann and Scheuer

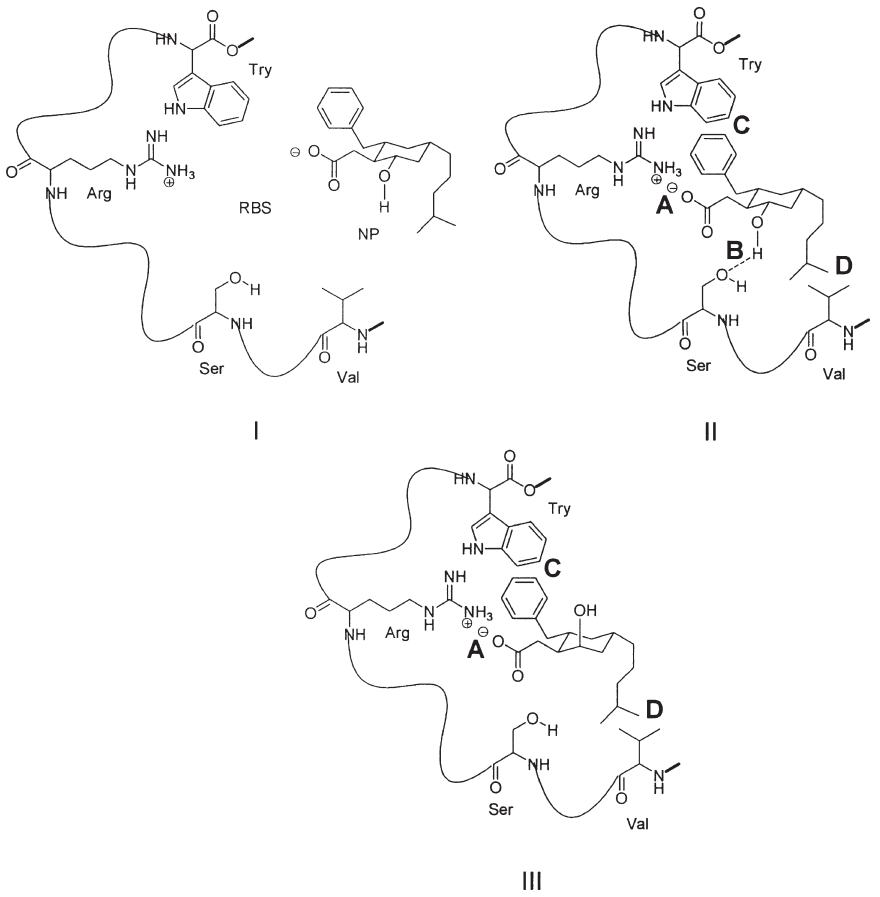

Fig. 1.1 Illustration of a hypothetical natural product (NP) interacting with a receptor binding site (RBS). I. Receptor is indicated by four amino acids (*Val* valine, *Ser* serine, *Arg* arginine, *Try* tryptophan) arrayed along a peptide chain (*wavy lines*); NP approaches RBS from open face. II. NP binds with four amino acids: A, carboxylate of NP forms ionic bond with *Arg* guanidinium group of RBS; B, alcohol group of NP forms hydrogen bond with alcohol group of *Ser*; C, phenyl ring of NP interacts with RBS via π-stacking with *Try* indole ring; D, lipophilic chains of NP and *Val* associate using van der Waals forces. III. The stereochemical configuration of NP functional groups is critical to strong binding; notice that the change in stereochemical configuration of NP alcohol results in the absence of a hydrogen bond in structure III, which would make III less stable than II

1993). Currently in phase II clinical trials as a treatment for melanoma, hepatocellular carcinoma, and non-small-cell lung cancer (Hamann 2004), the compound has a mode of action that is not well understood, though it has been deemed necrosis-like and characterized by cytoplasmic swelling and DNA clumping (Janmaat et al. 2005).

1.3 Compound Classes

1.3.1 General Overview

Secondary metabolites are classified according to the biosynthetic pathway from which they are derived. Biosynthetic and genetic studies have revealed that a limited number of core biosynthetic pathways, generalized in Fig. 1.2, are responsible for the production of most of the natural products (boxed in Fig. 1.2). The Bioinformatics Center of Kyoto University and the Human Genome Center of the University of Tokyo have created a remarkable bioinformatics resource called the Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al. 2004). This user-friendly database provides a graphical representation of biological pathways, replete with diagrams of molecular interactions, reactions, and relations, as well as structures of primary and secondary metabolites. Moore (2005, 2006) has authored two recent reviews on the biosynthesis of marine natural products from both micro- and macroorganisms.

While common biosynthetic pathways may provide the framework for the various classes of secondary metabolites, their functionality is imparted by specialized tailoring enzymes that are often unique to natural products (Walsh 2004; Hertweck et al. 2007). Whether it is the addition of alcohol groups, halogenation (Gribble 1998), oxidation, reduction, stereochemical manipulation, or cyclization, it is often these functionalities that make secondary metabolites unique and bioactive.

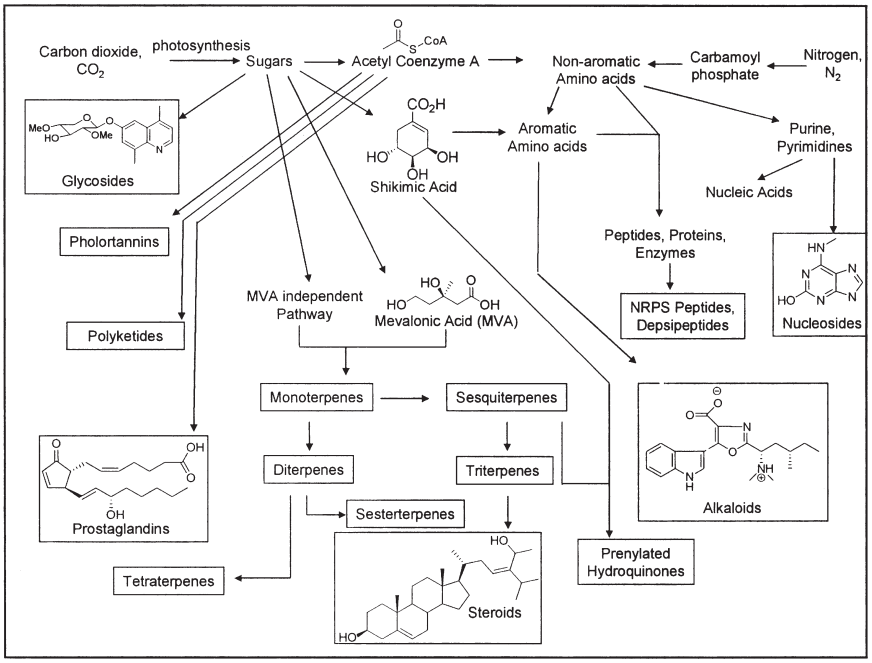

Fig. 1.2 Biosynthetic origin of the major classes of natural products

1.3.2 Terpenes

Terpenes are a large and diverse class of compounds produced by a wide variety of organisms, though plants are an especially prolific source. The terms terpenoid and isoprenoid can be used interchangeably with terpene, though, strictly, terpenes are hydrocarbons (composed only of carbon and hydrogen) while terpenoids and isoprenoids have been further functionalized.

More than half of the reported secondary metabolites from macroalgae are isoprenoids. Terpenes, steroids, carotenoids, prenylated quinines, and hydroquinones make up the isoprenoid class, which is understood to derive from either the classical mevalonate pathway, or the mevalonate-independent pathway (Stratmann et al. 1992). Melavonic acid (MVA) (Fig. 1.2) is the first committed metabolite of the terpene pathway. Dimethylallyl (dī měth əl ăləl) pyrophosphate (DMAPP) (Fig. 1.3) and its isomer isopentenyl pyrophosphate (IPP, Fig. 1.3) are intermediates of the MVA pathway and exist in nearly all life forms (Humphrey and Beale 2006). Geranyl (jə rən əl) (C_{10}) and farnesyl (C_{15}) units are generated by head-to-tail (Fig. 1.3) condensation of two (for C_{10}) or three (for C_{15}) 5-carbon DMA-like isoprene units, identifiable in final products by the characteristic fish-tail repeating units, as traced over the structure of a sesquiterpene in Fig. 1.3 (Humphrey and Beale 2006). Additional IPP condensation with farnesyl pyrophosphate (FPP)

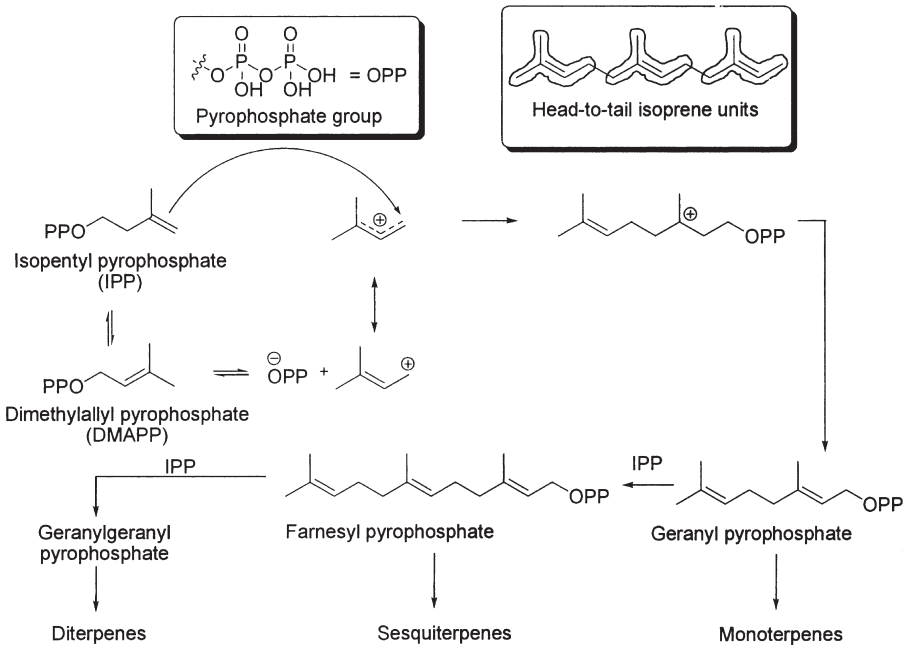

Fig. 1.3 Summary of terpene biosynthetic pathway

yields geranylgeranyl pyrophosphate, which is the precursor of all diterpenes (C_{20}), while the condensation of two FPP, in a head-to-head fashion, leads to squalene, the precursor of all triterpenes (C_{30}) and sterols (Brown 1998).

1.3.3 Polyketides

The second largest class of compounds reported from macroalgae is the polyketides, which comprise approximately a quarter of known algal compounds (Blunt et al. 2007). Polyketides are polymers of acetate (C_2) and occasionally propionate (C_3) and are very similar to fatty acids in their biosynthetic origin. Polyketides can be found in plants, animals, bacteria, and fungi. With a range of activities as broad as their structures, the polyketides are a diverse family of natural products classified based upon the polyketide synthases (PKSs) responsible for their biosynthesis, primarily type I and type II.

All polyketides use the same general mechanism for chain elongation. Acetyl coenzyme A provides acetate (C_2) units, which are condensed by a ketosynthase (KS). This in turn catalyzes condensation of the growing chain onto an acyl carrier protein (ACP), as generalized in Fig. 1.4. Enzymes such as ketoreductase (KR), enoyl reductase (ER), and dehydratase (DH) establish the oxidation state of carbon during translation, imparting structural diversity. Successive translation of each module leads to a chain of the required length that is eventually passed to thioesterase (TE), which releases the chain as a free acid or lactone.

Type I polyketides include linear and macrolide -type structures, including algal toxins such as the brevetoxins (Fig. 1.8a) as well as microbially derived antibiotics such as erythromycin (Staunton and Weissman 2001). Type II polyketide synthases yield condensed aromatic ring systems (Hertweck et al. 2007), such as those found in plant flavonoids, algal phlorotannins (Fig. 1.7), and, perhaps most well-known, microbe-derived antibiotics such as tetracyclin. General enzyme terminology is the same for type I and type II; however, the enzymes are fundamentally different. Type I PKSs are large, multifunctional enzyme complexes where intermediates translate along modules, whereas type II PKSs involve iterative multienzyme complexes of single proteins. Polyketides synthesized by type II PKS will typically have chain lengths of 16 (octaketides), 20 (decaketides), or 24 (dodecaketides) (Hertweck et al. 2007). Other PKSs have been described, and it is becoming apparent that the diversity of these systems is greater than previously recognized (Shen 2003).

Structurally related to polyketides are another class of linear, acetate-derived compounds, the acetogenins ("genesis in acetate"). These compounds are often indistinguishable from polyketides based purely on inspection of their chemical structure. However, with their origin in the fatty acid pathway, they are not biosynthesized by polyketide synthases but rather by fatty acid synthases (FASs). Algal acetogenins are often odd numbered (C_{11} and C_{15} are the major algal acetogenins) due to decarboxylative degradation of the parent fatty acid (Sect. 1.4.1).

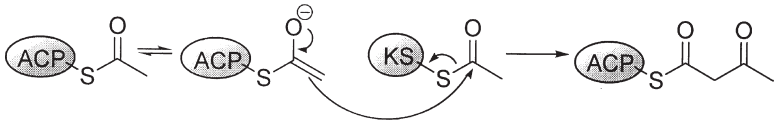

Fig. 1.4 Chain extension during polyketide biosynthesis

1.3.4 Amino-Acid-Derived Natural Products

Natural products derived from amino acids form a broad and divergent group, including simple amino acid derivatives, alkaloids, and small, often cyclic, polypeptides. Simple amino acid derivatives, which are not uncommon in algae, are often oxidation or rearrangement products of one of the 20 common amino acids. Alkaloids and polypeptides are more complex in their structural modifications.

Alkaloids

The term alkaloid has a long history and has been applied to compounds of many different pathways and structures. Originally, the term was used to describe any compound with a basic nitrogen (amine), often bitter tasting, and usually aromatic in structure. Alkaloids are more often recognized now as highly condensed amino acids, which may or may not have a *basic* nitrogen, bitter taste, or aromatic structure, but can also include lipophilic nitrogen containing compounds whose origin from amino acids is less clear. There is no unifying biosynthetic pathway for alkaloids, but rather many different classes of alkaloids have related pathways. The KEGG database describes two fundamentally different schemes of interrelationships, but even that only addresses a fraction of known alkaloids.

Nonribosomal Peptides

Peptide bonds are formed from the condensation of the carboxylic acid carbon of an amino acid with the α-nitrogen of a second amino acid. Large molecular weight polymers of amino acids constitute the proteins and enzymes of primary metabolism. A number of smaller polymers ("oligomers") are found in nature in a species-specific manner. Too small for structural roles or enzymatic activity, these peptides have demonstrated a number of bioactivities, including biomedical as well as ecological (Tan 2007). Recent research has shown that these small peptides are not produced by the translational biochemistry of the ribosome, where structural peptides and enzymes originate, but rather are biosynthesized by a group of enzymes referred to as nonribosomal peptide synthases (NRPS). NRPS products and polyketides share a similar model of biosynthesis. Both are created on modular enzymatic assembly lines, with their structural diversity governed by optional

enzymes within the enzyme complexes (Walsh 2004). In fact, it is not uncommon to find secondary metabolites of mixed PKS-NRPS biosynthetic origin (see curacin in Sect. 1.4.4). Products from NRPSs are secondary metabolites and often include ester linkages among the peptide bonds, producing natural products known as depsipeptides, such as the kahalalides, discussed previously. Most of the NRPS products found in algae are isolated from cyanobacteria and microalgae (Sect. 1.4.4).

1.3.5 Shikimates

Shikimates, which include phenylalanine, tyrosine, tryptophan, and their derivatives, are represented by many aromatic natural products, including hydroquinones found in brown algae such as *Sargassum* (Segawa and Shirahama 1987). Flavonoids are a structural class of shikimates found in plants, including isoflavonoids or neoflavonoids, as is the γ-pyrone (coumarin) core structure (Knaggs 2003).

1.3.6 Miscellaneous Classes of Algal Natural Products

Other natural product classes are found less frequently in algae. Nucleosides are constructed from nucleobases often linked to sugars derived from nucleic acids (Rosemeyer 2004). Terpenes, polyketides, and amino acid derivatives can be sugar-bound, or glycosylated, (Pfander and Stoll 1991). Glycosylated compounds are known as glycosides, the sugar is referred to as the glycone, and the remaining portion of the molecule is the aglycone. Arsenic-containing sugars are produced by brown algae (Usov et al. 2001). Prenylated quinones and hydroquinones are examples of mixed biogenesis, as their aromatic rings are derived from the shikimic acid pathway while isoprene units constitute their side chain. The term prenyl denotes an isoprene group.

1.4 Algal Chemistry

Macroalgae have accounted for almost 3,000 natural products representing ~20% of the chemistry reported from the marine realm. During the 1960s, the period when dedicated marine natural product laboratories were being established worldwide, more than 50% of newly reported natural products came from macroalgae, though that number has steadily decreased and now hovers around 10% annually (Munro and Blunt 2005; Blunt et al. 2007). Research into the chemistry of marine microalgae and cyanobacteria is thriving, representing almost 50% of the literature accumulated since 2000 (Blunt et al. 2007). Despite the recent shift away from macroalgal chemistry, interest in several macroalgal compounds is high for

pharmaceutical application (e.g., sulphated polysaccharides, kahalalides) (Smit 2004) and as antifouling agents (e.g. fimbrolides) (Bhadury and Wright 2004).

1.4.1 Natural Products Chemistry of Rhodophyta

With more than 1,500 compounds reported, the secondary metabolite chemistry of Rhodophyta is richer than those of other macroalgae, both in terms of abundance and diversity. With the exception of phlorotannins, all major classes of natural products are represented among Rhodophyta (Munro and Blunt 2005). Red algae elaborate predominantly isoprenoid and acetogenin derivatives, along with some amino acid, shikimate and nucleic acid derivatives. What truly distinguishes red algae is that they are impressive producers of halogenated compounds, with over 90% of those reported containing bromine or chlorine, compared with only 7% of green algal compounds and less than 1% of those from brown algae (Harper et al. 2001). These halogenated terpenoids have shown only a paucity of nonecological bioactivity, which may explain the decline in interest in red algal chemistry (Blunt et al. 2007).

More than half of the reports (57%) on Rhodophyta chemistry come from Family Rhodomelaceae and the vast majority (85%) of that represents chemistry of the genus *Laurencia*, which produces a wealth of halogenated sesquiterpenes and C_{15} acetogenins, along with a few higher terpenes (C_{20} and greater), as seen in Fig. 1.5. *Laurencia* sesquiterpenes, like those from several other rhodomelacean genera, are typically cyclized, often polycyclic, as seen in laurinterol (lō rĕn tûr ôl) (Fig. 1.5a) (Irie et al. 1966) and pacifenol (Fig. 1.5b) (Sims et al. 1971). Chemically unusual spiro-ring fusions, rings connected through just one atom, observed in elatol (Fig. 1.5c) (Sims et al. 1974), are not uncommon among *Laurencia* sesquiterpenes. Higher terpenes in Rhodophyta are largely limited to *Laurencia*, with some exceptions (see Kubanek et al. 2005). Diterpenes are primarily monobrominated polycycles, such as the irieol (Fig. 1.5d) (Fenical et al. 1975), while triterpenes are most often polyether in nature, such callicladol (Fig. 1.5e) (Suzuki et al. 1995). In fact, among all the families of Rhodophyta, polyhalogenated higher terpenes are rare, represented by a couple of dozen dibromominated polycycles and a few monobromo, monochloro *Laurencia* polycycles. Sesterterpenes are not represented in Rhodophyta.

Laurencia is nearly alone in elaborating a rather unique series of C_{15} acetogenins, such as laurepinnacin (Fig. 1.5f) (Fukuzawa and Masamune 1981), and laurallene (Fig. 1.5g) (Fukuzawa et al. 1979). The C_{15} carbon backbone (Fig. 1.5h) (Kigoshi et al. 1986) is hypothesized to originate from a C_{16} carboxylic acid precursor via apparent decarboxylation to the enyn e functionality commonly found terminating this family of largely halogenated compounds. Besides the enyne, or its isomeric form, the allene, the C_{15} acetogenins are generally adorned with bromine and chlorine (or both) with oxygenation on the adjacent carbon. This pattern of halogen with vicinal (adjacent) oxygenation reflects their assembly on the carbon skeleton via carbocation chemistry (Staunton and Weissman 2001).

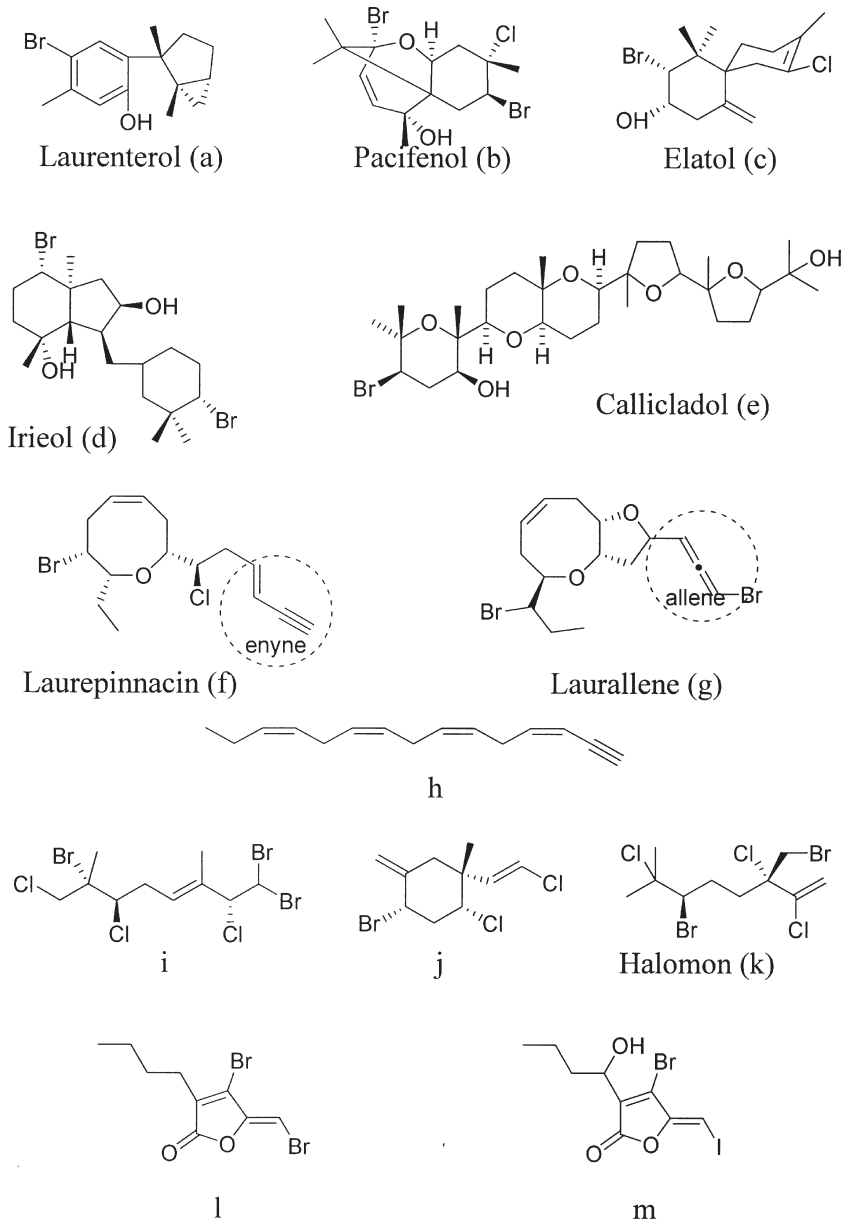

Fig. 1.5 Representative Rhodophyta chemistry

Three other Rhodophyta families, Rhizophyllidaceae, Plocamiaceae, and Delesseriaceae, distinguish themselves from the Rhodomelaceae by producing largely halogenated monoterpenes. The Rhizophyllidaceae genera *Chondrococcus*, *Desmia*, and *Ochtodes*, the plocamiacen *Plocamium*, and the delesseriacean *Pantoneura*, are,

with few exceptions, the only sources of monoterpenes among the red algae. These red algal monoterpenes are often highly halogenated linear (Fig. 1.5i) (Bates et al. 1979) or monocyclic (Fig. 1.5j) (Crews et al. 1978). One notable exception is the rhizophyllidacean *Portiera*, which elaborates one of the most intensely studied red algal anticancer metabolites, halomon (Fig. 1.5k) (Fuller et al. 1992).

A few other genera in Rhodomelaceae (for example, *Odonthalia*, *Polysiphonia*, *Rytiphloea*, *Vadalia*, *Symphyocladia*) elaborate brominated phenol s. The genus *Acanthophora* stands out as a producer of nonhalogenated steroids. Several genera in Bonnemaisoniaceae (*Delesea*, *Asparagopsis*, *Bonnemaisonia*, *Ptilonia*) are notable as producers of small, linear halongenated ketones and branched lactones. The fimbrolides, for example (Fig. 1.5l, m), are a series of halogenated furanones from *Delisea pulchra* (Kazlauskas et al. 1977) that have been shown to interfere with signaling in bacteria and provide an antifouling defense (Kjelleberg and Steinberg 2001) by functioning as an intracellular signal antagonist (Rasmussen et al. 2000).

1.4.2 Natural Products Chemistry of Phaeophyta

More than 1,140 secondary metabolites have been reported from Phaeophyceae. The characteristic compounds of these brown algae include diterpenes, phlorotannins, and small C_{11} acetogenins, all with very little halogenation (Blunt et al. 2007). Phlorotannins are the true niche compound from brown algae, often accounting for an astonishing 10–20% of dry weight (Ragan and Glombitzka 1986; Amsler and Fairhead 2006).

Almost a third of the reported brown algal chemistry comes from a single genus, *Dictyota*, which has elaborated a wealth of terpenes (>250) (Munro and Blunt 2005). Diterpenes dominate *Dictyota* chemistry and are typically di- and tricyclized, as seen in dictyol E (Fig. 1.6a) (Danise et al. 1977), amijiol (Fig. 1.6b) (Ochi et al. 1980), Fig. 1.6c (Tringali et al. 1984), and dictyoxetane (Fig. 1.6d) (Pullaiah et al. 1985).

Another dictyotalean genus, *Dictyopteris*, has been reported to produce an array of C_{11} cyclic or acyclic acetogenins derived from higher fatty acids (Stratmann et al. 1992). Examples include the hydrocarbons dictyopterene A (Fig. 1.6e) (Moore et al. 1968) and dictyopterene D'[B1] (Fig. 1.6f) (Moore and Pettus 1971), which act as pheromones in sexual reproduction (Stratmann et al. 1992). The compounds are short lived and undergo facile degradative oxidation to yield compounds such as dictyoprolene (Fig. 1.6g) (Yamada et al. 1979) and dihydrotropone (Fig. 1.6h) (Moore and Yost 1973). In a true exhibition of efficiency, these degradative products have also been shown to act as a chemical defense (Hay et al. 1998).

Phlorotannins, or polyphenols, are structural classes of polyketides found exclusively in brown algae and classified into six groups based upon variations in their assemblage from the polymerization of phloroglucinol (Fig. 1.6i) (1,3,5-trihydroxybenzene) units (Ragan and Glombitzka 1986; Targett and Arnold 2001). Fucols (Fig. 1.6j) (Geiselman and McConnell 1981), phlorethols, fucophlorethols, fuhalols, isofuhalols (Fig. 1.6k) (Grosse-Damhues and Glombitzka 1984), and eckols differ in

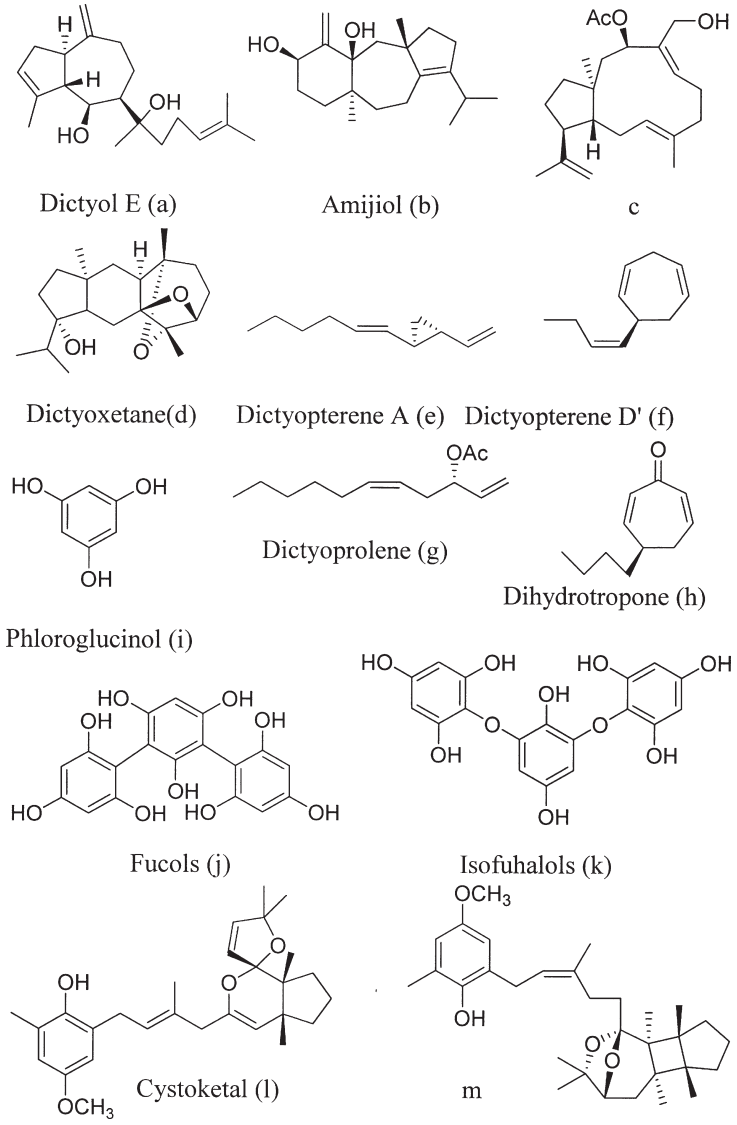

Fig. 1.6 Representative Phaeophyta chemistry

the number of hydroxyl groups present and in their bond linkages. All but the fucols are attached by ether bonds. The phloroglucinol units are often esterified or acylated, and can dimerize or polymerize into larger units. These compounds also exhibit a broad range in size, thought to polymerize as they age and thereby grow larger. They are typically 10–100 kDa, although their range spans from as small as 126 Da to as large as 650 kDa (Targett and Arnold 2001; Boettcher and Targett 1993). Stored within cells in vessels called physodes, phlorotannins have been reported from

almost all brown algal orders. There is specificity within genera as seen with the fucols in *Fucus* and eckols in *Eckonia*. Phlorotannins are easily quantifiable by common colorimetric techniques (Ragan and Glombitzka 1986; Targett and Arnold 1998; Amsler and Fairhead 2006) and have been proposed to play ecological roles in wound healing, herbivore deterrence, microbial infection, metal ion chelation, and UV protection, as well as having antialgal and antifungal activities (Sieburth and Conover 1965; Ragan and Glombitzka 1986; Lau and Quian 1997; Pavia et al. 1997; Targett and Arnold 1998; Amsler and Fairhead 2006).

The brown algal order Fucales accounts for just over a third of known chemistry, with the genus *Cystoseira* accounting for more than 100 reported structures (Munro and Blunt 2005). The compounds are primarily prenylated quinones and hydroquinones, ranging from simple and linear to often complex and polycyclic forms as seen in cystoketal (sĭs tō kē tăl) (Fig. 1.6l) (Amico et al. 1984) from *C. balearica* and from *C. stricta* (Fig. 1.6m)[B2] (Amico et al. 1987).

1.4.3 Natural Products Chemistry of Chlorophyta

Of all macroalgae, green algae are the least prolific producers of natural products, with less than 300 known compounds and only a handful of new secondary metabolites reported each year (Blunt et al. 2007). Marine green algae are known to produce compounds similar to those from red algae, primarily functionalized di- and sesquiterpenoids, but lacking the extensive halogenation of the red algal compounds. The characteristic chemistry of Chlorophyta is the presence of the "1,4-diaceoxybutadiene" dienolate ester found in many green algal terpenes (see Fig. 1.7a).

Less than half of the reported natural products of green algae come from the order Bryopsidales and the vast majority of its metabolites (>85%) are terpenoids (Munro and Blunt 2005). Families Udoteaceae, Caulerpaceae, and Halimedaceae produce more than 85% of known Bryopsidales compounds, many of which contain the earlier-mentioned 1,4-diaceoxybutadiene unit, examples of which include caulerpenyne from *Caulerpa flexilix* (Amico et al. 1978) and udoteal (Fig. 1.7b) found in *Udotea* sp. (Paul et al. 1982a). These enolate esters are often referred to as "masked aldehydes" because they can be enzymatically hydrolyzed to aldehydes (Paul and Van Alstyne 1992); they are occasionally found in their aldehyde state, as is the case for halimedatrial (Fig. 1.7c) from *Halimeda* sp. (Paul and Fenical 1983). Although not as prevalent, other terpenoid motifs have been reported, such as the norcycloartene triterpenoid (Fig. 1.7d) from *Tydemania expeditionis* (Paul et al. 1982b).

The kahalalides, such as kahalalide A (Fig. 1.7e), are unusual green alga compounds, since no other polypeptides are known from the phylum (Hamann et al. 1996).

Cymopolia barbata elaborates a unique series of (20 prenylated bromohydroquinones called the cymopols (Hoegberg et al. 1976). Monobromination is found exclusively para (directly across) to the site of prenylation as seen in cymopolone (Fig. 1.7f). The isoprene side chains all originate from C_{10} geranyl units with different

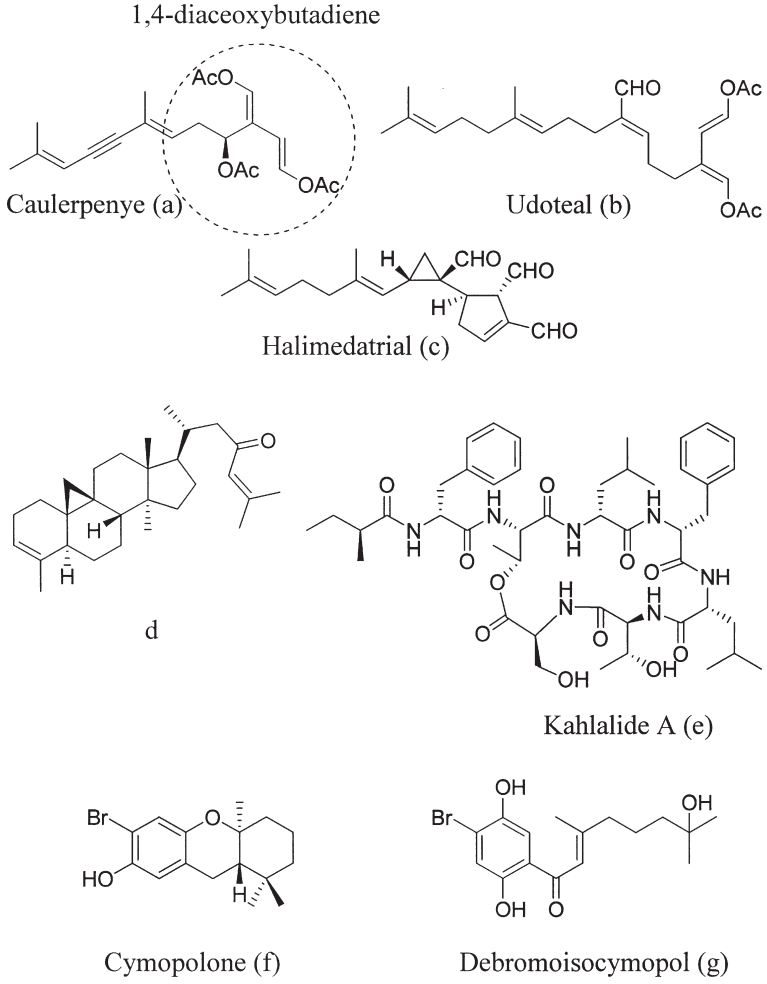

Fig. 1.7 Representative Chlorophyta chemistry

oxidation states, some of which cyclize back onto the aromatic hydroquinone as seen in debromoisocymopol (Fig. 1.7g).

1.4.4 Natural Products Chemistry of Cyanobacteria and Microalgae

With advances in methods of isolation and cultivation of microalgae and cyanobacteria, and the striking bioactivity of other microbial metabolites, research into the chemistry of culturable algae has escalated since the 1990s. Metabolites from these

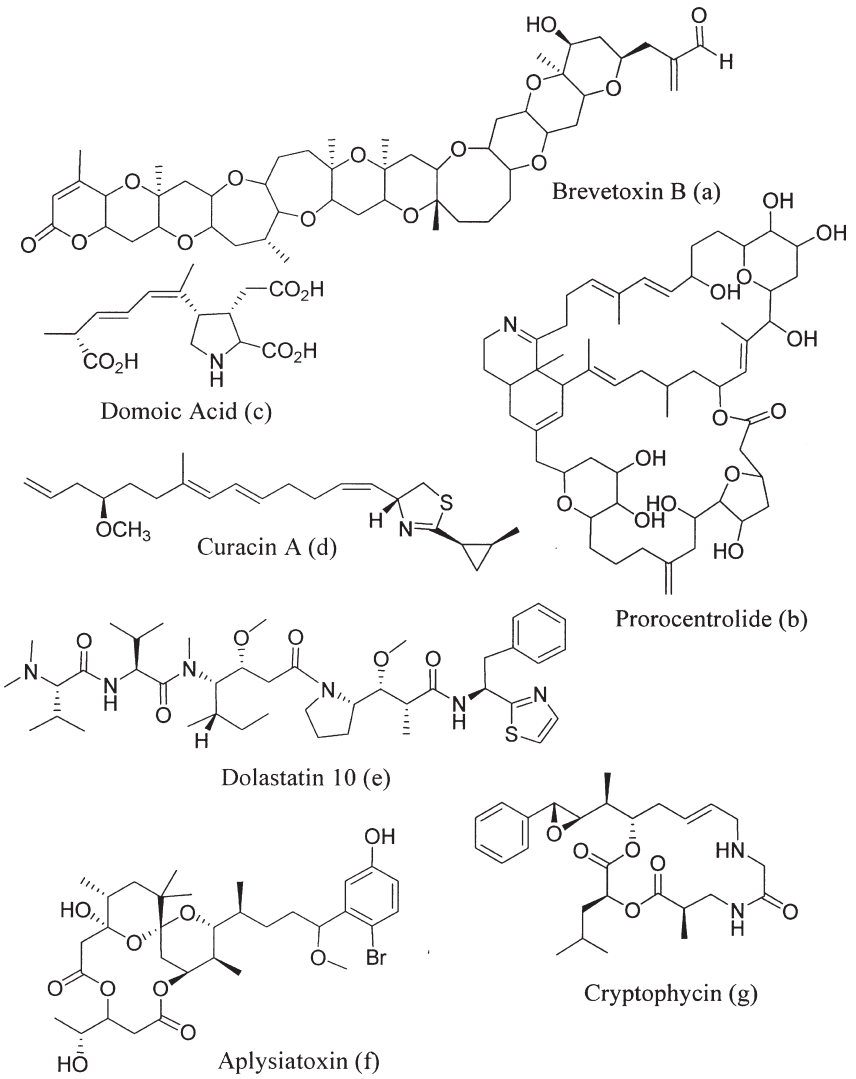

Fig. 1.8 Representative microalgal and cyanobacterial chemistry

organisms include NRPS products, alkaloids and polyketides, while terpenes are uncommon (Singh et al. 2005; Blunt et al. 2007).

Microalgae produce many potent natural products in the form of complex polycyclic polyethers, a type of polyketide. The ladder-like polyether brevetoxin B (Fig. 1.8a) (Lin et al. 1981) is representative of a host of such toxins, which include ciguatoxin (Scheuer et al. 1967), yessotoxin (Murata et al. 1987), maitotoxin (Murata et al. 1993), gambieric acids (Murata et al. 1992), and azaspiracid (Satake et al. 1998). Brevetoxin B, one of the causitive agents of red tide poisoning, can be isolated from

the cultured dinoflagellate *Karenia brevis* (formerly known as *Gymnodinium breve*, *Ptychodiscus brevis*) or from shellfish which accumulate red tide toxins from their dinoflagellate diet. The ladder-like carbon skeleton is lipophilic, contributing to its accumulation in the food chain. The neurological disease that results from human consumption of brevetoxins is know as neurotoxic shellfish posioning (NSP) and results from the toxins binding to voltage-gated sodium channels in nerve cells (Poli et al. 1989). Prorocentrolide (Fig. 1.8b) is a polyketide polyether with a basic imino-nitrogen (Torigoe et Al. 1988) and is representative of the nonladder dinoflagellate polyketide toxins, other members of which include amphidinolides (Kobayashi et al. 1986), zooxanthellatoxins (Nakamura et al. 1995), and luteophanols (Doi et al. 1997). Prorocentrolide is a macrolide from a marine dinoflagellate, *Prorocentrum lima*, composed of C_{49} polyketide chain that incorporates a C_{27} macrolide, a C_{26} carbocycle (a ring composed entirely of carbon), and a hexahydroisoquinoline ring system in its unique structure. Domoic acid (Fig. 1.8c) is characteristic of a number of alkaloid toxins from dinoflagellates, other members of which include saxitoxin and kainic acid. Originally isolated from a red alga (Daigo 1959) but later discovered to be produced by diatoms of the genus *Pseudonitzschi* (Shimizu et al. 1989), domoic acid is responsible for amnesic shellfish poisoning (ASP).

More than 550 cyanobacterial secondary metabolites have been reported, predominantly NRPS products but including substantial numbers of polyketide derivatives, which are often complex and bioactive (Burja et al. 2001; Tan 2007). Most of the reported compounds from the cyanobacteria (>40%) have come from the genus *Lyngbya*, which has been the source of over 200 compounds. Curacin A (Fig. 1.8d), a type I polyketide with a thiazoline (NRPS) moiety, is a potent antimitotic agent from *L. majuscula* (Gerwick et al. 1994). This filamentous cyanobacterium is known to produce an array of nitrogenous toxic metabolites, such as the linear peptide dolastatins (Fig. 1.8e) (Pettit et al. 1987), and cyclic polyketides, such as aplysiatoxin (ə plē sē əŏk sĭn) (Fig. 1.8f) (Kato and Scheuer 1974). Cyclic depsipeptides are another common NRPS product, illustrated here by one of the potent antitumor cryptophycins (Fig. 1.8g) from the Cyanophyta genus *Nostoc* (Trimurtulu et al. 1994).

1.5 Summary

Algal chemistry is both rich and diverse, spanning most natural product classes and including functional group characteristics found from no other source. Most important, algal chemistry is phylogenetically representative. The ecology of many algal natural products has been studied. In fact, algal chemistry was one of the early focuses of marine chemical ecology, yet little is known about the molecular interactions of algal bioactivity. Biomedical interests continue to drive the study of marine algae, though the focus has shifted from early studies of macroalgae toward microalgae, not only because of the biomedical potential of microalgae but also because of the reliability of supply inherent in a cultured source. Algae will continue to be an

important subject of chemical and chemical ecological study, in part because of interests on marine phototrophs in comparison with their terrestrial counterparts, and also because they are abundant and chemically rich components of marine ecosystems, both of which have implications in the trophodynamics of primary production.

Acknowledgments This work was supported in part by a grant from the National Science Foundation (OPP-0442857).

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2
Macroalgal Chemical Defenses and Their Roles in Structuring Tropical Marine Communities

R.C. Pereira(✉) and B.A.P. da Gama

2.1 Introduction

The impacts of herbivores on macroalgae have been relatively well studied in the last decades (e.g., Carpenter 1986; Choat 1991; Hixon and Brostoff 1996; Harborne et al. 2006; Vinueza et al. 2006), probably because of their profound effects in both temperate and tropical communities and their importance as major conduits of energy between autotrophs and the rest of the food web. Nonetheless, very little is understood about how macroalgal chemical defenses – against herbivores or against competitors, epibionts, pathogens, etc. – function and what their exact role is in structuring marine communities. Tropical marine communities are well known to possess a plethora of macroalgal species (see Lüning 1990; Kerswell 2006), as well as an equally high diversity of herbivores (Floeter et al. 2005), which exert an intense, constant, and unparalleled pressure on the former, presumably selecting, over evolutionary timescales, for the presence of diverse and effective chemical defenses. In this scenario of bottom-up control, a number of counteradaptations are supposed to have occurred in herbivores, including feeding specialization and sequestration of defenses, which represent potential steps toward a top-down control of macroalgal communities. However, experimental evidence to support these assumptions remains largely elusive. This chapter attempts to review, although not exhaustively, our current knowledge of different aspects of chemical defenses from tropical macroalgae and their potential effects on tropical marine benthic community structure. The focus will be on known examples that support or rebut theoretical assumptions about tropical communities in an effort to point the reader toward new trends or research priorities that emerge from our current knowledge about marine tropical systems.

This chapter primarily considers aspects of chemical defenses in tropical macroalgae belonging to the divisions Chlorophyta, Phaeophyta, and Rhodophyta, but

R.C. Pereira
Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, C.P. 100.644 – CEP 24001-970, Niterói, RJ, Brazil. email: renato.pereira@pesquisador.cnpq.br

B.A. Perez da Gama
Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, C.P. 100.644 – CEP 24001-970, Niterói, RJ, Brazil

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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also includes macroscopic and conspicuous tropical Cyanobacteria that can be important in structuring tropical marine benthic communities.

2.2 The Tropical Marine Environment

When thinking about tropical marine ecosystems, generally what comes to mind are the diverse and colorful coral reefs, but other ecologically important environments, such as mangrove forests, seagrass beds, and rocky shores, are also abundant at low latitudes. To define the tropical environment in which macroalgae flourish, it is necessary to consider that (1) macroalgae are essentially restricted to the upper euphotic zone, where light is strong enough to allow net positive photosynthesis; (2) the present boundaries of seaweed biogeographical regions are physiologically set by sea surface isotherms (i.e., lines of the same mean water temperature averaged over many years for a particular month); (3) an essential condition for the existence of most macroalgae species is the existence of hard substrata along any coast; and finally (4) the present distribution of benthic marine macroalgae is the result of their migration and of the displacement of coastlines along geological times (Lüning 1990). We could add to this the relatively recent (probably occurring over the last five centuries) human-mediated amplification of seaweed natural distributions or bioinvasions. These include both deliberate (e.g., aquaculture purposes) and accidental (e.g., hitchhikers on ship's hulls or floating debris) introductions (Sax et al. 2005). Further changes in seaweed distributions can occur in the future as a consequence of oceanic current changes due to global warming (Schmittner and Stoecker 1999) or because of phase shifts caused by increasing cultural eutrophication (see McCook 1999). Thus marine communities that have evolved over billions of years can change suddenly, literally from the bottom up, through changes in the benthic flora.

The tropical regions are considered to be limited to the north and south by the 20°C-winter isotherm (February isotherm in the Northern Hemisphere, August isotherm in the Southern Hemisphere) (Lüning 1990). Sea surface temperatures may warm up to 30°C in the central areas and to 25°C in the marginal areas of the tropical range. The distribution belt of reef-building (hermatypic) corals coincides with the boundary of the following four tropical regions distinguished by marine biogeographers (e.g., Briggs 1974): (1) eastern Atlantic tropical region, (2) western Atlantic tropical region, (3) Indo-West Pacific tropical region, and (4) eastern Pacific tropical region. Besides coral reefs, seagrasses and mangroves characterize many tropical coasts, and the peaks of diversity of the three groups occur in the Indo-West Pacific region, with secondary centers in the Caribbean (McCoy and Heck 1976; Lüning 1990).

The tropical regions represent the oldest marine habitats, where red and green algae dominate, while brown algae are more abundant toward cold temperate regions. The diversity of marine fauna reaches a maximum in tropical seas, and many hypotheses have been proposed to explain this (Krebs 2001; Willig et al. 2003;

Floeter et al. 2005). The diversity of marine flora does not peak, however, in tropical regions (Kerswell 2006). One possible reason for this could be the pantropical distribution of several tropical species, in contrast to the more endemic, restricted distribution of temperate or cold seaweed species (Lüning 1990). The idea that the high diversity and cover of hermatypic corals would leave little settling space for seaweeds does not seem to be, in our opinion, a plausible explanation for the lower diversity of macroalgae in the tropics, since other ecosystems, such as seagrass beds, rocky shores, and mangroves, not dominated by corals would be available for algal settlement. A widely accepted concept is that the high diversity of herbivorous animals and their constant and intense herbivory pressure (as opposed to the seasonally variable activities of cooler-water, higher-latitude herbivores) would exert a strong selective pressure for chemically defended species (e.g., Hay 1996). However, the assumption that herbivory is – or was – the main evolutionary force driving the production of macroalgal secondary metabolites still needs to be more clearly demonstrated. A number of alternative explanations (Gottlieb 1989, 1990; Williams et al. 1989; Kerswell 2006), as well as other possible driving forces, remain to be tested.

2.3 Tropical Macroalgal Natural Products

Contrary to species from other marine regions, tropical macroalgae have been shown to produce a greater diversity of structurally unique and biologically active compounds, mainly terpenoids (e.g., Smit 2004; Blunt et al. 2007 and previous reviews), than do their temperate counterparts. Many of these macroalgal natural products were initially presumed to act as chemical defenses against herbivores because of positive correlations between the results of feeding preference experiments and the presence of secondary metabolites reported in the chemical literature (e.g., Ogden 1976; Hay 1981; Lewis 1985). Over the last two decades numerous studies have evaluated this hypothesis as well as other ecological roles of these natural products in assays in the laboratory and/or the field (in situ experiments). These studies have been periodically reviewed during the development of the marine chemical ecology field (e.g., Hay and Fenical 1988; Hay and Steinberg 1992; Paul 1992; Paul et al. 2001, 2006; Paul and Puglisi 2004; Amsler and Fairhead 2006; Ianora et al. 2006). The production of deterrent metabolites can be vital to macroalgal survival in herbivore-rich coral reef environments.

The majority of natural products from tropical macroalgae are terpenoids (mainly sesqui- and diterpenoids), followed by acetogenins (acetate-derived compounds), and metabolites of mixed biosynthetic origin (such as meroditerpenes), frequently composed of terpenoid and aromatic portions (Blunt et al. 2007 and previous reviews; see Chap. 1).

Chlorophyta or green algae comprise one of the major groups of algae and include several exclusively marine orders and genera from tropical regions. Natural products chemistry research has identified more than 300 secondary metabolites from Chlorophyta, with most being sesquiterpenoid and diterpenoid compounds

produced mainly by species belonging to tropical Caulerpales. A few halogenated compounds occur in three genera (*Avrainvillea*, *Cymopolia*, and *Neomeris*) and others such as brominated sesquiterpenes, prenylquinones, and aromatic compounds are also found in green macroalgae (Harper et al. 2001; Blunt et al. 2007 and previous reviews; see Chap. 1).

The division Phaeophyta or brown algae occur almost exclusively in marine environments ranging from polar to tropical regions; in tropical areas, a few brown algal species can produce substantial biomass. The most common brown algal secondary metabolites found in tropical Phaeophyta (mainly from species belonging to the order Dictyotales) are terpenoids, acetogenins, and terpenoid-aromatic compounds of mixed biosynthetic origin (Blunt et al. 2007 and previous reviews). Polyphenols (phlorotannins) are found in tropical brown algae, but within the algae, only in this group. Unlike other algal secondary metabolites, which are most common in tropical macroalgal species, polyphenols occur in higher concentrations in species from temperate (Toth and Pavia 2006) and polar habitats (Fairhead et al. 2006) and because of this reason polyphenols will not be considered in this chapter (see Chaps. 3 and 4 for discussions of the ecological roles of polyphenols).

Rhodophyta or red algae are largely marine and occur predominantly along the coastal and continental shelf areas of tropical, temperate, and cold-water regions. A diversity of compounds characterize the red algae, including many that are halogenated, ranging from simple halogenated methanes, cetones, and phenolics to more complex terpenoids (Blunt et al. 2007 and previous reviews; see Chap. 1). Among the red macroalgae, the genus *Laurencia* continues to be a prolific source of new secondary metabolites (Blunt et al. 2007) and is an important component of many benthic communities in tropical regions.

Multicellular Cyanobacteria (also known as blue-green algae or Cyanophyta) exhibit considerable morphological plasticity and occur in a variety of marine benthic regions worldwide, including rocky shores, sandy shores, salt marshes, or, sometimes, floating on surface waters. Blooms of cyanobacteria are common and may include toxic species (harmful algal blooms), which are probably increasing in frequency worldwide (Paul et al. 2005; Watkinson et al. 2005). Cyanobacteria produce a wide variety of secondary metabolites, including many nitrogen-containing secondary metabolites such as peptides and lipopeptides, probably because of their capacity to fix nitrogen (Moore 1996; Blunt et al. 2007 and previous reviews; see Chap. 1). For example, benthic cyanobacteria such as *Lyngbya* spp. continue to intrigue natural products chemists as a rich source of nitrogenous secondary metabolites (Paul et al. 2006).

2.4 Tropical Chemically Defended Macroalgae

Chemical defenses of marine macroalgae have been extensively studied during the past 20 years (Paul and Puglisi 2004), ranging from species collected in Antarctic polar waters (e.g., Amsler et al. 2005; see Chap. 4) to those from the

tropics (Paul et al. 2001). The goal here is not to review all the literature on chemical defenses from tropical macroalgae, but rather to focus attention on the known and potential chemical mediation of ecological relationships of tropical macroalgae. Currently, a total of 55 species of tropical macroalgae representing 29 genera have been studied in the context of their chemical ecology. Their probable distributions around the world are listed in Table 2.1. Among the Cyanobacteria, a total of four species from tropical regions have been studied within a chemical ecology context. The green macroalgae have been investigated most extensively, including a total of 27 species distributed across 12 genera. Studies of tropical brown macroalgae are represented by only 6 genera and 16 species while studies of red macroalgae have included 7 genera and 8 species.

According to the biogeographic distribution of the species studied (Table 2.1), most of the chemically defended tropical macroalgae are broadly distributed (pantropical, see definitions and biogeographical regions in Lüning 1990); chemical defenses in tropical marine macroalgae are thus widespread, although there are large quantitative and even qualitative variations along distribution ranges (e.g., Bolser and Hay 1996; Pereira et al. 2004; see also Sect. 2.5.5). Therefore, provided these macroalgae are truly important to the structure of marine benthic communities, it is possible to postulate that chemically defended macroalgae exert broad effects in tropical regions worldwide.

Most of the research on the ecological roles of macroalgal metabolites during the past two decades has been focused on their roles in providing defense against generalist consumers, mainly fishes, sea urchins, and gastropods. Indeed, chemical defense against consumers was the initial focus of, and continues to be, an important area of research within macroalgal chemical ecology.

In general, we still know relatively little about the allelopathic, antifouling, and antipathogenic bioactivities of seaweed natural products; there are several recent reviews on macroalgal antifouling agents, but these studies are not included here because the majority of them take an applied (e.g. Bhadury and Wright 2004; Fusetani 2004; Dahms et al. 2006) rather than an ecological approach. If secondary metabolites have multiple functions (Schmitt et al. 1995; Pereira et al. 2003; see Chap. 3), future studies taking a broad ecological approach could help us to more fully understand the ecological and evolutionary importance of these compounds to tropical macroalgae.

2.5 Tropical Macroalgal Chemical Defenses and Community Structure

Many herbivorous fishes are found on coral reefs (Horn 1989; Choat 1991) and their grazing activities are commonly the dominant factor affecting the distribution and abundance of macroalgae (e.g., Morrison 1988; Hixon and Brostoff 1996; Marques et al. 2006) as well as the structure and function of marine communities and ecosystems in general (Duffy and Hay 2001). For example, the exclusion of

Table 2.1 Chemically defended tropical macroalgae, with geographic distribution, ecological role attributed, and references

Genus and studied species	Occurrence and geographic distribution[a]	Chemical ecology studies and references
CYANOBACTERIA		
Hormothamnion	One species was described from Pacific Is., the second one (type species) was recorded from Caribbean district, Canary Is. and Mauritius	
H. enteromorphoides	North America, Africa, Indian Ocean Is., South-west Asia, Australia and New Zealand, Pacific Is.	The crude extract from Guam specimens inhibits fishes and a mixture of cyclic peptides containing laxaphycin A deters feeding by the fish *Scarus schiegeii*, the sea urchin *Diadema savignyi*, and the crab *Leptodius* sp. (Pennings et al. 1997)
Lyngbya	Common all over the world	
L. majuscula	Ireland, Europe, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, South-east Asia, Australia and New Zealand, Pacific Is.	From Guam specimens: Extract inhibits the fish *S. schlegeli* (Nagle and Paul 1999); pitipeptolide deters the sea urchin *Echinometra mathaei*, the amphipods *Parhyale hawaiensis* and *Cymadusa imbroglio* and the crab *Menaethius monoceros* (Cruz-Rivera and Paul 2007); microcolin A and B, ypaoamide, malyngolide, curacin A, debromoaplysiatoxin, and malyngamide A and B inhibit the mollusk *Stylocheilus longicauda* (Nagle et al. 1998); malyngamide A and B, and malyngolide inhibit the fishes *Siganus spinus* and *S. schlegeli* (Thacker et al. 1997)
Microcoleus	Worldwide distribution	
M. lyngbyaceus	Ireland, Europe, North America, Africa, Australia and New Zealand, Pacific Is.	Malyngamide A from Guam specimens inhibits the fish *Zebrasoma flavescens* (Wylie and Paul 1988)
Schizothrix	Worldwide distribution	
S. calcicola	Europe, Arctic, Ireland, Europe, Atlantic Is., North America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, South-east Asia, Australia and New Zealand, Pacific Is.	The compound ypaoamide from Guam specimens deters feeding by the rabbitfishes *Siganus argenteus* and *S. spinus*, the parrotfish *S. schlegeli*, and the sea urchin *E. mathaei* (Nagle and Paul 1998)

CHLOROPHYTA		
Avrainvillea	Pantropical to subtropical	
A. elliottii	North America, Caribbean Is., Indian Ocean Is., Caribbean Is.	Specimens from southern Brazil are chemically defended against the sea urchin *Lytechinus variegatus* (Lima et al., submitted)
A. longicaulis	Atlantic Is., North America, Central America, Caribbean Is., South America, South-east Asia	Low susceptibility to Florida reef fishes due to secondary metabolites (Paul and Hay 1986). Specimens of Carrie Bow Cay, Belize, produce a brominated diphenylmethane derivative (avrainvilleol), which significantly deters feeding by reef fishes (Hay et al. 1990)
A. obscura	Africa, South-west Asia, Asia, South-east Asia, Australia and New Zealand, Pacific Is.	Crude extract and avrainvilleol from Guam specimens inhibit the fish *Naso lituratus* (Meyer et al. 1994)
Bryopsis	Global distribution spans polar to tropical latitudes	
Bryopsis pennata	Europe, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, Pacific Is	Crude extract from Guam specimens inhibits the fishes *Z. flavescens* (Wylie and Paul 1988) and *N. lituratus* (Meyer et al. 1994)
Caulerpa	Global distribution, from tropical to subtropical, with some species extending into the Mediterranean Sea and temperate regions of Australia	
C. filiformis	South America and Africa	Extracts from Australian specimens inhibit feeding by the gastropod *Turbo torquatus* and the sea urchin *Heliocidaris tuberculata* (Davis et al. 2005)
C. prolifera	Europe, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, South-west Asia, South-east Asia	Low susceptibility to reef fishes in Florida and produce secondary metabolites (Paul and Hay 1986)
C. racemosa	Australia and New Zealand, South America, Europe, Atlantic Is., North America, Central America, Caribbean Is., Africa, Indian Ocean Is., South-west Asia, South-east Asia, Pacific Is., and Asia	Extract and caulerpenyne from Guam specimens inhibit feeding by the mollusk *Dolabella auricularia* (Pennings and Paul 1992)
Chlorodesmis	Most species are widespread, growing in tropical to subtropical waters, with extensions into temperate latitudes as well	

(continued)

Table 2.1 (continued)

Genus and studied species	Occurrence and geographic distribution[a]	Chemical ecology studies and references
CHLOROPHYTA		
C. fastigiata	Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Crude extract and chlorodesmin, a major compound found in Guam specimens, inhibit the herbivory by reef fishes (Paul 1987). Chlorodesmin from Guam specimens inhibits the fish *Z. flavescens* (Wylie and Paul 1988)
Cymopolia	Patchy pantropical distribution	
C. barbata	Atlantic Is., North America, Caribbean Is., and South America	The compound debromoisocymobarbatol from Florida Keys specimens inhibits parrotfishes, the fish *Lagodon rhomboides*, and the amphipod *Hyale macrodactyla* (Park et al. 1992). Cymopol from Florida inhibits the gastropod *Littorina littorea* (Targett and McConnnell 1982). Cymopol from Caribbean inhibits reef fishes (Hay et al. 1987)
Halimeda	Widely distributed globally in warm waters	
H. copiosa	Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay, 1986)
H. discoidea	North America, Central America, South America, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
H. goreauii	North America, Central America, Caribbean Is., and South America	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
H. incrassata	Pacific Is., Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, Pacific Is., Atlantic Is., North America, Central America, and Caribbean Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)

H. macroloba	Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	From Guam specimens: Halimedatetraacetate is deterrent to reef fishes (Paul 1987); extract inhibits the fishes *Scarus sordidus* and *Ctenochaetus striatus* (Schupp and Paul 1994); crude extract inhibits the fish *N. lituratus* (Meyer et al. 1994)
H. opuntia	Europe, North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
H. scabra	North America, Caribbean Is. and South America	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
H. tuna	Europe, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand and Pacific Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
Neomeris	Cosmopolitan in tropical and subtropical seas	
N. annulata	Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	From Guam specimens: Crude extract inhibits the parrotfish *S. sordidus* (Pennings et al. 1996); bromitated sesquiterpenes inhibit the fishes *S. sordidus* and *S. schlegeli* and the sea urchin *D. savignyi* (Lumbang and Paul 1996); crude extract and a brominated sesquiterpene inhibit the fish *N. lituratus* (Meyer et al. 1994)
Penicillus	Geographical distribution is tropical to somewhat subtropical	
P. capitatus	Europe, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, South-west Asia, and Europe	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
P. dumentosus	Atlantic Is., North America, Central America, Caribbean Is., and South America	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
Pseudochlorodesmis	Geographic distribution in tropical to warm temperate waters	
P. furcellata	Atlantic Is., Europe, Atlantic Is., Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	An epoxylactone found in Guam specimens inhibits the herbivory by reef fishes (Paul 1987)
Rhipocephalus	Geographic distribution is tropical western Atlantic	

(continued)

Table 2.1 (continued)

Genus and studied species	Occurrence and geographic distribution[a]	Chemical ecology studies and references
CHLOROPHYTA		
R. phoenix	North America, Central America, Caribbean Is., and South America	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986). Rhipocephalin from Florida inhibits the sea urchin *Diadema antillarum* and parrotfishes (Hay et al. 1994)
Tydemania	Geographical distribution is Indo-Pacific, and essentially tropical	
T. expedictionis	Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Crude extract from Guam specimens inhibits the fish *N. lituratus* (Meyer et al. 1994)
Udotea	Occurs globally in tropical to somewhat subtropical latitudes	
U. argentea	Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Crude extract and major compound udoteal from Guam specimens inhibit herbivory by reef fishes (Paul 1987)
U. conglutinata	Atlantic Is., North America, Central America, Caribbean Is., and South America	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
U. cyathiformis	Atlantic Is., North America, Central America, Caribbean Is., and South America	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
U. flabellum	Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, and Australia and New Zealand	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986)
PHAEOPHYTA		
Canistrocarpus		
C. cervicornis (=Dictyota cervicornis)	Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986). Crude extract from Guam specimens inhibits herbivory by reef fishes (Paul 1987); crude extract and a mixture of two diterpenes inhibits herbivory by the gastropod *Astraea latispina* (Pereira et al. 2002)
Dictyota	Cosmopolitan distribution	

D. acutiloba	South America, Australia and New Zealand, and Pacific Is.	From Hawaii: Acutilol A acetate inhibits the fishes *L. rhomboides*, *S. schlegeli*, and *S. sordidus* and the sea urchins *Arbacia punctulata* and *D. savignyi*; acutilol A inhibit *A. punctulata*, *L. rhomboids*, and *S. schlegeli* and acutilol B inhibits *S. schlegeli* (Cronin et al. 1997)
D. bartayresiana	Atlantic Is., Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, Pacific Is., North America, and Central America	Crude extract from Bahamas specimens inhibits the sea urchin *A. punctulata* (Bolser and Hay 1996). Pachydictyol A from Guam specimens (as *Dictyota bartayresii*) inhibits the fish *Z. flavescens* (Wylie and Paul 1988)
D. ciliolata	Africa, Atlantic Is., North America, Central America, Caribbean Is., South America, Indian Ocean Is., South-west Asia, South-east Asia, Australia and New Zealand, Pacific Is., South-west Asia, Asia, South-east Asia, and Australia and New Zealand	Specimens from Radio Is., North Carolina (not a true tropical system, see text for details): Pachydictyol A inhibits the sea urchin *A. punctulata* and dictyol A acetate inhibits this sea urchin species, the amphipod *Ampithoe longimana*, and the pinfish *L. rhomboides* (Bolser and Hay 1996). Pachydictyol A, dictyol B acetate, dictyol E, and dictyodial, also obtained from the same specimens, cause larval mortality, abnormal development, and reduced growth in bryozoans and hydroid species (Schmitt et al. 1998)
D. dichotoma	Species reported from all around the world but is possibly restricted to the Atlantic Ocean	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986). Specimens from Radio Is., North Carolina: Pachydictyol A inhibits the fish *Diplodus holbrooki*. This compound and dictyol B inhibit the sea urchin *A. punctulata* (Hay et al. 1987); both compounds from North Carolina also inhibit the fish *L. rhomboides* (Hay et al. 1988b)
D. menstrualis	Atlantic Is., North America, Caribbean Is., South America, Central America, and Caribbean Is.	From Brazil: Extract inhibits the amphipod *P. hawaiensis* and the crab *Pachygrapsus transversus* (Pereira et al. 2000b), a diterpenoid inhibits *P. hawaiensis* (Pereira et al. 2000a). From North Carolina: Pachydictyol A and dictyol E act as antifoulants (Schmitt et al. 1995), dictyol B acetate and dictyodial cause larval mortality, abnormal development, and reduced growth in bryozoans and hydroids (Schmitt et al. 1998); extract inhibits the urchin *A. punctulata* (Bolser and Hay 1996)

(continued)

Table 2.1 (continued)

Genus and studied species	Occurrence and geographic distribution[a]	Chemical ecology studies and references
PHAEOPHYTA		
D. mertensii	Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Asia, South-east Asia, and Pacific Is.	Crude extract from Brazilian specimens inhibits feeding by the amphipod *P. hawaiensis* and the crab *Pachygrapsus transversus* (Pereira et al. 2000b)
D. pfaffii	Atlantic Is., Central America, Caribbean Is., South America, and Australia and New Zealand	Crude extract and a diterpenoid inhibit fishes and the sea urchin *Lytechinus variegatus* (Barbosa et al. 2004)
D. pulchella	Atlantic Is., North America, Central America, and Caribbean Is.	Crude extract from Bahamas specimens inhibits the sea urchin *A. punctulata* (Bolser and Hay 1996)
Dictyopteris	Widely distributed in tropical to temperate seas	
D. delicatula	Central America, South America, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, South Africa, Indian Ocean Is., South-west Asia, South-east Asia, Australia and New Zealand, Pacific Is., and Atlantic Is.	A mixture of the dictyopterenes A and B from specimens collected at Mayrean Is., West Indies, inhibits mainly Scaridae and Acanthuridae fishes (Hay et al. 1988a)
D. justii	South America, Atlantic Is., North America, Caribbean Is., and South America	Extract from Bahamas inhibits the sea urchin *A. punctulata* (Bolser and Hay 1996). An unusual sterol from Brazilian specimens inhibits feeding by *P. transversus* (Teixeira et al. 2006)
D. membranacea	Ireland, Europe, Atlantic Is., North America, Caribbean Is., South America, Africa, and South-east Asia	C_{11} sulfur compounds in specimens from Villefranche-sur-mer, France, strongly inhibit feeding by the amphipod *A. logimana* (Schnitzler et al. 1998)
Lobophora	Occurrence from tropical to temperate seas	
L. variegata	Atlantic Is., South America, Africa, South-west Asia, Asia, Pacific Is., North America, Central America, Caribbean Is., Australia and New Zealand, Europe, and Indian Ocean Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986). Extract from Bahamas inhibits *A. punctulata* feeding (Bolser and Hay 1996)
Sargassum	Widespread in temperate and tropical waters.	
S. filipendula	Atlantic Is., North America, Caribbean Is., South America, Africa, South-west Asia, and South-east Asia	Specimens collected at Radio Is., North Carolina, protect associated algae from herbivores (Pfister and Hay 1988)
Sporochnus	Distributed in tropical and temperate seas	

S. bolleanus	Atlantic Is., North America, Caribbean Is., South America, and Africa	Sporochnol A, a major compound found in specimens from Belize, inhibits the sea urchin *D. antillarum* and Scaridae fishes (Shen et al. 1993)
Stypopodium	South Africa, India, Pakistan, Japan, Indonesia, Australia, Micronesia, Caribbean, Venezuela, Brazil	
S. zonale	Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986). Stypotriol inhibits reef fishes in the Caribbean (Hay et al. 1987). Extract and the compounds stypotriol and stypoldione inhibit feeding by the crab *P. transversus* and the sea urchin *L. variegatus* (Pereira et al. 2004)
RHODOPHYTA		
Amphiroa	Widespread in tropical and subtropical region	
Amphiroa fragilissima	Europe, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Crude extract from Bahamas specimens inhibits feeding by the sea urchin *A. punctulata* (Bolser and Hay 1996)
Encyothalia	Western Australia	
E. cliftonii	Australia and New Zealand	A phenolic compound from Western Australian specimens deterred the sea urchin *Tripneustes esculentus* (Roussis et al. 1993)
Laurencia	Occurring on temperate to tropical shores of the world, littoral to sublittoral up to 65 m deep	
L. obtusa	Ireland, Europe, Atlantic Is., North America, Central America, Caribbean Is., South America, Africa, Indian Ocean Is., South-west Asia, Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	The crude extract and the sesquiterpene elatol from Brazilian specimens inhibit the feeding by the crab *P. transversus* and the sea urchin *L. variegatus* (Pereira et al. 2003)
Liagora	Temperate to tropical distribution	
L. farinosa	Europe, Atlantic Is., North America, Caribbean Is., South America, Africa, South-west Asia, South-east Asia, Australia and New Zealand, and Pacific Is.	Low susceptibility to reef fishes in Florida and produces secondary metabolites (Paul and Hay 1986). A fatty acid compound from Guam specimens inhibits the fish *Z. flavescens* (Wylie and Paul 1988)

(continued)

Table 2.1 (continued)

Genus and studied species	Occurrence and geographic distribution[a]	Chemical ecology studies and references
RHODOPHYTA		
Ochtodes	Type species from the West Indies and also occurs in Brazil; a second species, *O. crockeri*, in Galapagos Is.	
O. secundiramea	Central America, Caribbean Is., and South America	Collected at Point Dunkerque on the Martinique Is., Caribbean, produces the monoterpene ochtodene as chemical defense against reef fishes (Paul et al. 1987). Crude extract from Guam inhibits the fish *N. lituratus* (Meyer et al. 1994)
Plocamium	Primarily a cool temperate genus	
P. brasiliense	North America, Caribbean Is., and South America	Crude extract from Brazilian specimens inhibits feeding by the amphipod *P. hawaiensis* and the crab *P. transversus* (Pereira et al. 2000b)
P. hamatum	Australia and New Zealand	Allelopathic effect on competitor soft coral *Sinularia* in Australia (De Nys et al. 1991)
Portieria	Red Sea and widespread localities in the Indian and eastern and central Pacific Oceans	
P. hornemannii	Africa, South-east Asia, Pacific Is., South-west Asia, Asia, Australia and New Zealand, and Indian Ocean Is.	Ochtodene from Guam specimens (as *Desmia hornemanni*) inhibits the fish *Z. flavescens* (Wylie and Paul 1988)

[a]Source: algaebase (www.algaebase.org)

fishes from shallow reef areas in Belize for a period of just 10 weeks was sufficient to permit an increase in the abundance of palatable and several less palatable macroalgae capable of overgrowing and killing corals (Lewis 1986). In fact, rates of herbivory on coral reef systems exceed those observed in any other marine or terrestrial habitats (Hay and Steinberg 1992, Carpenter 1986). This strong and persistent effect of herbivory selects for chemical defenses among macroalgae and these defenses probably affect community structure on both ecological and evolutionary timescales (Duffy and Hay 2001). To our knowledge, there are no published studies that quantify the importance of chemically defended macroalgae in determining community structure in tropical marine environments. However, it is possible to infer some cascading effects of defended macroalgae as they relate to community structure as a whole.

2.5.1 Are Tropical Macroalgae Better Defended Than Their Counterparts?

In general, it is accepted that chemical defenses are elaborated to a greater extent and are more important in tropical than in temperate or cold seas (Bakus and Green 1974; Gaines and Lubchenco 1982; Hay and Fenical 1988). For example, Bolser and Hay (1996) verified that macroalgal species from tropical regions are considerably less palatable to herbivores than are species from warm temperate regions. We estimated the number of natural products that could play a defensive role, using information from the extensive account of Harper et al. (2001). We then separated algae by their biogeographical regions (tropical, warm temperate, cold temperate, Arctic/Antarctic), as given in Algaebase (www.algaebase.org), and surprisingly, tropical seaweeds were not the most prolific in terms of the diversity of natural products (data not shown). Rather, the most diverse array of secondary metabolites is produced by temperate seaweeds, in accordance with peak seaweed species diversity in temperate rather than in tropical waters (Kerswell 2006). What still remains to be shown, given that the diversity of herbivores is indeed greater in the tropics, is whether individual species of tropical seaweeds produce a wider array of secondary metabolites, targeted to diverse herbivore groups, than their extratropical congeners.

2.5.2 The Causes and Effects of Ecological Dominance

Chemically defended macroalgae often dominate their environments (e.g., Marques et al. 2006). However, how these macroalgal species became dominant and what the mechanisms that maintain their dominance are, are questions that remain largely unanswered. Generally, herbivores that would consume more palatable, undefended seaweed species could be assumed to be the cause of chemically defended seaweed dominance, but experimental studies at the community level to

test this assumption are still lacking. For example, selective consumption of palatable macroalgae by herbivorous fishes can remove potential competitors and favor the establishment and/or proliferation of unpalatable or chemically defended macroalgae. But how will this story play out? Littler et al. (1986) suggested that undefended algae persist by living associated with chemically defended algae, such as *Stypopodium zonale*, in what has been called an "associational refuge," but more than 20 years later this idea has not yet been tested properly.

For decades, manipulative experimentation in intertidal and subtidal environments has been extensively used to evaluate the processes of competition (Connell 1961), predation (Paine 1974), grazing (Lubchenco 1978), recruitment (Gaines and Roughgarden 1985), and disturbance (Sousa 1979). Despite the problems in experimental intertidal ecology (Underwood 2000), manipulative experiments could be a good first approach to investigate the effects of the dominance of chemically defended macroalgae on benthic community structure. For example, an appropriate experimental design might include the following: (1) scraping the substrate clean of all associated organisms in order to determine patterns of initial colonization; (2) removing all of the chemically defended macroalgae to identify the changes occurring in the community in their absence; (3) removing all macroorganisms except the chemically defended macroalga to examine whether this macroalga is capable of inhibiting establishment of the removed or other organisms and whether the initially associated species will recruit there again; (4) removing the chemically defended macroalgal canopy, but leaving its corresponding holdfasts to determine whether the dominance of this macroalga is associated with its establishment on the substrate or is due to effects on the remaining species such as shading or exudation of allelopathic compounds by fronds; and (5) untreated control – the reference undisturbed community to compare with the different treatments.

2.5.3 Associational Defenses

Associational defenses occur when a species gains protection from a natural enemy by associating with a protected species (the earlier-mentioned associational refuge). In fact, the associational defense can be an important possibility if defensive chemicals produced by macroalgae can have a greater influence on community structure than does competition or predation. For example, indirect commensal or mutualistic interactions are very important in community organization (Hay 1986). The few studies providing evidence for this are restricted to tropical environments, but one example comes from a temperate region with tropical affinities, including similar flora: coastal North Carolina. Palatable algae seem to gain protection by living close to the unpalatable species *Sargassum filipendula* (Hay 1986). During high herbivory periods, the abundance of *Gracilaria tikvahiae* is positively correlated with that of *S. filipendula*. This brown macroalga decreased feeding by the sea urchin *Arbacia punctulata* on *G. tikvahiae*, probably by means of its defensive chemicals (Pfister and Hay 1988).

Along a broad littoral area of southeastern Brazil, a common association can be found between the brown macroalgae *Sargassum* and *Dictyota*, mainly during the summer period. *Sargassum* grows in monospecific stands in mid- and sublittoral zones, but is sometimes epiphytized by *Dictyota* species. When offered to the sea urchin *Lytechinus variegatus*, a very common generalist consumer, *Sargassum* specimens are heavily consumed while *Dictyota* is barely consumed (Pereira et al. in prep.). Complementary experiments verified that when both these species are offered simultaneously to this sea urchin, less *Sargassum* is consumed, but this consumption is significantly lower when both species are offered together (Pereira et al. in prep.). This protection is chemically mediated, since *Sargassum* is easily consumed by *L. variegatus* when tied to plastic mimics of epiphytic *Dictyota*. These results confirmed previous evidence that several Brazilian *Dictyota* species are chemically defended (e.g., Barbosa et al. 2004) while *Sargassum* produces levels of polyphenols too low to be used as defenses (Pereira and Yoneshigue-Valentin 1999) and, in general, that polyphenols do not deter tropical herbivores (e.g., Steinberg and Paul 1990; Van Alstyne and Paul 1990). This is a typical associational defense that can be very important in the structure and dynamics of benthic communities in the southeastern Brazilian littoral.

On the other hand, association with more palatable seaweeds may have a negative impact on the chemically defended partner. For example, *Halimeda* specimens from Conch Reef, Florida Keys, with more than 50% of their thalli covered by *Dictyota* grow significantly slower than unepiphytized thalli (Beach et al. 2003). This study also verified that epiphytic *Dictyota* negatively affects metabolic rates of *Halimeda tuna* in part by shading their thalli, but probably also by chemical means, because the exposure to *Dictyota*-conditioned water elevated respiration rates in a manner similar to when *H. tuna* is naturally epiphytized by *Dictyota*.

These associational defenses are examples of a mechanism that is to date underexplored in tropical regions and that probably represents one of the most important cascading effects of chemically defended macroalgae on community structure. Thus, chemically defended macroalgae can act as local "biodiversity centers," by increasing the coexistence of species under their chemical defense umbrellas.

2.5.4 Chemically Defended Isomorphic Macroalgal Life Stages

In marine macroalgae, a wide diversity of complex life cycles is observed, changing the relative temporal and/or spatial importance of each alternating free or independent living haploid and diploid stage (Valero et al. 1992). Life cycles with haploid, haploid-diploid, and diploid phases are widespread in red, most brown, and many green macroalgae (Klinger 1993). The morphological similarity between the two phases varies dramatically in haploid-diploid seaweeds: in some species these different phases can be morphologically identical (isomorphic), and in others, strikingly different (heteromorphic).

Alternating heteromorphic life stages of some seaweeds exhibit their different morphologies supposedly as a response to differential herbivory pressure, or because of

other environmental stressors (Clayton 1988). Contrary to heteromorphic life history stages, isomorphic life history stages are very intriguing, since both phases can be found living simultaneously in the same habitat, or can also differ in their distribution, suggesting that some ecological specialization may occur among these stages. For example, in a study designed to compare the secondary metabolites produced by isomorphic life stages of the brown seaweed *Dictyota ciliolata* from North Carolina (a temperate/subtropical system but with floristic affinities with tropical regions, rather than a true tropical region), and their efficiency against herbivory, Cronin and Hay (1996) determined that both amphipods and sea urchins consumed similar amounts of diploid sporophytes and haploid female and male gametophytes. These different life stages also had similar levels of defensive chemicals (Cronin and Hay 1996).

In general, macroalgae of the order Dictyotales serve as a good model to evaluate this question because their species possess isomorphic life stages and are rich in secondary metabolites. However, because of the difficulty in distinguishing morphologically similar (e.g., isomorphic haploid and diploid) individuals, most studies have been limited to surveying only reproductive individuals.

On the Brazilian coast, observations in the field indicate that different haploid-diploid ratios of the alternating life stages of the tropical *D. menstrualis* can be found throughout the year, but that they exhibit temporal and/or spatial variation in the importance of each alternating stage. Defensive chemicals from the three life stages of this macroalga were analyzed by gas chromatography coupled to mass spectrometry – HRGC-MS analyses (Pereira et al., in preparation). Natural concentrations of the crude extracts from the male and female gametophytes and the sporophytes of *D. menstrualis* significantly inhibited consumption by the sea urchin *L. variegatus*, but with different efficiencies associated with the concentration of the diterpene (6*R*)-6-hydroxydichotoma-3,14-diene-1,17-dial (Pereira et al. in preparation), a known defensive metabolite of the Brazilian specimens of *D. menstrualis* (Pereira et al. 2000a).

The different susceptibility and/or survival of individuals in a macrolagal population may result in very distinct equilibrium population structures (Engel et al. 2001). For example, different susceptibility of the haploid and diploid stages to herbivory could be sufficient to cause deviations from the 1:1 haploid-diploid ratio that has been used to infer the advantages of haploidy or diploidy (e.g., Dick and De Wreede 1995; Van der Strate et al. 2002). Thus, if the balance of ploidy ratios is mainly due to survival aspects, defensive chemicals may be of vital importance in seaweed life cycles. For example, different chemicals produced by isomorphic life stages could be a mechanism by which the generations differentially exploit environmental resources and escape from herbivory, whereas different stages utilizing the same compounds probably indicates that they are under the same selective pressures by herbivores.

2.5.5 Intrapopulational Variation

Knowledge of both the amount of and variation in secondary metabolites in marine organisms is an essential element for assessing studies in chemical ecology and for placing them into an ecological and evolutionary context (de Nys et al. 1995, 1998;

Schmitt et al. 1995; Hay et al. 1998). However, little attention has been given to evaluating these factors, particularly with respect to their consequences for consumers or their repercussions for population and community organization. There are several reasons why it is important to understand the intrapopulational variation in defensive chemicals. Although this is an aspect largely undocumented and underappreciated in marine systems, it is this variation upon which natural selection acts and as such it is important for understanding the genetic versus phenotypic components shaping marine biodiversity (Hay 1996). In addition, the variation in secondary metabolites has important implications in understanding both basic issues such as population regulation and applied aspects such as the development of pharmaceuticals and other useful chemical products from the sea (Hay and Fenical 1996).

In a recent study on the tropical seaweed *Laurencia obtusa*, it was determined that the mean within-thallus concentration of the defensive compound elatol was 9.89 mg g^{-1} dry weight (d.w.), varying from 0.1 to 2.2%. Conversely, the mean surface elatol contents reached much lower values (0.006 mg g^{-1} d.w.), varying from 5×10^{-6} to 5×10^{-3}% d.w. (Sudatti et al. 2006). In an ecological context, the surface concentration ranges are very low from the perspective of being sufficient to provide defense against herbivory or fouling (Sudatti et al., submitted). However, the range of concentrations observed may be important in the evolutionary context in that it provides variation for natural selection for counteradaptation in the sympatric mesoherbivores.

Several studies on macroalgal chemical defenses in tropical regions have verified that species containing compounds that deter fish feeding are often selectively consumed by small, relatively sedentary herbivores such as amphipods, polychaetes, and ascoglossan gastropods – mesograzers (Hay 1992). In fact, these small herbivores living on and consuming chemically defended macroalgae experience less predation pressure by generalist consumers than those living on undefended algae (Hay 1992). In this way, intrapopulational quantitative variation in chemicals would be an important aspect for the small and more sedentary consumers and, consequently could exert a significant impact on community structure. This possibility remains to be explored.

In addition, the relationship between specialized consumers and macroalgae that selects for defensive secondary metabolites can produce additional cascading effects. For example, about 60% of the sesquiterpenes found in mollusks (genus *Aplysia*) do not occur in *Laurencia* species (Pereira and Teixeira 1999), suggesting that *Alpysia* species are capable of chemically modifying precursor metabolites obtained in their natural diets. This in turn can promote the diversification of metabolites acting at higher trophic levels within food webs, potentially producing cascading effects on community organization.

2.5.6 Surface Ecology

Although herbivore pressure is more intense and is exerted by a more diverse assemblage of consumers in the tropics (Lüning 1990), it is now known that herbivory fails to fully explain the production of secondary metabolites, and that these

compounds can play multiple functional roles (Schmitt et al. 1995; Pereira et al. 2003), which increases their adaptive value. Moreover, the diversity of epibionts and the colonization of substrata also occur more rapidly and intensely in tropical regions (e.g., Railkin 2004). Therefore, competitors for space and epibionts should exert a pressure that may also select for the production of seaweed natural products. There is, indeed, an extensive recent record of seaweed secondary metabolites that possess some sort of antifouling activity (see Chap. 11 and Sect. 3.4.1), but these will not be reviewed here. Instead, we will concentrate on other aspects that seem to have been neglected in the literature and that point to possible selective pressures exerted by epibionts and competitors in tropical macroalgae.

A growing body of morphological and methodological evidence suggests that secondary metabolites are present at or near the surfaces of macroalgae, where they could play ecological roles as antifouling defenses, settlement cues, or in allelopathic interactions. One example is physodes, which are well-known membrane-bound, refractive vesicles in the cytoplasm of brown algae that contain phlorotannins (Ragan and Glombitza 1986). These phenolic compounds are known to have antifouling bioactivity along with other functions (Ragan and Glombitza 1986, Amsler and Fairhead 2006). Physodes may occur in most tissues, but commonly occur in the outermost layers (Tugwell and Branch 1989; Amsler and Fairhead 2006). Another example can be seen in red algae of the genus *Laurencia*, the most prolific natural-product-producing alga, with about 700 different secondary metabolites known to date (Harper et al. 2001; see Chap. 1). These also contain refractile, membrane-bound vesicles known as *corps en cerise* (cherry bodies) in the outer cell layer (the cortical layer). These have long been postulated to be the place of synthesis and storage of halogenated metabolites (Young et al. 1980), although this has only recently been demonstrated (Sudatti et al. 2006; submitted). Some *Laurencia* species are known to produce and release halogenated antifouling compounds such as elatol (Da Gama et al. 2003; Sudatti et al. 2006; submitted). Finally, the red algae *Delisea pulchra* and *Asparagopsis armata* have been shown to possess gland cells that release secondary metabolites, some of which clearly have an antifouling activity (e.g., Dworjanyn et al. 2006; Nylund et al. 2007).

There are several criteria necessary to demonstrate that seaweeds use antifouling chemicals (Davis et al. 1989; Schmitt et al. 1995; Clare 1996; Hay 1996): (1) The macroalga should be naturally devoid of fouling in the field; (2) The putative antifouling compound inhibits fouling at the concentration that it is naturally found at the macroalgal surface; and (3) The bioassays conducted to verify antifouling properties employ ecologically relevant fouling organisms. In addition, it is also useful to know the following: What compounds are presented by the seaweed (on the thallus surface or in the surrounding water)? What if any interactions occur between or among the compounds produced by the host seaweed? and By what mechanism are these compounds translocated to the macroalgal surface?

To date, within the constraints of the these criteria, it is primarily temperate species that have been adequately studied. For tropical species, the only study that meets these criteria appears to be a recent evaluation of the transport and ecological

roles of secondary metabolites in the red alga *L. obtusa* (Sudatti et al., submitted). This macroalga is usually epibiont-free. Two important sympatric biofoulers, the mussel *Perna perna* and the barnacle *Amphibalanus amphitrite*, were evaluated, and elatol, the major secondary metabolite, was found on the surface of *L. obtusa*. In addition, the existence of connections between the *corps en cerise* and the cell walls of *L. obtusa* was observed (Sudatti et al. 2006; submitted). Nonetheless, the natural concentration (0.5 to 10 ng cm^{-2}) of elatol found on the macroalgal surface is too low to serve as an effective chemical defense against fouling organisms (cyprid settlement and mussel attachment) and herbivory by the sea urchin *L. variegatus* (Sudatti et al., submitted).

Collectively, these studies indicate that storage cell structures and transport of compounds to thallus surfaces can provide essential cues about surface-mediated chemical defenses. In addition, these studies reveal that the concentrations of the compounds on macroalgal surfaces are probably not absolute characteristics of the species, but may vary according to environmental conditions. Advances in this "surface ecology" will be fundamental to understanding and evaluating the importance of surface chemicals in the interactions between macroalgal species as they relate to benthic community structure. For example, epibiont cover may attract consumers that otherwise would not feed significantly on the host plant (Karez et al. 2000) and may also increase grazing rates (Wahl and Hay 1995; Karez et al. 2000). Detection of chemicals on macroalgal surfaces (i.e., surface extracts) can be an indication of an actual antifouling role (e.g., Nylund et al. 2007). However, in some instances whole-plant extracts may be as relevant in antifouling research as surface extracts, since in the field, among the thousands of organisms representing a wide phyletic diversity that can settle on algal surfaces, some certainly experience contact with internal tissues (see Leonardi et al. 2006).

2.5.7 Is Inducible Resistance to Herbivores Common Among Tropical Macroalgae?

Despite the growing number of studies reporting inducible defenses in brown, temperate macroalgae (reviewed by Amsler and Fairhead 2006; Paul et al. 2006; see Chaps. 3, 7) and the large number of chemical ecology studies with tropical seaweeds (see Paul et al. 2001), a significant gap remains in our knowledge of inducible defenses in tropical macroalgae, as well as about the dynamics of production/induction of virtually any type of chemical defense against herbivores other than phlorotannins (Paul and Puglisi 2004). The ease of quantifying this class of compounds by colorimetric assays may explain this (Paul et al. 2006; see Van Alstyne 1995, for problems with phlorotannin quantification methods). Current theory also assumes that inducible defenses should be more common in temperate than in tropical macroalgae, given that intense and constant herbivore pressure in low-latitude ecosystems should favor the selection of constitutive rather than inducible defenses in warmer waters (e.g., Hay and Fenical 1992).

However, one of the few recent tests of inducible defenses from tropical macroalgae (Weidner et al. 2004) found that eight of the nine Brazilian species tested showed some degree of inducible defense after direct consumption by amphipod grazers. Moreover, Molis et al. (in preparation), after conducting a meta-analysis of 62 seaweed-grazer combinations from sets of identical induction experiments performed in nine different sites around the world, concluded that the prevalence of inducibility was twice as high in macroalgae from tropical than from subtropical or temperate habitats. In addition, defense induction was as common in red as in brown macroalgae. However, the strong geographical differences in the frequency of inducible algae were not statistically significant.

Another gap in our current knowledge of inducible defenses is the fact that despite the large number of studies showing that phlorotannin levels increase in response to herbivory, tropical algae appear to be an exception to this pattern and produce only low constitutive levels of phlorotannins (e.g., Steinberg and Paul 1990; Van Alstyne and Paul 1990). This clearly suggests that herbivory is not the "raison d'être" of phlorotannin production in tropical macroalgae (see also Amler and Fairhead 2006 for a review of other functions of phlorotannins and Chap. 3 for a discussion of their roles in temperate macroalgae). Further experimental studies are needed to cast additional light upon the dynamics of defense production in tropical seaweeds, as well as the relative importance of herbivory as a driving force for the production of chemical defenses.

2.5.8 The Invasive Potential of Chemically Defended Tropical Macroalgae

Invasion success has been correlated in many cases with several abiotic (Moyle and Light 1996) and biotic (Chapin et al. 1998) features of the host habitat. However, some features of the invasive organism, including genetic variability, body size, physiological tolerance, and reproductive strategy, may be equally important in promoting successful invasions (Reichard and Hamilton 1997). Nonetheless, some recent studies strongly suggest that the invasive success of many species is more a result of their capacity to respond to natural selection than to their having wide tolerance or physiological plasticity (e.g., Lee 2002).

The defensive chemistry of exotic marine species has been less studied, but secondary metabolites commonly produced by tropical macroalgae may be an important contributing factor to the success of introduced species, further altering the native community structure. In this context, chemical defenses against consumers and competitors (allelopathic defenses) are of special interest, since they may be used by exotic species as a strategy for colonization, perpetuation and expansion and, consequently, for successful invasion. Several genera of tropical macroalgae are known to use chemical defenses to protect themselves from various types of consumers (see Table 2.1), and the use of chemical defenses is not restricted to adult organisms, but may be found in propagating agents such as spores and in some initial developmental stages such as embryos (Hay et al. 1998). Considering

that (8,000 species of marine macroalgae are known to produce secondary metabolites (Blunt et al. 2007; Faulkner 2002, and previous reviews of both authors), the integration of defensive chemistry with the life history may be of great ecological significance.

Extracts of the likely invasive green alga *Caulerpa filiformis* in Australia inhibit feeding by the gastropod *Turbo torquatus* and the sea urchin *Heliocidaris tuberculata* (Davis et al. 2005). These observations suggest that secondary metabolites may well contribute to its success as an invasive species. In fact, many *Caulerpa* species are highly invasive in other regions, such as North America (Jousson et al. 2000) and a number of Mediterranean countries (Meneisz et al. 1993; Piazzi et al. 1994).

The macroalga *Caulerpa taxifolia* is an example of a chemically defended species that invaded several areas of the northeastern coast of the Mediterranean from Monaco. In some cases, this species can be found hundreds of kilometers from the site where its accidental introduction took place (Meinesz and Hesse 1991; Meinesz et al. 1993). One lineage descended from this Mediterranean population has already reached the coast of California (Jousson et al. 2000). This macroalga is known to possess a sesquiterpene (caulerpenine), also found in other *Caulerpa* species, which makes it unpalatable to sea urchins and which may also be toxic to fish (Amade and Lemée 1998; Boudouresque et al. 1996; Uchimura et al. 1999). Although other factors may be important in order for *Caulerpa* species to become invasive (e.g., types of algae present in the native community; see Ceccherelli et al. 2002), defensive chemistry may play a leading role. The presence of caulerpenine in this genus may confer a strong invasive potential due to its "defensive properties." For instance, *C. racemosa*, which is believed to have immigrated from the Red Sea, has dispersed over the southeast Mediterranean and has recently also reached its western shores (Modena et al. 2000; Verlaque et al. 2000). Invasion by this species is presumably facilitated by the same defensive chemistry as found in *C. taxifolia*. Nevertheless, it is important to note that defensive chemistry would not necessarily be the only important characteristic contributing to the success of exotic secondary metabolite-producing species.

Although the production of chemical defenses is commonly associated with consumer pressure (herbivory and predation), there are no studies to date that conclusively demonstrate that herbivores or predators exert selective pressures resulting in the current pattern of abundance and distribution of a given macroalga. Thus, if invasion is indeed a rapid evolutionary process (see Lee 2002), chemically defended marine invasive species provide us with an excellent opportunity to identify adaptive responses of secondary metabolite production under the selective pressures of a new environment. Recently, a first-cut, innovative study was conducted to compare defensive chemical production in an invasive macroalga in its native habitat with that in a new region using the temperate brown macroalga *Fucus evanescens* (Wikström et al. 2006). Although this species was the preferred host for herbivores in its native habitat, the invading population supported a less diverse herbivore fauna because of reduced palatability due to the presence of higher concentrations of polyphenols in it relative to the native species in the host community

(Wikström et al. 2006). We need similar studies to assess the impact of chemically defended macroalgae on the structure and dynamics of tropical marine benthic communities.

2.6 Conclusions

Less studied tropical ecosystems such as seagrass beds, mangrove forests, and rocky shores that may support even more luxuriant macroalgal communities than do coral reefs, should receive the same attention as do coral reefs to better understand driving forces other than consumer pressure that have selected for secondary metabolites in tropical seaweeds. Moreover, studies are needed to evaluate the actual roles these compounds play in structuring marine communities. This does not mean, however, that the forces structuring coral reef ecosystems are fully understood. Indeed, although competition is considered to be one of the key biological factors structuring these ecosystems, surprisingly little is known about how coral reef macroalgae compete for space, including the role of secondary metabolites as allelopathic agents in interference competition (McCook et al. 2001; see also de Nys et al. 1991; Suzuki et al. 1998). This knowledge is essential for a more thorough understanding of how algal secondary metabolites influence marine community structure and function.

Since tropical communities possess the highest known faunal diversity (Krebs 2001; Willig et al. 2003), and competition for limited space is thus intense, the ecology of interactions at algal surfaces assumes a special interest and has attracted much recent attention (e.g., Nylund et al. 2007). As epibionts and competitors are presumably inhibited by macroalgal secondary metabolites at the settling stage (antifouling defenses), this may act as a selective force with an intensity equivalent to herbivory in driving defense production. There has been some recent debate concerning the ecological validity of using whole-plant extracts instead of surface extracts to assess antifouling activity (e.g., Sudatti et al. 2006; Nylund et al. 2007). Although new methodologies and studies in this young, important field are surely welcome, it is noteworthy that many plant epibionts do penetrate and damage the algal surfaces (e.g., Leonardi et al. 2006), thus coming into contact with compounds stored within thalli. Intensive efforts are indeed needed to assess the role that seaweed antifouling defenses play in marine communities as a whole.

Although inducible defenses have been thought to generally occur in temperate brown algae, recent evidence suggests that tropical algae, including red seaweeds, respond to herbivory with inducible defenses more frequently than their extratropical counterparts (Molis et al. in prep.). Much work is needed in tropical systems to understand the largely unknown dynamics of defense production (and release) of defensive compounds other than phlorotannins, which seem to be of little value as defenses in tropical macroalgae. In addition, assessing the inducibility of defenses in response to other triggering agents, such as epibionts, competitors and pathogens, would help us to understand the roles of factors other than herbivory regulating

defense production in tropical macroalgae. Finally, experimental manipulations to test the effects of secondary metabolites of tropical seaweeds in mediating community level interactions remain virtually absent. Such studies surely represent one of the next frontiers in tropical marine chemical ecology.

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3
Macroalgal Chemical Defenses and Their Roles in Structuring Temperate Marine Communities

V. Jormalainen(✉) and T. Honkanen

3.1 Interactions in Diverse Macroalgal Communities

Temperate zone macroalgal communities in general and littoral communities in particular consist of a diverse producer assemblage, a rich mesograzer fauna occasionally complemented with larger herbivores, and predators. Diversity in the producer assemblage is generated by small-scale heterogeneity in substrate characteristics, seasonal variation in growing conditions, and the depth gradient. Abiotic disturbances, mainly in the form of wave energy, counteract competitive exclusion, thereby enhancing species richness (Sousa 2001). The diverse producer assemblage comprises a habitat and food resource for a species-rich fauna (reviewed in Underwood 2000; Menge and Branch 2001). In such a diverse community, interactions within and between trophic levels become important for the regulation of the structure and function of both the producer and consumer assemblages (Duffy 2002; Worm and Duffy 2003).

Macroalgae compete for substrates, nutrients, and light. Blooms of filamentous species, capable of taking advantage of nutrient pulses, and epibiotism (using other organisms as substrates) illustrate competition for nutrients and substrate (e.g., Wahl and Mark 1999; Lotze et al. 2000). Competition occurs not only between different macroalgal species, but also between macroalgae, sessile animals, and the periphytic community. Ecophysiological capabilities such as nutrient uptake rates, timing and duration of spore dispersal, and the existence of spore bank largely determine the competitive abilities of the species (Lotze et al. 1999; Lotze and Schramm 2000; Worm et al. 2001). Competition is a major selective agent for coexisting species, implying that a range of traits have evolved in the context of avoiding and succeeding in competition. Periodic sloughing of outer cell wall layers to remove epibiota, chemical resistance to surface colonization, and allelopathic compounds employed against competitors for space are examples of such traits (Davis et al. 1989; Gross 2003).

V. Jormalainen
Section of Ecology, FIN-20014 University of Turku, Finland. e-mail: veijo.jormalainen@utu.fi

T. Honkanen
Section of Ecology, FIN-20014 University of Turku, Finland. e-mail: tuija.honkanen@utu.fi

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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Herbivory is intense in macroalgal communities, as evidenced by its profound role in the organization and biomass accumulation of macroalgal communities (e.g., Duffy and Hay 2000; Lotze et al. 2001; Korpinen et al. 2007c). The community effects of grazing are particularly important at the colonization and early life-history stages of macroalgae (Lotze et al. 2001; Korpinen et al. 2007c). When grazers have a strong impact on the producers, the higher trophic levels (above herbivores) may have a strong cascading effect on producers, which is often found in macroalgal communities (reviewed in Shurin et al. 2002). For example, in the kelp forests of the North Pacific Ocean, top-down regulation of sea urchins by sea otters cascades to kelps (Estes et al. 2004; Halpern et al. 2006) as sea otter predation on urchins transforms sea-urchin-dominated rocky reefs into kelp-dominated communities. Trophic cascades not only affect population regulation but also influence the evolution of traits related to plant-herbivore interactions (Steinberg et al. 1995).

In this chapter, we examine macroalgal chemical defenses by considering their consequences for temperate communities, i.e., how defenses may modify the biotic interactions in the communities. We first review studies on macroalgal defenses focusing on induced defenses and within-plant variation of defenses, both of which have been actively studied in temperate macroalgae. We then proceed to summarize the consequences of macroalgal chemical defenses for grazers. Finally, we review how chemical defenses function in allelopathic interactions. Studies have clearly pointed out the importance of chemical defenses against epibiota as well as the complicity of the interactions among host algae, epibiota, and grazers. The brown alga *Fucus vesiculosus* and its phenolic defensive metabolites, phlorotannins, make a case study we use as an example throughout the chapter.

3.2 Defense Strategies

In marine littoral habitats, typically a very high percentage (50–100%) of macroalgal production is consumed by herbivores (Hay 1991; Cyr and Pace 1993). This is on average thrice higher than in terrestrial communities (Cyr and Pace 1993). Grazing pressure varies spatially, e.g., with water temperatures, and is considered higher in tropical coral reefs than in temperate habitats (Paul et al. 2001). But if low growth rates in temperate environments are taken into account, options to tolerate grazing by growth are smaller and grazing effects may become relatively more important there. Negative effects of grazing on algae are seldom quantified, but often evident. Grazing, in addition to the direct loss of the consumed biomass (Toth et al. 2007), may increase tissue loss due to weakening and breakage of the thallus (Viejo and Åberg 2003; Toth and Pavia 2006), increase pathogenic infections through grazing wounds, and decrease growth rates because of partial dependency of meristematic areas on the total thallus area (Schmitz and Lobban 1976; Honkanen and Jormalainen 2002). Grazing pressure can sometimes be extremely strong, as shown, for example, by extensive defoliations of *F. vesiculosus* belts by the isopod *Idotea baltica* in the Baltic Sea (see Sect. 3.5) or by deforestations of kelp communities by

sea urchins (reviewed in Steneck et al. 2002). Thus, grazing can be assumed to be a major selective agent for macroalgal traits.

A number of hypotheses have been proposed as frameworks for the study of patterns of plant resistance to herbivory (reviewed in Cronin 2001; Stamp 2003). The fundamental objective of these hypotheses is to explain and understand the variability in plants' allocation patterns among survival and other needs, growth and reproduction (Herms and Mattson 1992). There are excellent reviews on the utility and application, and also on controversies concerning these hypotheses (Cronin 2001; Hamilton et al. 2001; Koricheva 2002b; Lerdau and Coley 2002; Stamp 2003; Amsler and Fairhead 2006; see Chap. 7), and so we guide interested readers to those sources. Here, we adopt the most general framework, the optimal defense theory (Feeny 1976; Rhoades 1979), as the contextual frame for reviewing the empirical research on macroalgal chemical defenses.

The optimal defense theory provides an adaptive explanation for the evolution of defenses. It explains the variability in defense allocation patterns among-species, within-species, and within-individuals on the basis of fitness maximization (reviewed in Stamp 2003). Several kinds of defensive patterns, depending on the variation and intensity of herbivory, can be adaptive. According to the theory, defenses are assumed to be costly because they divert resources from other needs, and these costs fall in the absence of herbivores. In addition to this "allocation cost," defense costs often manifest themselves in interactions with other enemies or competitors, referred to as "ecological" or "opportunity" costs, respectively (Koricheva 2002a). Because the assumption of costs is fundamental to the optimization of defenses, costs have been measured extensively; highly variable results suggest that the expression of costs is condition-dependent (reviewed in Bergelson and Purrington 1996; Koricheva 2002a; Strauss et al. 2002).

Costs of defenses have been measured in macroalgae too. Through a meta-analysis of seven published studies, it has been found that the phenotypic correlations between antiherbivore defense and algal fitness are, on average, negative (Koricheva 2002a). Thus, there is some support for the cost of defense in macroalgae, although it is restricted to only one type of secondary metabolite, brown algal phlorotannins, and mainly to phenotypic relationships. In a rare example of a correlation between growth and defensive metabolites at the genetic level, Dworjanyn et al. (2006) found a positive genetic correlation between growth and furanone concentration in the red alga *Delisea pulchra*, but a negative one between fecundity and furanones. Thus, the expression of the allocation cost may also depend on the fitness component measured (Dworjanyn et al. 2006).

Evolution of chemical defenses according to the optimal defense theory presumes, in addition to costly defenses, that there is genetic variation for the defensive metabolites, that herbivory is the major selective agent for such metabolites, and that the chemical trait in question is efficient in reducing herbivory (Stamp 2003). Research on macroalgal chemical defenses has strongly emphasized the last precondition, which has mainly been studied by testing the deterrence effects of secondary metabolites in bioassays. The defensive role for the trait has been assumed on the basis of deterrence it provides. Very little research on the first two

preconditions exists. Genetic variation of defensive metabolites has been well documented in terrestrial plants (Berenbaum and Zangerl 1992), but, in macroalgae, it has been quantified in two cases only: in furanones of the red alga *D. pulchra* (Wright et al. 2004) and in phlorotannins of the brown alga *F. vesiculosus* (see Sect. 3.5). Thus, the existence of heritable variation in concentrations of secondary compounds, and the consequent potential for their evolutionary responses to natural selection, has been documented also in macroalgae.

Owing to the costs of defenses, natural selection will optimize defenses. There are various aspects in this optimization, including defense allocation with respect to temporal variation in grazing pressure and within-plant defense allocation with respect to grazing risk and the value of different plant parts (reviewed in Stamp 2003). Here we will focus on both the induced defenses and within-plant allocation of defenses, because these aspects have been widely studied in macroalgae.

3.2.1 Induced Defenses Against Herbivory

Defense allocation can be divided into the defenses constantly produced at effective levels, called constitutive defenses, or those produced at active concentrations only upon a cue indicating an increased risk of becoming grazed, called induced defenses. A sufficient cue can be the grazing damage itself. The selective environment determines the benefits of constitutive versus induced defenses. Constitutive defenses are beneficial when herbivores are more or less constantly present, but on the other hand, there is a cost of maintaining the defense at a high constitutive level. When predation pressure varies temporally, induced defenses are beneficial and less costly, because they are generated only when needed and relaxed when grazing ceases (Karban and Baldwin 1997). High overall herbivory selects for constitutive defenses while low or temporally variable herbivory selects for inducible defenses (Karban and Baldwin 1997). Constitutive and induced defenses can be alternatives, but they do not need to be mutually exclusive; there can be a certain constitutive level of defenses, above which the induced defense rapidly rises when needed (Stamp 2003). Macroalgae also possess so called activated defenses (see Chap. 8), where the damage as such results in the conversion of a stored secondary metabolite into a product with higher feeding deterrent potent. These are basically constitutive defenses because the precursors are constantly stored in the thallus.

Marine mesograzers are mobile, generalist feeders, which can occur at high densities and are capable of exerting intense grazing pressure on the host algae (Hay 1991). Mesograzers have been hypothesized to select for induced rather than constitutive defenses because this kind of consumer feeds over temporal and spatial scales that would allow induced responses to be effective (Hay 1996). So far, evidence for this hypothesis has been mixed; initially very few studies on macroalgae supported the existence of inducible responses (reviewed in Hay 1996; Cronin 2001), but recently, observations on inducible responses have started to accumulate (reviewed in Ianora et al. 2006).

Meta-analysis of Induced Defenses in Macroalgae

In order to summarize knowledge on induced defenses in macroalgae, we conducted a quantitative synthesis of the published research by means of meta-analyses (Gurevitch and Hedges 1993). The existing studies in macroalgae allowed us to compile two induction databases: one for the studies where induced resistance was tested using herbivore feeding preference in a bioassay, and one for the studies where induction of phenolic compounds was measured. We concentrated on phenolic compounds, since they are the most extensively studied secondary compounds in macroalgae, and thus enough studies for a meaningful meta-analysis exist. For the feeding preference database, we used meta-analyses to assess (1) whether natural herbivory and artificial clipping of macroalgae differ as inducers, (2) whether the occurrence of the induction differs between test situations where the induced and the control algae were offered to herbivores either as fresh algae or as powdered algae embedded in agar, (3) whether the occurrence of induction differs among the herbivore taxa (Gastropoda, Isopoda, Amphipoda, and Echinoidea) used as inducers and in the subsequent bioassay, and (4) whether the induced responses differ among the algal taxa (brown, red, and green algae). For the phenol induction database, we analyzed (5) whether there is an overall significant effect of induction treatment on the subsequent concentration of phenolic compounds and (6) whether natural herbivory and artificial clipping differ as inducers. Because the statistical significance of the average effect size of phenolics induction was only marginal, we conducted a cumulative meta-analysis of the studies to evaluate (7) whether the average effect size has converged and stabilized to the current level.

The databases were compiled by conducting key-word searches in the Science Citation Index, and by examining the literature-cited sections of the papers obtained. The phenolic database consisted of 22 papers (22 indicated with "*" in the reference list) available in the literature in early 2007 (and one submitted study of our own), and a total of 48 records were derived from those studies. Accordingly, the preference database consisted of 18 papers (indicated with "#" in the reference list) and a total of 86 records. The majority of the studies were conducted on cold or warm temperate species, but we did include also the two existing studies on tropical species and one study on Antartic species. We did not include studies on activated defenses because we considered those as constitutive defenses. In order to ensure independency of the records derived from the same study, we applied the following rules when selecting the data from each study: (1) When results from several algal and/or herbivore species were reported in the same study, we included the data for each algal and/or herbivore species. (2) When several levels of grazing/clipping were applied within the same study, we used the most severe treatment. (3) When results were presented for several sampling dates, we chose the measurement resulting in the largest effect size. (4) When several parts of the same species were tested in the same study, we chose only the part showing the largest effect size. (5) When the same control group was used for several induction treatments, we only used the treatment showing the largest effect size.

For each study and response variable, we calculated an estimate of the magnitude of the induced response (effect size, Hedge's *d*) as the difference between the

means of the experimental and control groups divided by the pooled standard deviation, and weighted by a correction term that eliminates small-sample-size bias (Gurevitch and Hedges 1993). We used the MetaWin statistical program (Rosenberg et al. 2000) to calculate the mean effect sizes and their confidence intervals for the pooled data and the earlier-mentioned fixed subgroups. The mean effect size was considered to be statistically significant if the 95% confidence interval of the mean effect size did not overlap 0 (Gurevitch and Hedges 1993). The homogeneity of the results from different studies was evaluated on the basis of the within-group heterogeneity statistics (Q_W) and the differences between the fixed groups by calculating the between-group heterogeneity (Q_B) and testing them against the χ^2 distribution (Gurevitch and Hedges 1993). To control for the publication bias toward statistically significant differences, we used the fail-safe number (Rosenthal's method) calculated by the MetaWin. A fail-safe number is the number of nonsignificant, unpublished, or missing studies that need to be added to the meta-analysis in order to change its result from a significant to a nonsignificant one.

Meta-analysis Outcomes

On the basis of a total of 86 tests in 40 algal species that used herbivore feeding preferences to indicate induction, we found a statistically significant induced resistance of a moderate magnitude (Fig. 3.1). Negative effect sizes indicate decreased preference for induced algae, i.e., induction of deterrence. The fail-safe number for the pooled mean effect size was high, indicating that the meta-analysis result is insensitive to a possible publication bias or shortage of studies. However, the different studies had significantly heterogeneous effect sizes (Fig. 3.1), which justified the subgrouping of the studies and the search for differences among them. Both natural herbivory and artificial clipping significantly decreased the preference of herbivores; the induction of resistance was stronger in the group of artificially induced algae than in the one with natural induction (Fig. 3.1a). Similarly, induced resistance was found both in bioassays conducted using fresh algae and in algae embedded in agar-matrix, and the effect tended to be stronger when fresh algae were used (Fig. 3.1b). The type of the herbivore did not significantly explain the variation of the effect sizes. However, the mean effect-size implied statistically significant induced resistance only in studies using amphipod mesograzers (Fig. 3.1c). Because induction itself was independent of whether the cue was artificial or natural, we interpret this indicating that amphipod grazers were the most sensitive to the induced resistance. Comparison among the main algal taxa, brown, red, and green algae, revealed that brown algae showed a clear induced resistance while red and green algae did not show statistically significant induction (Fig 3.1d). The main results from a recently published meta-analysis on induced resistance in macroalgae (Toth and Pavia 2007), which used more permissive criteria for including records into the analysis, paralleled the ones reported here.

Meta-analysis of 48 tests, where induction of phenolic compounds after artificial or natural wounding was measured, revealed a marginally significant, positive mean effect size (Fig. 3.2a). Positive effect sizes indicate larger concentrations of phenolics in the wounded algae, i.e., induction of phenolics. The mean effect sizes

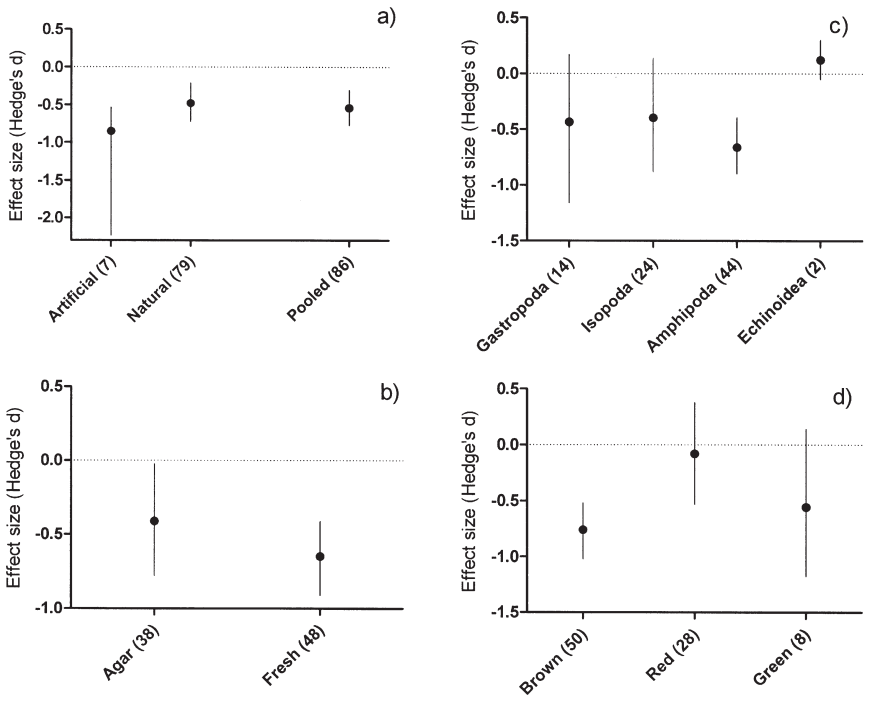

Fig. 3.1 Effect sizes (Hedge's *d*; mean ± bootstrapped 95% confidence intervals) from the meta-analysis of induced resistance in macroalgae. Induced resistance was inferred on the basis of feeding deterrence of grazers in preference bioassays. Negative effect sizes indicate preference for the control over the previously induced algae, i.e., existence of induced resistance. Numbers in parentheses along the horizontal axis indicate the number of tests. (**a**) Comparison of induction between studies using either artificial or natural induction cues. Total heterogeneity: $Q_T = 426$, df = 85, $P < 0.0001$; between-group heterogeneity: $Q_B = 5.06$, df = 1, $P < 0.05$; within-group heterogeneities: $Q_{W(artificial)} = 45.9$, df = 6, $P < 0.0001$; $Q_{W(natural)} = 375$, df = 78, $P < 0.0001$; fail-safe number for the pooled effect size = 3,506. (**b**) Comparison of induction detected in bioassays using either algal extracts embedded in agar-matrix or fresh algae. Between-group heterogeneity: $Q_B = 3.52$, df = 1, P = 0.06; within-group heterogeneities: $Q_{W(Agar)} = 180$, df = 37, $P < 0.0001$; $Q_{W(fresh)} = 242$, df = 47, $P < 0.0001$. (**c**) Comparison of induction among studies conducted using gastropods, isopods, amphipods, or echinoids as inducers and grazers. Between-group heterogeneity: $Q_B = 5.20$, df = 3, NS; within-group heterogeneities: $Q_{W(Gastropoda)} = 120$, df = 13, $P < 0.0001$; $Q_{W(Isopoda)} = 157$, df = 23, $P < 0.0001$; $Q_{W(Amphipoda)} = 140$, df = 43, $P < 0.0001$; $Q_{W(Echinoidea)} = 0.09$, df = 1, NS. (**d**) Comparison of induction among studies conducted using brown, red, and green algae. Between-group heterogeneity: $Q_B = 23$, df = 2, $P < 0.0001$; within-group heterogeneities: $Q_{W(brown)} = 276$, df = 49, $P < 0.0001$; $Q_{W(red)} = 106$, df = 27, $P < 0.0001$; $Q_{W(green)} = 20.0$, df = 7, $P < 0.01$

did not differ between the tests where the induction was conducted manually or by using natural grazers (Fig. 3.2a). However, even within the groups of artificial and manual clipping, effect sizes were heterogeneous among the studies (Fig. 3.2a). In particular, studies on kelps had highly variable responses to induction treatment: in different species, both 40% decreases (Toth and Pavia 2002b) and 90% increases (Hammerström et al. 1998) in phlorotannins were found. The cumulative analysis

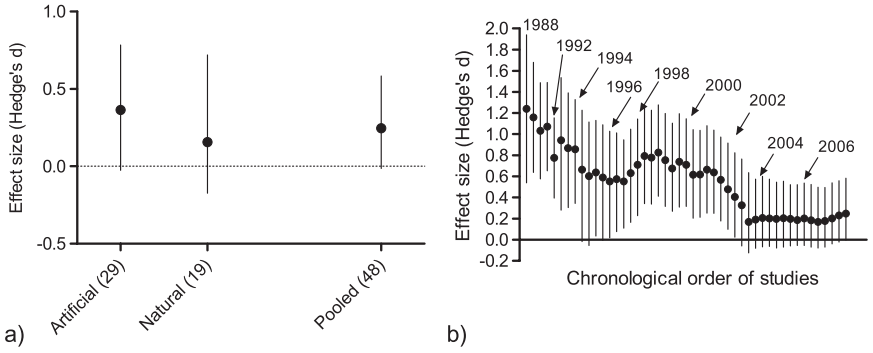

Fig. 3.2 (**a**) Effect sizes (Hedge's *d*; mean ± bootstrapped 95% confidence intervals) from the meta-analysis of induced response in the concentration of phenolic metabolites in brown algae. Positive effect sizes indicate higher concentration of phenolics in the wounded than in control algae, i.e., induction of phenolics. Numbers in parentheses along the horizontal axis indicate the number of tests in studies using either artificial or natural induction cues. Total heterogeneity: $Q_T = 311$, df = 47, $P < 0.0001$; between-group heterogeneity: $Q_B = 2.82$, df = 1, P = 0.09; within-group heterogeneities: $Q_{W(artificial)} = 160$, df = 28, $P < 0.0001$; $Q_{W(natural)} = 148$, df = 18, $P < 0.0001$. (**b**) Cumulative meta-analysis of induced response in the concentration of phenolic metabolites in brown algae. Studies are arranged in chronological order (within publication years, in random order). Confidence intervals represent parametric 95% confidence limits for the first five steps in the analysis and bootstrapped 95% confidence limits for steps 6–46

of the induction of phenolic compounds revealed high induction levels reported in the earliest studies, followed by a decreasing trend in the cumulative mean effect sizes and stabilization at the current level during the last five years (Fig. 3.2b). The effect size remaining continuously positive and its tendency to stabilize provide further evidence for the generality of the phlorotannin induction. The studies on phlorotannin induction involved a total of 15 brown algal species.

Cronin (2001) concluded that there are only few cases of induction of chemical defenses in seaweeds. However, both our analysis here and the meta-analysis by Toth and Pavia (2007) showed that, as among terrestrial plants, induced resistance is common in macroalgae. Although vascular terrestrial plants and nonvascular seaweeds have different biologies (e.g., translocation systems in seaweeds are probably not as efficient as those in vascular plants), induced responses to herbivory seem to occur similarly. It has been suggested that physical damage alone would be too unspecific a cue to induce resistance and, to get the induction, a natural grazer would be needed (Pavia and Toth 2000; reviewed in Amsler 2001; Borell et al. 2004). Such a natural cue could be for example salivary enzymes secreted by snails, which have been shown to induce resistance to grazing in *Ascophyllum nodosum* (Coleman et al. 2007b). However, induced resistance occurred in the current data set independently of whether the induction was done manually or by natural grazers, indicating that a nonspecific cue is often sufficient for the induction. We found differences in the induced response among the algal taxa: only brown algae displayed clear induced resistance. Although this may indicate real differences among taxa, we cannot exclude the possibility that the result reflects the amount of available tests, where particularly the green algae are poorly represented.

The studies of induced defenses in macroalgae have most often analyzed the induction of the previously grazed or artificially wounded part of the thallus, i.e., local induction. Local response at the site of wounding has also been nicely documented in the brown alga *Ecklonia radiata*. In this species, small-scale accumulation of phlorotannin vesicles occurs within a few days following wounding (Lüder and Clayton 2004). This indicates that phlorotannins contribute to wound healing and may provide resistance to further grazing. However, spreading of induction from the point of damage to the whole plant would be potentially beneficial, as it could diminish biomass loss to herbivory. Studies on the spreading of induction have found variable patterns, from lack of spreading (Hemmi et al. 2004) to spreading to adjacent undamaged branches (Van Alstyne 1988) or to neighboring individuals (Toth and Pavia 2000). However, even if the induced resistance were local, it can be beneficial because it may lead to spreading the damage instead of letting the grazer cut through the thallus, causing breakage and potentially large biomass loss (Hemmi et al. 2004).

To Help Yourself or Cry for Help?

Survival and continuity of littoral algal populations despite the intense grazing pressure may be due to their generally poor quality as food (e.g., due to high level of defenses), or, alternatively, due to regulation of grazer populations by higher trophic level organisms (e.g., Halpern et al. 2006; Korpinen et al. 2007b). Both bottom-up and top-down influences have been shown to be important in certain communities (reviewed in Hay 1996; Underwood 2000; Menge 2000; Shurin et al. 2002). Plant defenses against herbivory do not evolve in isolation; the selective environment includes the whole community ranging from parasites to carnivores. In multitrophic systems with strong trophic cascades, plants may obtain indirect defense from the enemy of their enemy. This may select for the evolution of plant traits that facilitate the enemies of herbivores when the plant is facing increased grazing (Hay et al. 2004). Such traits are referred to as indirect defenses. Evidence for them comes from terrestrial plant-herbivore systems (reviewed in Dicke and van Loon 2000; Dicke et al. 2003b; Cory and Hoover 2006). There the facilitation of herbivores' enemies (carnivores, parasites, and/or parasitoids) is usually based on volatile (Dudareva et al. 2006) signals of grazing. While the terrestrial studies have shown that enemies of herbivores can and do respond to plant volatiles and that plants can thereby gain indirect defense, it has remained more controversial whether plants themselves can respond to the plant-emitted or other long-distance cues indicative of grazing (Dicke et al. 2003a; Kost and Heil 2006).

Studies on temperate macroalgae have tested the responses of algae to waterborne cues of grazing, with interesting but inconsistent results. The study by Toth and Pavia (2000) in the brown alga *A. nodosum* and the periwinkle *Littorina obtusata* was the first to suggest that seaweeds could respond to such grazing cues. They maintained *A. nodosum* in through-flow aquaria that received water containing the grazing cue from other aquaria containing periwinkles grazing on conspecifics.

In those algae, a 15% higher phlorotannin concentration and deterrence to periwinkle grazing were found, indicating induced resistance. The nature of the cue, whether it originated from periwinkles, algae, or some change in water quality associated with the presence of periwinkles, could not be determined. Another study using a similar approach in a different fucoid species, *Sargassum filipendula,* and an amphipod grazer, did not find any evidence of an induced response to indirect grazing cues (Sotka et al. 2002). However, resistance did induce as a response to direct grazing (Sotka et al. 2002). The next study, again in a fucoid species, *F. vesiculosus*, found that an indirect cue from grazing of the neighboring individuals by the isopod *I. baltica* induced resistance but that by the gastropod *Littorina littorea* did not (Rohde et al. 2004). Direct grazing of both herbivore species induced resistance. The presence of the grazer alone did not cause the response, and so the cue had to originate from the grazing activity as such or from the wounded algae. Induction was measured on the basis of feeding preferences in bioassays and thus could not be traced to changes in concentration of any specific metabolite. In addition to these studies, inducible resistance based on water-borne cues from the presence of grazers has been found in the brown alga *Glossophora kunthii* (Macaya et al. 2005) and possibly in the green alga *Codium platylobium* (Diaz et al. 2006), but, although tested, was not found in *Codium decortatum* (Weidner et al. 2004).

The earlier-mentioned studies provide some support for the idea that macroalgae are able to respond to water-borne cues of grazing by inducing resistance. However, all the evidence comes from small-scale laboratory experiments, and a critical test of whether such responses occur in natural populations is missing (see Toth and Pavia 2000). Also, the origin and the chemical nature of the cues remain ambiguous. It is clear that water-borne substances are both passively released and actively exuded from macroalgae (Sieburt and Jensen 1969; Ragan and Jensen 1979; Swanson and Druehl 2002; Koivikko et al. 2005) and such compounds may well have importance with respect to natural enemies. Tritrophic interactions, positive responses of carnivores and parasites to algal exudates, may be one potential role for such exuded metabolites. A recent study by Coleman et al. (2007a) showed that two predator species, a crab and a fish, are able to sense cues from grazing of *A. nodosum* by the snail *L. obtusata*, thus highlighting the importance of tritrophic interactions. Furthermore, most mesograzers are mobile enough to be able to respond to water-borne cues from algae or grazing. Further research on ecological roles of water-borne substances from macroalgae is needed and may provide interesting new insights into the interaction of bottom-up and top-down regulation of algal communities.

3.2.2 Within-Plant Variation in Defenses: Watch Your Valuables!

The optimal defense theory predicts that the benefit/cost ratio of defenses will be maximized by natural selection. When a plant consists of different kinds of tissues, within-plant variation in defenses is predicted to depend on the value of the plant part for fitness and on its risk of becoming grazed. The more valuable the tissue is

in contributing to plant fitness and the higher its grazing risk, the higher the predicted level of defenses (Rhoades 1979). This prediction particularly concerns the level of constitutive defenses. The prediction on the strength of inducible defenses is equivocal; highly inducible defenses have been suggested to occur not only in the less valuable or low-grazing-risk parts (Zangerl and Rutledge 1996) but also in the highly valuable parts (Toth et al. 2005).

Conventionally, meristematic tissues and reproductive organs have been considered valuable for fitness because of their straightforward contribution to growth and reproduction (e.g., Cronin and Hay 1996; Van Alstyne et al. 1999; Stamp 2003). However, defining the fitness value of different plant parts is a difficult task and seldom done. Terrestrial plants are especially difficult in this respect because they have highly differentiated tissues and the risk of herbivory below and above ground is very different. Macroalgae are a good model system for studying within-plant variation in defenses because they lack the below ground root system, the risk of grazing for the different parts is more uniform owing to the prevalence of mobile generalist grazers (Taylor et al. 2002), and the different tissues often differ in their contents of secondary metabolites (Tuomi et al. 1989; Hammerström et al. 1998; Van Alstyne et al. 1999; Pavia et al. 2002). Studies on macroalgae have shown that the conventional reasoning concerning fitness values of different tissues is too simplistic.

Pavia et al. (2002) applied population matrix models, so-called sensitivity analysis, to determine the fitness values for different parts of the brown alga *A. nodosum*. The analysis gives the relative contribution of different, predetermined life-cycle stages to the population growth rate. By assuming that the contributions of different life-history processes, growth, stasis, and fertility, can be applied to the specific parts of the plant – annual shoots, stipes and receptacles, respectively – they were able to quantify the fitness value of each algal part. On the basis of these determined fitness values and the optimal defense theory, they predicted the defense level being highest in the stipes, intermediate in annual shoots, and lowest in receptacles. They tested this prediction by determining phlorotannin concentrations and feeding preferences of the gastropod *L. obtusata* for the different parts. The results supported the prediction: the level of resistance, on the basis of both phlorotannin concentration and feeding deterrence, was the highest in stipes, intermediate in annual shoots, and lowest in receptacles. These differences represented constitutive levels of defenses. However, in *A. nodosum*, the phlorotannin concentration increases as an induced response to grazing and this induced resistance is also more pronounced in the stipes than in annual shoots (Toth et al. 2005). Comparable differences among different thallus parts in their phlorotannin contents, feeding deterrence, and quality for herbivores have also been found in another fucoid, *F. vesiculosus* (see Sect. 3.5).

Although it may seem contradictory that reproductive organs have lower fitness value than stipes, the morphology of fucoids can explain the pattern: the high fitness value of stipes is based on their role in supporting the whole plant. In fucoids with a dichotomous branching pattern, the number of supported apices increases exponentially with the number of passed branching points when moving from the apex toward the holdfast. Because each apical meristem can either continue to grow or produce a reproductive organ, fitness of an alga is closely coupled with the number apices (Jormalainen and Honkanen 2004) and, consequently, the fitness

value of the supporting thallus increases with its age. This also holds true for other growth patterns as long as growth is based on apical meristems. Thus, observations of higher levels of feeding deterrent secondary metabolites in older parts of a thallus, or in stipes or holdfasts, than in young or reproductive parts (Hammerström et al. 1998; Hemmi et al. 2005; Fairhead et al. 2005; Connan et al. 2006) fit well with predictions from the optimal defense theory. However, there are also cases with no such differences (Toth and Pavia 2002a; Toth and Pavia 2002b) or with opposite patterns (Amade and Lemee 1998; Van Alstyne et al. 1999). In the Laminariales, where the meristematic region is basal and supports the blade that provides photosynthates for the meristem (Schmitz and Lobban 1976), meristematic regions also often contain high concentrations of phlorotannins (Van Alstyne et al. 1999; Toth and Pavia 2002b).

The issue of within-plant distributions of chemical resistance is complicated by the variety of traits providing defense; structural defenses may often be involved as constitutive defenses of the supporting structures. For example, chemical and structural traits contribute to the defense of different tissues in the brown seaweed *S. filipendula*. Taylor et al. (2002) studied variation in resistance to amphipod feeding among apical blades, basal blades, and stipes of *S. filipendula*. They found that the apical blades were the least resistant to grazing, apical stipes and basal blades intermediate, and basal stipes the most resistant. They reasoned that the basal stipes were defended constitutively by virtue of their toughness rather than by deterrent chemistry. On the other hand, apical stipes, which contain the meristematic tissue responsible for future growth, showed induced chemical defenses. In this case, the within-plant resistance pattern with high constitutive resistance in the most valuable basal stipes was consistent with predictions of the optimal defense theory.

3.3 Consequences of Algal Defenses to Grazers

3.3.1 From Defenses to Herbivore Population Dynamics

Population dynamic consequences of algal defenses on grazers have not been directly studied, but the quality of food is assumed, and also shown, to affect herbivore performance. For example, eutrophication-related changes in the quality of host algae have been shown to increase growth and reproduction of crustacean mesograzers (Hemmi and Jormalainen 2002; Hemmi and Jormalainen 2004a; Kraufvelin et al. 2006). Such studies have highlighted the effects of intraspecific variation in host quality on herbivore performance and thus the potential of bottom-up regulation of mesograzer populations via variation in host quality. Faster maturation and higher reproductive effort of grazers found in the studies are likely to transform to a higher population growth rate. This, in turn, will lead to increased grazing pressure on aquatic macrophytes, which has also been found in some cases (see Sect. 3.5). Although these studies were not focused on determining the chemical basis of the variation in algal quality, they revealed the potential

influence that variations in chemical quality of algae may have on mesograzer performance and population dynamics.

Although the feeding preferences of grazers with respect to both induced responses (e.g., Fig. 3.1) and variation in the content of secondary metabolites (reviewed in Paul et al. 2001) have been extensively studied, very few studies on fitness consequences of these on marine grazers exist. In an alga-grazer interaction, the consequences of induced resistance on herbivore fitness have been measured in only one study: Toth et al. (2005) showed that induced resistance in *A. nodosum*, which is based on phlorotannins, decreased the egg production of the periwinkle *L. obtusata*. Thus, in this system, induced resistance affects both the preference (Pavia et al. 2002) and performance of the grazer and may thereby contribute to the regulation of the periwinkle population in a density-dependent way.

Macroalgal defenses are assumed to negatively affect consumer fitness, which is the basis for the evolution of feeding preferences. Some studies have illuminated the chemical basis of such fitness effects in herbivores. The most extensively studied are the effects of brown algal phlorotannins. Negative effects on consumer performance have been found repeatedly, although these are far from straightforward and depend upon the identity of both the grazer and alga species, polymer-size of the phlorotannins, and the nutrient content of the food (reviewed in Targett and Arnold 2001; Amsler and Fairhead 2006, see Sect. 3.5 for an example). Fewer studies exist on the effects of nonphenolic metabolites on grazer performance (reviewed in Paul et al. 2001). Of these, among the best known are the diterpene alcohols (dictyols) from the brown algal genus *Dictyota*; these reduced growth of a herbivorous fish (Hay et al. 1987) and decreased survival, growth, or reproduction of several amphipod species (Cruz-Rivera and Hay 2003). Also the fitness effects of dictyols are species-specific: the isopod *Paracerceis caudate*, for example, was not affected negatively by the same dictyols that were harmful to amphipods (Cruz-Rivera and Hay 2003).

3.3.2 Defenses as Selective Agents

In plant-herbivore interactions, both parties generate selection for each others traits leading to coevolution of the interaction. Herbivory selects for the defenses, while the defenses in turn select for the herbivores' ability to overcome them (Thompson 2005 and references therein). When the evolutionary potential of the herbivore is higher than that of the plant, e.g., owing to the shorter generation time, the herbivore is expected to evolve faster and may overcome the defense (Bergelson et al. 2001). Therefore, grazers may adapt to exploit even heavily defended hosts (e.g., Stachowicz and Hay 1996; Pavia et al. 1997; Jormalainen et al. 2001; Cruz-Rivera and Hay 2003), and local adaptations to exploit the available host assemblage may evolve (reviewed in Sotka 2005). Because of the coevolutionary nature of the interaction, efficiency of defenses and herbivores' ability to tolerate them must be seen in the context of the evolutionary history of the interacting species. This means that

a great deal of species specificity is expected, and also found (reviewed in Targett and Arnold 2001; Cruz-Rivera and Hay 2003). Instead of regarding such variability as an ambiguity in a metabolite's ecological role, it is more fruitful to consider it reflecting the specificity of adaptations and counter adaptations in the interactions being studied.

Generalism is the predominant strategy in marine mesograzers (Hay 1991); for example, amphipods are often actively seeking mixed diets (Poore and Hill 2006). One potential consequence of high levels of defensive secondary metabolites in algae may be forcing grazers towards mixed diets. By mixing several host species, toxic effects of a particular secondary metabolite can be diluted (Bernays and Minkenberg 1997). For example, this may be the strategy of the isopod *I. baltica*, the performance of which is better on a mixed diet than on the most preferred host alone (see Sect. 3.5). Studies on the fitness effects of single-host versus mixed diets show that mixed diet is either equally good as the best of the single-host diets provided (Cruz-Rivera and Hay 2000; Cruz-Rivera and Hay 2001; Hemmi and Jormalainen 2004a) or exceeds all the single-host diets provided (Pennings et al. 1993; Cruz-Rivera and Hay 2000). Although broad diets may also have benefits other than diluting secondary metabolites, e.g., balancing nutrition or ensuring food availability, we consider it likely that macroalgal chemical defenses contribute to the predominance of generalism in marine mesograzers.

Macroalgal chemical defenses may also encourage host-plant specialization. Relatively small-sized mesograzers are living between plants and predators, and the selection generated by predators may become important for the evolution of host associations. This led Hay et al. (1989) to suggest that chemically defended algae may serve as safe habitats for small-sized herbivores, enemy-free spaces against omnivorous predatory fishes, and that this may be a dominant factor selecting for specialization. This may explain why several motile mesograzers are closely associated with chemically defended algae (Hay 1992; Paul et al. 2001 and references therein) and it may further intensify selection for the herbivores' ability to tolerate the algal defenses. Thus, algal chemical defenses may affect the evolution of grazer feeding strategies in various ways.

Piggybacking Algal Defenses

An illuminating example of a community consequence of plant defensive metabolites, and of a grazer's adaptation to exploit its host plant, is the ability of some herbivores to sequester plant-derived secondary metabolites for their own defenses (e.g., Avila 1995; Marin and Ros 2004). In terrestrial systems, several specialist insects can sequester defensive chemicals from their host plants (e.g., Nishida 2002), but in aquatic plant-herbivore systems with a prevalence of generalist herbivores this is less common. However, sea hares (Anaspidea) and some other herbivorous opisthobranch molluscs are known to be able to sequester defenses from their algal foods (Avila 1995; Marin and Ros 2004). For the opisthobranch species that are known to obtain metabolites from their food, algae were the

second largest host group (26%), following sponges (49%) (Avila 1995). The sequestered compounds include mono-, di-, tri- and sesquiterpenes, steroids, halogenated furanones, nitrogenated compounds, and phenolics (Avila 1995; Rogers et al. 2000). Known algal hosts include mainly red and green algae, but also some brown algae and cyanobacteria (Avila 1995).

Sea hares have been suggested to use algal-derived compounds for defensive functions such as antimicrobial activity, chemical camouflage, and rendering themselves unpalatable to predators. The efficiency of diet-derived secondary metabolites in defense against predators has remained somewhat equivocal because in different studies the diet-derived metabolites have been both found (de Nys et al. 1996; Ginsburg and Paul 2001) and not found (Pennings et al. 2001; Rogers et al. 2002) to diminish susceptibility to predators. However, algal-derived metabolites have been localized in the following regions of the herbivore, consistent with a suggested role of defense: in mucous and opaline secretions (Rogers et al. 2000), within egg masses (Avila 1995), and within the ink opisthobranchs spray toward the attacking predator (Johnson et al. 2006).

In addition to opisthobranches, piggybacking of macroalgal defenses has been reported in the amphipod *Pseudamphithoides incurvaria*, which constructs a bivalved domicile with the pieces of thallus from the chemically defended brown alga *Dictyota bartayresii* (Hay et al. 1990). The amphipod uses this algal species for domicile construction even when the alga is rare. The alga produces fish-feeding deterrents and amphipods inside their domiciles are rejected by fish while amphipods removed from the domiciles are readily eaten (Hay et al. 1990).

3.4 Allelopathy in Space Competition and in Resisting Epibiotism

Competition for space is intense among benthic organisms (e.g., Menge 1991; Underwood 2000) and epibiotism is a manifestation of that. In marine environments, the space competitors include, in addition to macrophytes, sedentary animals and the periphytic community of microalgae, cyanobacteria, heterotrophic microbes, and protists. Allelopathy originally refers to the harmful effect of a plant upon another by secretion of toxic substances, allelochemicals. Allelochemicals are thus means to compete for space by interfering with settlement and/or growth of competitors. In aquatic environments, allelopathy must be extended to include negative influences between plants and nonautotrophs as well as antifouling substances (Gross 2003).

One of the best known examples of allelopathy between macroalgae in temperate systems is seen in crustose algae, in which allelopathy may be an important means to prevent overgrowth by canopy-forming macroalgae (Gross 2003). The crustose coralline alga, *Lithophyllum* spp., produces an allelopathic, nonpolar substance that destroys zoospores of the brown alga, *Laminaria religiosa* (Suzuki et al. 1998). Suzuki et al. (1998) suggested that the reduction of epiphytes due to allelopathy

may contribute to the predominance of crustose coralline algae in the coastal region of the Northern Japan Sea. In another study, extracts from the crustose coralline alga *Lithophyllum yessoense* inhibited spore settlement of 14, and germination of spores of 13, of the total of 17 tested macroalgal species (Kim et al. 2004). Kim et al. (2004) suggested that *L. yessoense* probably has multiple allelochemicals, including water-soluble exudates, to cope with macroalgal recruits.

Allelopathy may be involved in competitive success of bloom-forming macroalgae (Nelson et al. 2003; Råberg et al. 2005). Nelson et al. (2003) studied toxic properties of two common "green tide" seaweeds *Ulva fenestrata* and *Ulvaria obscura*. They found that zygote development of *Fucus gardneri* was inhibited, accumulation of epiphytic algae was suppressed, and development of oyster larvae was arrested by extracts from both the species. Furthermore, growth of *Ulva* and *Ulvaria* was inhibited by ecologically relevant concentrations of both intra- and interspecific extracts obtained by incubating sun-dried thalli either in seawater or buffer medium. The study suggested that exudates modify competitive interactions by inhibiting germination or development of algae and invertebrates and may thereby substantially alter community structure in the vicinity of ulvoid blooms (Nelson et al. 2003). Polar metabolites of *Ulva* species have also been found to depress growth of several epiphytic microalgae (Jin and Dong 2003; Nan et al. 2004; Jin et al. 2005).

Annual mass occurrences of the brown alga *Pilayella littoralis* take place in the eutrophic conditions of the Baltic Sea. When *P. littoralis* is present on the substrate prior to seeding of *F. vesiculosus* eggs, the recruitment of *F. vesiculosus* germlings is reduced (Råberg et al. 2005). Allelopathy may be involved as exudates from *P. littoralis* affect *F. vesiculosus* negatively: a low concentration of exudates reduce the attachment rate, while higher concentrations hamper germination and rhizoid development (Råberg et al. 2005). Such detrimental exudates may have ample community level effects through their release during the bloom and later during decomposition. For example, in addition to lowering recruit survival of canopy-forming macroalgae (Eklund et al. 2005; Råberg et al. 2005), intact and/or degrading filamentous algae may decrease survival of fish eggs (Aneer 1987) and reduce the abundance of benthic animals (Norkko and Bonsdorff 1996).

3.4.1 Epibiotism as a Natural Enemy

Epibiosis is ubiquitous in marine environments and may have major effects on the species involved (Wahl and Mark 1999). Epibiotism is generally considered harmful for the host alga for several reasons: epibiota compete with the host for light and nutrients, thereby decreasing growth and reproduction (D'Antonio 1985; Cebrian et al. 1999; Honkanen and Jormalainen 2005); they raise mortality by increasing drag (D'Antonio 1985; Hemmi et al. 2005); and they may attract grazers to the host alga (Bernstein and Jung 1979; Wahl and Hay 1995; Wahl et al. 1997). Such negative effects on macroalgal fitness can be expected to select for efficient antifouling defenses.

Research on antifouling defenses has focused mainly on identifying the mechanisms responsible for fouling resistance (reviewed in Davis et al. 1989; Steinberg and de Nys 2002). Chemical resistance against various epibiotic organisms, e.g., bacteria, microalgae, periphyton, fungi, invertebrate larvae, and epiphytic macroalgae, has been suggested for all major macroalgal groups (see Chaps. 11 and 12). In a typical test of chemical antifouling, the potential epibionts are exposed to a crude extract, polar or nonpolar, in a bioassay in the laboratory and the settlement, growth, or survival of the epibionts is monitored. Typically, negative effects of macroalgal extracts on epibiota have been found. The metabolite responsible for the resistance to epibiotism has been separated and identified in a minority of these studies (see Chaps. 11, 12).

Although a wealth of examples on antifouling properties of algal extracts exists, the generality of antifouling metabolites has been questioned. A screening of nonpolar secondary metabolites extracted from the thallus surface of 12 macroalgal species did not support the ubiquitous role of such metabolites as antifoulants: metabolites from only one species, *D. pulchra*, deterred the bryozoan larvae that were used to test the antifouling role of the compounds (Steinberg et al. 2001). In order to be effective in providing resistance to surface colonization in the ecological context, the active compound must be a nonpolar, surface active metabolite, or if it is a polar metabolite, it has to be continually exuded into surrounding water (Steinberg and de Nys 2002; Harder et al. 2004; Nylund 2005). The problem with most of the existing studies is that, although they clearly show macroalgae commonly containing metabolites with antifouling properties, it is not known whether the compounds have an antifouling role in the real ecological interaction with their natural enemies (Steinberg et al. 2001). The metabolites may have evolved in other contexts, e.g., as antiherbivory compounds, and their antifouling properties may be artifacts generated by breaking-up the cell structure and exposing the compounds to fouling organisms. For example, in the red alga *Delesseria sanguinea*, crude extracts decrease settlement in assays where substrates are coated with metabolites even though the intact alga does not inhibit settlement (Nylund and Pavia 2003). Although the ecologically relevant examples of natural antifoulants are still few, their number is increasing (see Chap. 11), indicating that chemical characteristics of macroalgae do affect the epibiotic community.

Fouling resistance may not be an interaction between the host alga and the eukaryotic epibionts alone, but the microbial community on the thallus may influence the propensity of becoming fouled. Many marine macrofoulers, e.g., invertebrate larvae and algal spores, use biofilm properties as indicators of substratum suitability (Wieczorek and Todd 1998; Patel et al. 2003; Paul et al. 2006). Furthermore, some bacteria are known to produce metabolites with antifouling activity (Boyd et al. 1999; Dobretsov et al. 2006b). Therefore, capability to inhibit or modify the very first fouling stages by bacteria may be especially important, as it may prevent or reduce successive epibiotism. Macroalgae commonly contain antimicrobial metabolites (e.g., Hellio et al. 2001) and the microbial community on algal frond surfaces has been found to differ from that of inanimate, undefended substrates (Dobretsov et al. 2006a), indicating that macroalgae may be able to

affect the formation of bacterial biofilm and thereby influencing successive fouling by macroorganisms. Oxidative bursts, i.e., rapid production of active oxygen species such as hydrogen peroxide and hydroxyl radicals from cells when they come into contact with pathogens, may be involved in encounters with epiphytic bacteria, fungi, or other organisms (reviewed in Steinberg and de Nys 2002; Potin et al. 2002; see also Chap. 12). Thus, antifouling strategies of marine macroalgae may not be based on constitutive defense strategies and secondary metabolites alone, but may involve rapid responses following challenge by epibiotic organisms.

Despite the ubiquitous occurrence of epibiotic interactions in aquatic communities, the ecological and evolutionary consequences of epibiotism have remained little studied. For example, we lack a comprehensive view of the strength of selection for antifouling defenses because demonstrations of direct or indirect effects of epibiota on the host are still rare (Steinberg et al. 2001). Furthermore, in order to demonstrate evolution of any putative antifouling trait by natural selection, the existence of genetic variation and heritability of the trait and its relationship with host fitness should be shown. So far, this has not been done for any presumed antifouling trait. The only case we are aware of where genetic variation for resistance to epibiotism has been shown is in the brown alga *F. vesiculosus* (see Sect. 3.5). The role of epibiotism in affecting macroalgal communities is increasing in many coastal areas and estuaries because of anthropogenic eutrophication (e.g., Rönnberg and Bonsdorff 2004; Russell et al. 2005). Therefore, understanding the ecology and evolution of macroalgal antifouling strategies has become increasingly important. When the trait provides resistance to biofoulers in nature, it is realistic to assume that the trait has evolved under selection generated by the epibiotic organisms. However, understanding the evolution of antifouling traits requires information on genetic variation of and selection for the presumed resistance traits.

3.4.2 Community Context Matters: Interactions among Hosts, Epibiota, and Grazers

The consequences of epibiotism for the host algae are not necessarily negative, but instead depend on the community context. For example, a nonobligate, mutualistic relationship between seaweeds and gastropod grazers is well known: Stachowicz and Whitlatch (2005) studied the effects of the gastropods *Anachis lafresnayi* and *Mitrella lunata* on the red alga *Chondrus crispus*. These snails are commonly associated with the alga in southern New England. The abundance of *C. crispus* depends positively on the occurrence of these snails. Furthermore, the amount of epibiota on the host alga decreases with the abundance of the snails. An experimental removal of snails resulted in an increase of epibiotism and loss of algal biomass. In this study, the benefits of the two snail species were complementary because the species removed different epibiotic organisms. Such positive effects of fouling removal by snails on the host alga may be of considerable magnitude. For example, when *Theodoxus fluviatilis* snails removed epibiota from the brown alga *F. vesiculosus* in a mesocosm experiment, the growth rate of the host alga doubled (Honkanen and

Jormalainen 2005). Other examples of such positive effects of epibiont removal by grazers include the prevention of overgrowth of the crustose alga *Ralfsia verrucosa* (McQuaid and Froneman 1993) and elimination of epibiota on the red alga *Gracilaria verrucosa* (Mancinelli and Rossi 2001). Thus, in many cases grazers, often gastropods, can drastically counteract the negative influences of epibiotism.

Epibiota on the host alga may modify the host's susceptibility to herbivores such that it would differ from susceptibility without the epibiota. Epibiota may either decrease or increase susceptibility to herbivores. Wahl and Hay (1995) took the host species as the reference point, and classified epibiosis-caused decreases in herbivory as "associational resistance" and epibiosis-caused increases in herbivory as "shared doom." The same phenomena have also been called "protective coating" and "co-consumption," respectively (Karez et al. 2000). Both these epibiosis-related modifications of susceptibility to herbivory have been found in macroalgae. Wahl and Hay (1995) studied feeding preferences of the omnivorous sea urchin, *Arbacia punctulata*, among several hosts and epiphytic algae, and compared how different epibiotic species on the host modify the preference for the host. The preference for the host-epibiont association was different from that for the nonfouled algae in most cases. When epibionts were more attractive than their host, consumer pressure on the host increased. When epibionts were less attractive than their host or repellent, consumer pressure on the host decreased. Thus, the rank of the epibiont in the preference hierarchy compared to that of the host-modified grazing pressure on the host, high ranking epibionts increasing consumer pressure and low ranking ones decreasing it. Similar effects of epibiosis on susceptibility to predation have been found in marine invertebrates (Wahl et al. 1997 and references therein). Karez et al. (2000) studied the effect of an epiphytic brown alga, *Elachista fucicola*, on grazing pressure on the host *F. vesiculosus*. The amphipod *Gammarus locusta* was behaviorally deterred by *E. fucicola*, and therefore the epiphyte provided some protection for the host against this grazer. The isopod *Idotea granulosa*, on the contrary, was attracted to the epiphyte, and also consumption of the host increased when the epibiont was present. The study showed that the role of epibionts in affecting grazing pressure depends on the grazer species. Furthermore, the host-epibiont-grazer interactions vary with the identity of the host and epibiont species. Pavia et al. (1999) studied the habitat and food preferences of the same two grazer species, but used another fucoid host, *A. nodosum*, with different epiphyte species, the brown alga *P. littoralis* and the red alga *Ceramium nodulosum*. They found that both the grazer species were much more abundant on epiphytized hosts and that the grazers fed on both the host and the epiphytes, suggesting that epiphytism increased grazing of the host. Studies on the role of bryozoan epifauna in affecting susceptibility of kelps for herbivores further highlight the species specificity of such influences: Encrustation of juveniles of the giant kelp *Macrocystis pyrifera* by *Membranipora membranacea* increases grazing on the host by the omnivorous fish *Oxyjulis californica* (Bernstein and Jung 1979). On the contrary, the bryozoan *Lichenopora novaezelandiae* growing on the kelp *Agarum fimbriatum* deters herbivory by the snail *Tegula pulligo* (Durante and Chia 1991). Thus, the role of epibiota in modifying susceptibility of the host to grazing can be highly variable depending on the species identities of the parties, which makes generalizations on

the prevalence of associational resistance versus shared doom in host-epibiont interactions unattainable.

In these examples, the most likely explanation for the changes in the susceptibility of the host was the herbivore food preference pattern for epibiota, and consequent co-consumption or refusal to eat the host. Co-consumption can be expected when the herbivore is a generalist feeder, as are most marine mesograzers (Hay 1991). However, it is possible that epibiotism changes the quality of the host so that it becomes a better and more preferred food for herbivores. For example, in *F. vesiculosus* fouling load on the thallus causes a decrease in the concentration of phlorotannins (see Sect. 3.5). In this species, fouling load and the probability of becoming grazed by the isopod *I. baltica* are positively correlated (unpublished data by authors), and so co-consumption occurs, but we do not know whether this follows from the increased attraction due to epibiota or due to a change in the chemical quality of the host. Thus, critical tests of the mechanisms explaining co-consumption are needed; in particular, data on changes in host quality due to epibiota and consequent community consequences.

The community context where macroalgae are susceptible to multiple natural enemies, including herbivores and epibionts, and where the susceptibility to one enemy may depend on the occurrence of others, implies that the patterns of natural selection for resistance to epibiotism and resistance to herbivory may be correlated. When enemies tend to coexist on the same hosts, generalized defenses, i.e., traits that provide resistance to assorted enemies, are expected to evolve (Rausher 1996). There are some data supporting such multiple functions of macroalgal secondary metabolites: In *Dictyota menstrualis,* the diterpene alcohols pachydictyol A and dictyol E, which provide resistance to herbivores (reviewed in Hay and Fenical 1992), also prevent fouling organisms from colonizing the surface (Schmitt et al. 1995). Similarly, brown algal phlorotannins have been suggested to have multiple functions, e.g., to act both against herbivores and epibiota (reviewed in Amsler and Fairhead 2006). It is not known whether generalized, multiple-function defense traits or specialized defenses are more widespread among macroalgae. Owing to the highly variable coexistence patterns of enemies and the species specificity of the consequences of coexistence, generalizations may not be possible but variable patterns of resistance to multiple enemies are to be expected. What is clear is that epibiosis and grazing are often intertwined and that this may influence both community dynamics and the evolution of resistance traits.

3.5 Case Study of *F. vesiculosus* in the Eutrophic Northern Baltic Sea: Genotypically Variable, Plastic Phlorotannins as Chemical Defenses

In this section, we illustrate some patterns of variation in temperate macroalgal chemical ecology by focusing on one model system, the bladderwrack *F. vesiculosus* and its phlorotannins in the Northern Baltic Sea. In this region, *F. vesiculosus* is the

only perennial, belt-forming brown alga, which makes it a key species in creating habitat structure and providing shelter for invertebrate and fish fauna. The species has declined (from 1970s) because of eutrophication and associated changes in competitive interactions and grazing pressure (reviewed in Korpinen et al. 2007a). *F. vesiculosus* forms populations in the rocky littoral of the islands; pelagic areas spatially isolate these local populations. Competition with organisms benefiting from eutrophication, such as filamentous, fast-growing macroalgae, sessile fauna, and periphytic microorganisms, results in high loads of epibiota on the *F. vesiculosus* (Worm and Sommer 2000; Wikström and Kautsky 2004). Grazing pressure on the alga is generally high; at the colonization stage, grazers may consume up to 80% of the juveniles (Korpinen et al. 2007c), and in experimental setups, where predation on grazers was excluded, grazers consumed all the algal production (our unpublished data). Grazing on eggs and early juvenile stages is mainly due to gastropod grazers (Malm et al. 1999) while adult thallus is consumed almost solely by the isopod grazer *I. baltica*. Extensive grazing damage of entire *F. vesiculosus* belts during mass occurrences of *I. baltica* occurs (our personal observations; Engkvist et al. 2000). Consequently, both the epibiotism and herbivory can be assumed to generate strong selection for resistance. Resistance to both epibiotism and herbivory, measured as the inverse of fouling load or grazing loss, vary among *F. vesiculosus* genotypes (unpublished data by authors) and therefore evolutionary responses to selection are expected.

Baltic Sea populations of *F. vesiculosus* contain relatively large amounts of phlorotannins, phenolic secondary metabolites characteristic to brown algae, compared with some oceanic populations (e.g., Kubanek et al. 2004). Studies comparing populations in the Archipelago Sea (Northern Baltic Sea), at the same time of the year, found that phlorotannin averages among populations varied between 8 and 14% phlorotannins of the algal dry weight (Hemmi and Jormalainen 2004b; our unpublished data). Both genetic and environmental variations in phlorotannins contribute to this spatial variation. Experiments using cloned genotypes have shown high genetic variation in phlorotannin concentration; the broad sense heritability values vary from 0.31 to 0.70 depending on the growing environment (Jormalainen et al. 2003; Jormalainen and Honkanen 2004). Among-population differences in phlorotannin contents remain even after rearing individuals for several months in the same environment (our unpublished data), indicating that these differences have a genetic component. However, phlorotannin concentrations also show temporal variation and plasticity with respect to several environmental factors, such as salinity, UV irradiation, and light and nutrient availability (Tuomi et al. 1989; Jormalainen and Honkanen 2001; Hemmi et al. 2004). For example, phlorotannins increase with light availability but decrease with increasing nutrient availability. In the region, during the growing season phlorotannin concentrations correlate negatively with the environmental variation in nutrient availability (Ilvessalo and Tuomi 1989). These plastic responses are often compatible with the predictions from the resource-based hypotheses on defense allocation (carbon-nutrient balance and growth-differentiation balance hypotheses; see Chap. 7 and Amsler and Fairhead 2006), especially if the increase in the epibiotic periphyton (due to increased nutrient availability) and the

consequent shading effect on the *F. vesiculosus* is taken into account. This indicates that phenolic compounds of the *F. vesiculosus* vary to some extent with respect to resource availability, which does not, by any means, contradict the idea that phlorotannins are providing resistance to natural enemies. The biosynthesis of secondary compounds is likely to be sensitive to variation in nutrient and carbon availability (reviewed in Stamp 2003).

Although induced responses of phlorotannins to herbivory have been found in several brown algae, including members of the Fucales (e.g., Van Alstyne 1988; Pavia and Toth 2000), we have not found them in *F. vesiculosus*. We have looked for them in seminatural through-flow systems, using either artificial wounding (Hemmi et al. 2004) or isopod grazers (unpublished data by authors) as inducers, with no induction of phlorotannins. We have found phlorotannins to increase as a response to feeding by the gastropod *T. fluviatilis*, but this may be due to the specific type of resource manipulation by this species rather than to an induced defense response: the snail does not eat the algal thallus, but removes epibiotic periphyton from the thallus surface and cuts hyaline hairs that the alga uses for nutrient uptake (Jormalainen et al. 2003). Increased light availability because of fewer epibionts, together with decreased nutrient uptake because of the lost hairs, may explain the increase in phlorotannin concentration. Thus, the data so far suggest that consumption of the alga does not induce increase of phlorotannins in *F. vesiculosus*. However, this does not preclude the possibility that induced resistance to herbivory occurs in *F. vesiculosus*, but is based on metabolites other than phlorotannins. Hemmi et al. (2004) found that simulated grazing induced resistance to feeding by *I. baltica* rapidly after wounding, and the induced resistance faded after 10 days. The localized nature of the induction implied that such resistance, while not efficient as a general defense against herbivory, may benefit the alga by dispersing the damage and thereby aiding to avoid breakage of whole algal fronds. In addition, a recent study on *F. vesiculosus* from the Southern Baltic Sea found induced resistance as a response to grazing damage by *I. baltica* and *Litorina littorea* (Rohde et al. 2004).

Phlorotannin contents vary among different parts of the *F. vesiculosus*. Basal parts have consistently higher phlorotannin contents than do the apical parts grown during the same season, while the reproductive organs (receptacles) have the lowest concentrations, with only about half of that observed in the vegetative apices (Tuomi et al. 1989; Hemmi et al. 2005). Honkanen et al. (2002) found that the vegetative apices of *F. vesiculosus* are higher quality food for *I. baltica* than are the basal parts. Such differences in palatability may be due to several plant traits such as differences in thallus morphology or nutritive value, but, in this case, differences in phlorotannin content are the likely explanation for the following reasons: Vegetative apices had, in all the populations studied, 10–25% lower phlorotannin concentrations than did the basal parts. Low nutritive value of an alga, which could be an alternative reason for its low quality, is often associated with observed compensatory consumption. However, there was no compensatory consumption of the *F. vesiculosus* by *I. baltica*, supporting avoidance due to chemical deterrence rather than low nutrition. Thus within-plant differences in quality may well reflect differential allocation of defenses within the plant. Such a pattern of defense allocation between basal and apical parts is consistent

with the predictions from the carbon-nutrient balance- and growth-differentiation balance hypotheses (see Chap. 7): actively growing parts allocate carbon to growth and have lower levels of quantitative defenses, while the basal parts are differentiated and can allocate photosynthates to defense. However, similar allocation pattern can also be predicted by the optimal defense theory, if assuming that the fitness value of the basal parts is higher than that of the apical meristems or receptacles (Honkanen et al. 2002). Receptacles, however, are tougher than vegetative apices, which led Tuomi et al. (1989) to suggest that receptacles may be primarily mechanically rather than chemically defended.

The variation in phlorotannins described earlier, as well as in all ecological studies on the role of phlorotannins, is based on quantification of soluble total phlorotannins found in vacuoles inside the cells. Phlorotannins, however, are polymers of phloroglucinol subunits, and different polymers may have different characteristics. In *F. vesiculosus*, 16 different HPLC peaks in the fraction containing soluble phlorotannins have been recognized, indicating at least as many individual phlorotannins (Koivikko et al. 2007). Thus it remains possible that there exists more variation in the amounts of separate phlorotannins than what has been revealed by the quantification of total phlorotannins alone. In addition to occurring within the cells, phlorotannins may be located in the cell walls or exuded outside the thallus. Koivikko et al. (2005) determined both the contents of soluble and cell-wall-bound phlorotannins from *F. vesiculosus*. They reported that the majority of phlorotannins were found in a soluble form while a rather constant, minor amount of phlorotannins was bound to the cell walls. In addition, *F. vesiculosus* exudes phlorotannins into water, and this exudation increases as a consequence of herbivory (Koivikko et al. 2005). Based on these findings, it was concluded that phlorotannins have, at least quantitatively, a minor role as primary metabolites and their main adaptive function may be in interactions with other organisms.

Phlorotannins of *F. vesiculosus* are not selectively neutral. In a field experiment, the relationship of total phlorotannins with algal fitness varied depending on the selective environment; no selection, balancing selection for, or directional selection against phlorotannins was found (Jormalainen and Honkanen 2004). The high heritability of phlorotannins suggest that there is potential for evolutionary response under directional selection (Jormalainen and Honkanen 2004). On the other hand, the variable patterns of selection as such are likely to maintain genetic variation in phlorotannins. Although in the earlier-mentioned study we could not identify the selective agents, there is evidence that phlorotannins are important in the interactions with both the herbivores and epibiota.

The isopod *I. baltica* is adapted to use *F. vesiculosus* as food as shown by its capability to grow and reproduce when fed with this alga alone. *I. baltica* prefers *F. vesiculosus* as a structural host and feeds readily on it. However, *F. vesiculosus* is not the best single-host diet species; growth of this herbivore on a sole *F. vesiculosus* diet was inferior to many other hosts and to a mixed diet (Jormalainen et al. 2001; Hemmi and Jormalainen 2004a). Phlorotannins from *F. vesiculosus* were harmful to the performance of this herbivore: Increasing concentrations of phlorotannins in extracts from *F. vesiculosus* decreased both the total

assimilation efficiency and the nitrogen assimilation efficiency of *I. baltica* (Jormalainen et al. 2005). Different *F. vesiculosus* populations also varied in their quality as food for *I. baltica*, as measured in terms of growth rate of the herbivore (Honkanen et al. 2002), and the herbivore growth rate correlated negatively with the phlorotannin content of the host population (Jormalainen et al. 2007). Furthermore, when this herbivore was reared on different *F. vesiculosus* genotypes, the genetic variation in the phlorotannin contents of the *F. vesiculosus* correlated negatively with the growth rate of the herbivore (Jormalainen and Koivikko, unpublished data). Thus, phlorotannins are clearly harmful to the main herbivore of *F. vesiculosus* and thereby provide defense against herbivory. Phlorotannins are also likely to be the reason *I. baltica* includes other macrophytes in its diet (Jormalainen et al. 2005).

F. vesiculosus shows considerable genetic variation in resistance to epibiotism, which is found in experiments conducted both in mesocosms (Jormalainen et al. 2003) and in the field (Honkanen and Jormalainen 2005). In these studies, resistance was defined on the basis of fouling load of all epibiota on algae, high fouling loads representing low resistance and vice versa; it thus represents general resistance against colonization of the thallus surface. In a mesocosm experiment, there was a negative genetic correlation between the phlorotannin concentration in the thallus and the amount of epibiota (Jormalainen, Wikström and Honkanen, unpublished data). This indicates that phlorotannins may contribute to the resistance to epibiotism. However, the presence of epibiota decreased phlorotannins about 20%, compared with a control with epibiota removed (Honkanen and Jormalainen 2005), implying that phlorotannins do not provide inducible resistance but, instead, they may provide constitutive resistance to epibiotism. However, further studies linking the mechanism of action, surface activity or exudation, to fouling resistance are needed to convincingly show the role of phlorotannins as an antifouling trait.

3.6 Conclusions

A meta-analysis of the existing studies has shown that induced resistance to herbivory was common particularly in brown algae but less so or missing in red and green algae. Although this induced resistance was chemical by nature, the metabolites responsible for the induction have seldom been recognized, with the exception of brown algal phenolic compounds. Concentrations of these increased as a response to both natural and artificial induction treatments, although the responses were highly variable; there are cases where the phlorotannin concentration increases, and also cases where it decreases, as a response to induction. While induced resistance seems to be common in macroalgae, the chemical mechanisms behind as well as the nature of the induction cues (direct vs. water-borne) are generally poorly understood. Furthermore, the possible role of indirect, tritrophic defenses in macroalgal communities is virtually unknown.

Resistance to herbivory varies among different parts of algae. This variation is consistent with the idea of optimal allocation of defenses among parts of differing

fitness value. However, other models of within-plant defense allocation often give parallel predictions. Thus, thorough understanding of within-plant allocation of defenses necessitates critical distinction between the alternative models.

Macroalgal chemical defenses act as selective agents for the herbivore traits related to the interaction. Little is known about the effects of chemical defenses on herbivore population dynamics but the existing data suggest that bottom-up influences on herbivores may be important.

Macroalgae produce allelopathic metabolites that act against sessile invertebrates, canopy-forming macroalgal competitors, and epibiotic organisms in particular. However, relatively few studies have tested the antifouling properties in ecologically relevant contexts. To understand the evolution of macroalgae's antifouling strategies, studies on the strength of selection for and on the genetic variation of the putative antifouling traits are needed.

Consequences of epibiotism to the host alga depend on the community composition and, vice versa, epibiotism may modify the susceptibility of the host to herbivory. The community context hence largely determines the influences of both herbivory and epibiotism on the host algae, and should be taken into account more explicitly in studies of chemical resistance to natural enemies.

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4
Macroalgal Chemical Defenses in Polar Marine Communities

C.D. Amsler(✉), J.B. McClintock, and B.J. Baker

4.1 Introduction

Macroalgae are considerably less diverse at polar latitudes than in most temperate and tropical regions but they can still be very important components of benthic marine communities in polar waters (Dayton 1990; Wiencke et al. 2007). In fact, at some locations they can rival the biomass present in temperate kelp forests (e.g., Amsler et al. 1995). Wiencke et al. (2007) recently reviewed the ecophysiology and ecology of polar macroalgae and provided a brief overview of the state of our knowledge about their chemical defenses. The present chapter significantly expands upon that overview. Previous in-depth reviews of Antarctic marine chemical ecology (Amsler et al. 2001a, b) included macroalgae, but our knowledge of Antarctic macroalgal chemical ecology, although still relatively sparse when compared with lower latitudes, has expanded greatly since 2001. Indeed, of the 14 published or unpublished studies of polar macroalgal chemical defenses featured herein, only 3 were completed before 2001. Likewise, potential ecological roles of some Antarctic macroalgal secondary metabolites have been determined in recent years (Ankisetty et al. 2004; Lebar et al. 2007), but there are still relatively few secondary metabolites known from polar macroalgae. Not counting mycosporine-like amino acids (MAAs; see Chap. 13) or volatile halogenated organic compounds (VHOCs) (see Chap. 12), both of which include specific compounds produced by a wide diversity of algae, there are only 64 macroalgal secondary metabolites known from Antarctica and none from the Arctic Ocean (other than a presumption of the presence of phlorotannins in Arctic brown algae; Amsler et al. 2001a; Blunt et al. 2006; Lebar et al. 2007). There are, however, 18 macroalgal secondary metabolites known from northern areas of the Atlantic and Pacific Oceans that experience ice cover during some times of the year (Blunt et al. 2006; Lebar et al. 2007).

J.B. McClintock
Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170 USA

B.J. Baker
Department of Chemistry, University of South Florida, Tampa, FL 33620 USA

C.D. Amsler
Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294-1170 USA
e-mail: amsler@uab.edu

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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4.2 Western Antarctic Peninsula

Macroalgae occur all around the continent of Antarctica but are by far most abundant and diverse along the western side of the Antarctic Peninsula (Heywood and Whitaker 1984; Wiencke and Clayton 2002). Coincidently, a large percentage of coastal Antarctic research stations and other marine research activities are concentrated in this region; so it should not be surprising that we know more about Antarctic macroalgae in general and their chemical ecology in particular from the western Antarctic Peninsula region than from the rest of Antarctica combined.

It has been suggested for many years that macroalgae may play important roles in providing carbon to detrital food webs along the western Antarctic Peninsula (e.g., Neushul 1965; Dawson et al. 1985; Reichardt 1987; Fischer and Wiencke 1992; Dunton 2001). It has also been recognized that at least some Antarctic animals utilize macroalgae directly as food (e.g., Richardson 1975; Brand 1976, 1980; Iken 1996; Iken et al. 1997; Graeve et al. 2001; Kim 2001; Huang et al. 2006), but very little is known about the relative importance of herbivory or chemical defenses against herbivory in these communities. The first published experiments examining the potential for chemical defenses in macroalgae from the Antarctic Peninsula were those of Iken (1999) conducted at King George Island. (62°14′S, 58°40′W). She prepared water-soluble extracts from five macroalgal species that were unpalatable as fresh thallus to the snail *Laevilacunaria antarctica* and one palatable species by homogenizing them in seawater and then added equal volumes of these extracts of the palatable and unpalatable species into agar blocks. Consumption was measured by fecal pellet production. Although all extract-containing blocks were consumed at greater rates than plain agar blocks, none of the extracts of unpalatable algae significantly decreased feeding relative to the palatable alga control (and in one case, *Ascoseira mirabilis*, consumption was significantly higher than in the control). However, this experimental design was particularly conservative as a measure of chemical defenses, since the extracts were presented in the agar at less than one-twelfth their concentration in the algae on a volumetric basis and because only compounds quickly extractable into seawater could have been present.

The most extensive studies of macroalgal chemical defenses in any polar algae have been made off southwest Anvers Island (64°46′S, 64°03′W). Amsler et al. (2005a) took a comprehensive approach to evaluating the predominance of chemical defenses at this location. In order to prevent bias as to which species were or were not evaluated for chemical defenses, all species that could be collected in sufficient quantities were utilized in palatability bioassays with sympatric, omnivorous fish and sea stars. Palatability bioassays were performed on 35 subtidal, nonencrusting algal species collected during three, 2–4-month expeditions in 3 consecutive years. Since 41 such species were collected overall at the study site and ~100 such species are known for Antarctica as a whole, the algal species studies represent a significant subset. Of the species assayed, 83% were unpalatable to the fish and 63% were unpalatable to the sea stars. All macroalgal species unpalatable to the fish or sea

stars were then bioassayed as extracts using these animal species and also a herbivorous amphipod species. In the bioassays, the animals were presented with artificial foods containing tissue-level concentrations of both lipophilic and hydrophilic organic extracts of the macroalgae. Approximately 45% of the macroalgal species in the community studied were found to be chemically defended, including all of the large brown macroalgae that are ecologically dominant in terms of structure and biomass as well as most of the more common red macroalgae. It appears that chemical defenses are probably very important in allowing the dominant macroalgae to persist along the western Antarctic Peninsula. This is in contrast to many temperate kelp forests where the dominant macroalgae are palatable to consumers but persist because of top-down interactions between herbivores and their predators (Elner and Vadas 1990; Foster 1992). Physical toughness (Amsler et al. 2005a) as well as elemental and nutritive chemical composition (Amsler et al. 2005a; Peters et al. 2005) of macroalgae both appeared not to be important when compared to the role of chemical defenses in determining overall palatability, although it is possible that toughness is important in the unpalatability of some individual species. Extracts of many of these same species were examined for their potential as antifoulants against sympatric diatoms (using only nonecological, in vitro assays), and although there was a great deal of overlap between antiherbivore and potential antidiatom activity, several species that were not defended against herbivores were cytotoxic to diatoms (Amsler et al. 2005b).

In a more focused examination of eight of the more common macroalgal species at Anvers Island, Huang et al. (2006) were able to show that fresh thallus of the red alga *Palmaria decipiens* was much more palatable to two common, herbivorous amphipod species than were the other seven algal species examined. Unfortunately, presumably because the bioassay method used was not well suited for comparisons of foods with different overall morphologies, differences in palatability of the remaining seven species could not be resolved. However, in artificial food bioassays utilizing dried, ground thalli so as to remove morphological differences between species (Huang et al. 2006), the feeding preferences of both amphipod species for the eight macroalgal species were very similar to the patterns observed in bioassays with organic extracts (Amsler et al. 2005a).

The secondary metabolites responsible for unpalatability in these Antarctic macroalgae are known in a few cases. In the red alga *Plocamium cartilagineum,* the halogenated monoterpenes anverene and epi-plocamene D both deterred amphipod feeding but were not deterrent against sea stars (Ankisetty et al. 2004). In addition, furanones known to deter herbivory in cold temperate populations of the red alga *Delisea pulchra* (Wright et al. 2004) were identified from this species at Anvers Island but were not bioassayed against sympatric, Antarctic herbivores (Ankisetty et al. 2004). One of the ecologically dominant brown macroalgae, *Desmarestia menziesii*, elaborates the hydroquinone, menzoquinone, which deters feeding in bioassays with sympatric sea stars (Ankisetty et al. 2004) but not with amphipods (Amsler, unpublished observation). Another class of organic molecules known to be released from Antarctic macroalgae in significant quantities is VHOCs such as bromoform or dibromochloromethane (Laturnus 1995, 2001; Laturnus et al. 1996,

1997). We have determined that bromoform can deter feeding of Antarctic amphipods, but only at concentrations that are orders of magnitude higher than present in water samples taken from macroalgal beds or released by damaged algae (our unpublished observations).

Eukaryotic marine macroalgae produce relatively few nitrogen-containing secondary metabolites such as alkaloids or cyclic peptides even though many other marine organisms produce a diversity of such compounds (Baker 1996; McClintock and Baker 2001). Some authors have speculated that this could be because nitrogen is commonly a growth-limiting nutrient for macroalgae in marine waters and so there would be selection for producing nonnitrogenous compounds for defense (Hay and Fenical 1988; Hay and Steinberg 1992; Cronin 2001). As discussed by Amsler et al. (2005a), one place in the world where nitrogen and other inorganic nutrients are rarely, if ever, growth-limiting for macroalgae over very broad spatial scales is the coastal waters surrounding Antarctica, and this has probably been true for millions of years. Amsler et al. (2005a) prepared extracts targeting nitrogen-containing secondary metabolites from 24 common macroalgal species at Anvers Island and probed thin-layer chromatograms of the extracts with stains specific for nitrogenous organic molecules. No nitrogen-containing metabolites were identified. In addition, none of the 64 non-MAA secondary metabolites currently known from Antarctica contain nitrogen (Amsler et al. 2001a; Blunt et al. 2006; Lebar et al. 2007). This does not mean that there are no nitrogenous secondary metabolites in Antarctic macroalgae, but does indicate that if present, they are relatively rare just as in macroalgae from lower latitudes. This also indicates that the worldwide paucity of such compounds cannot be explained simply as an evolutionary response to nutrient limitation in the ocean. Neither can phylogenetics provide an explanation. Brown macroalgae are only very distantly related to green and red macroalgae, and green macroalgae in particular are relatively closely related to vascular plants (Baldauf 2003), which produce a huge diversity of alkaloids and related compounds (Raffauf 1996).

Brown algae commonly produce significant quantities of phlorotannins, which are polyphenolics with multiple primary and secondary metabolic roles (Amsler and Fairhead 2006). Phlorotannins can function as chemical defenses against herbivores but this is not always the case (Amsler and Fairhead 2006). Iken et al. (2007) measured phlorotannin concentrations in nine common brown macroalgal species at Anvers Island and reported levels comparable to or higher than those reported from other regions of the world. There were clear patterns of differential allocation to different thallus regions within many species but these patterns were not consistent across different species. The bioactivity of phlorotannins is in part a function of their degree of polymerization (Boettcher and Targett 1993). Iken et al. (2007) also examined the relative distribution of phlorotannin size classes and reported that most species were either dominated by large (>10 kDa) or small (<10 kDa) molecular size classes of phlorotannins, with a few species having an equal distribution and some species having different distribution in different thallus portions. In another study, Iken et al. (unpublished) showed that extracts enriched in phlorotannins usually significantly deterred amphipod, fish, and sea star feeding

in bioassays. In contrast, in bioassays conducted using purified phlorotannins only four of seven species' phlorotannins significantly deterred feeding, and none of these were deterrent to all three herbivore types.

The large, nonacidic, perennial desmarestialean algae *Desmarestia anceps, Desmarestia menziesii*, and *Himantothallus grandifolius* are the three most dominant overstory brown algae at Anvers Island and along most of the rest of the western Antarctic Peninsula (DeLaca and Lipps 1976; Amsler et al. 1995; Wiencke and Clayton 2002). All three species are strongly chemically defended based on bioassays with lipophilic extracts, hydrophilic extracts, or both (Amsler et al. 2005a). *D. anceps* and *D. menziesii*, which dominate in shallower waters, have been the targets of several more focused investigations. Fairhead et al. (2005a) examined within-thallus variations in both chemical and physical defenses in these species in the context of the Optimal Defense Theory (ODT; Rhoades 1979; see Chap. 7). Both species were divided into holdfast, primary axes, and lateral branches. All three thallus parts were chemically defended. Nonetheless, differences in relative levels of chemical defenses were detected using palatability bioassays with sympatric amphipods. *D. anceps*, which usually has a single and very distinct primary axis supporting all the lateral branches, strongly defended the main axis, as it was the most unpalatable thallus part in extract bioassays and was also physically tougher than the laterals. The holdfasts, which are composed of multiple, intertwining haptera, were not as strongly chemically defended as the primary axes but were also physically tougher than laterals. The laterals were, overall, more moderately defended both chemically and physically, compared with the axes and holdfasts. Since damage to either the primary axis or holdfast could lead to catastrophic loss of most or all of the lateral branches (where most photosynthesis and reproduction occur) and since small damage, such as could be caused by amphipods, is more likely to lead to catastrophic breakage in the flexible primary axis rather than in the more rigid and intertwined haptera of the holdfast, this pattern is consistent with ODT predictions (Fairhead et al. 2005a). *D. menziesii* differs from *D. anceps* in having multiple "primary" axes and axes that are only slightly distinct from the lateral branches in terms of their morphology. As with *D. anceps*, both the holdfasts and axes were significantly tougher than the lateral branches, but chemical extracts of all three tissue types were equally unpalatable. This somewhat lower relative investment in defenses in the axes of *D. menziesii*, compared with *D. anceps*, is probably a reflection of the difference in the respective relative contribution to fitness of any one of the many axes in *D. menziesii* versus a single main axis in *D. anceps*.

Fairhead et al. (2005b) examined small-scale within-thallus variations in the phlorotannin contents of *D. anceps* and *D. menziesii* and also compared individuals collected from different sites at Anvers Island as well as from the upper and lower limits of their depth distributions at those sites. Thirteen defined thallus parts within each individual were sampled, and although there was considerable variation within individuals at that spatial scale, there were no consistent patterns. When fine scale parts within the individuals were grouped, the main axis of *D. anceps* had significantly lower phlorotannin levels than did lateral branches. In *D. menziesii*, the distal-most tips of the lateral branches had significantly higher phlorotannin levels

than did the primary axes, portions of the lateral branches proximal to them, or portions of the laterals midway between the axes and branch tips. It is noteworthy that these patterns of phlorotannin levels do not correspond with the differences in crude extract palatability described by Fairhead et al. (2005a) for the same species sampled in the same area during the same season. For example, although the primary axis in *D. anceps* was less palatable than either the holdfast or lateral branches, it had significantly lower levels of phlorotannins than did the laterals and lower overall levels than did the holdfast, although that difference was not statistically significant.

However, using a somewhat different sampling design and extraction methods coupled with sampling from only a single depth at a single site in two previous years in the same vicinity, Iken et al. (2007) reported different patterns. Phlorotannin levels were significantly lower in the holdfasts of *D. anceps* than in the main axis or laterals and significantly higher in the holdfasts of *D. menziesii* than in a combined sample of the axes and lateral portions. The differences between the two studies could be due to interannual variations, methodological differences in thallus subsampling, extraction, or phlorotannin quantification, and/or to the samples (of Iken et al. (2007)) coming from a single depth and location in each year. Fairhead et al. (2005b) did report significant variations between phlorotannin levels in individuals of both species collected from different locations (all within a few kilometers of each other) and, in *D. anceps*, from the upper and lower limits of their depth ranges. Regardless, the patterns of phlorotannin allocation reported by Iken et al. (2007) still do not correspond with the differences in relative palatability reported for either species by Fairhead et al. (2005a).

The roles of phlorotannins in *D. anceps* and *D. menziesii* with respect to herbivory are ambiguous. Both Fairhead et al. (2006) and Iken et al. (unpublished observations) found that extracts of both species that were enriched in phlorotannins were deterrent to amphipods, and Iken et al. (unpublished) also found this to be true with fish and sea stars. But when phlorotannins were purified from both species by Iken et al., only those from *D. menziesii* were deterrent to the amphipods and just the opposite was observed in feeding bioassays with omnivorous fish and sea stars, where only purified phlorotannins from *D. anceps* were deterrent. However, Fairhead et al. (2006) reported that purified phlorotannins from *D. anceps* were deterrent to the same species of amphipod used by Iken et al. Furthermore, Fairhead et al. (2006) reported that experimental manipulations that increased light levels resulted in higher phlorotannin levels in these species. However, when amphipods were offered a choice between artificial foods containing ground algae from before and after the experimental treatments, they preferred the higher phlorotannin containing ground *D. anceps* and they had no preference at all for higher or lower phlorotannin containing ground *D. menziesii* (Fairhead et al. 2006). So at least under these circumstances, any feeding deterrent effects of phlorotannins on amphipods were negated.

Phlorotannins have also been implicated as a defense against ultraviolet radiation (UVR), and their concentrations have been reported to increase with increasing UVR (Pavia et al. 1997). Within the context of recent, anthropogenic increases in UVR in polar regions (Frederick et al. 1998), Fairhead et al. (2006) examined

the potential for *D. anceps* and *D. menziesii* to increase phlorotannin accumulation in response to increasing UVR. These authors increased UVR levels in situ by transplanting individuals from 15 m depth to 5 m depth (increasing ultraviolet-B radiation from 0.05% of surface levels to 5% of surface levels), but blocked this increased UVR with plexiglass filters in some treatments. Although phlorotannin levels increased in all treatments, presumably because of increased photosynthetically active radiation, there were no treatment effects attributable to differences in UVR for either phlorotannin levels or the palatability of fresh thallus to herbivorous amphipods.

Fairhead et al. (2006) also examined the potential of herbivory by two species of amphipods and one species of snail, and/or mechanical wounding to induce an increase in unpalatability and/or in phlorotannin levels in *D. menziesii*. No such increases were found during a 4-week mesocosm experiment. The Induced Defense Model (IDM; Karban and Meyers 1989; Harvell 1990; see Chap. 7) predicts that there should be selection for defenses that can be induced by the onset of predation if the predation is variable in space or time and if the predator consumes the prey slowly enough for a defensive response to be produced. Hay (1996) observed that small herbivores such as amphipods and snails, which are known as mesoherbivores or mesograzers, often display the characteristics that this model would predict to select for inducible defenses. That Fairhead et al. (2006) did not detect induced defenses in response to amphipod or snail grazing is most likely because mesoherbivores, particularly amphipods, are very abundant in macroalgal forests along the western Antarctic Peninsula, with densities on larger overstory species such as *Desmarestia* spp. ranging to well over tens-of-thousands of individuals per squared meter (Richardson 1971, 1977; Jazdzewski et al. 1991; Huang et al. 2007). For example, at Anvers Island, Huang et al. (2007) found that amphipod abundances were particularly high on finely branched species such as the brown algae *Desmarestia menziesii* (20 individuals/g wet wt.) and *D. anceps* (2 individuals/g) and the red alga *P. cartilagineum* (6 individuals/g). Combining these mean amphipod density values with raw algal biomass data collected by Amsler et al. (1995) in the same communities, the calculated (estimated) amphipod densities range up to 308,000, 32,000, and 26,000 individuals per squared meter for stands of *D. menziesii*, *D. anceps*, and *P. cartilagineum*, respectively. Clearly, grazing pressure by mesoherbivores is likely to be constantly high, which the IDM would predict to select for defenses that are produced constitutively.

Peters (2003) noted that the subtidal macroalgal flora at King George Island is nearly devoid of filamentous epiphytes but that filamentous algae are very common growing endophytically within the larger macroalgae. He suggested that this was probably an adaptation of the filamentous algae to the very high density of mesoherbivores in the community. We have observed the same pattern of few filamentous algae growing subtidally except within the thalli of the larger chemically defended macroalgae at Anvers Island. Because of their great abundance coupled with a relatively low density of macroherbivores such as sea urchins or fish, the roles of amphipods and other mesoherbivores in macroalgal community dynamics along the western Antarctic Peninsula are a very important topic for future investigations.

4.3 McMurdo Sound, Antarctica

Although many Antarctic macroalgal species have circumpolar distributions (Neushul 1968; Wiencke and Clayton 2002), they become less abundant and diverse south of Anvers Island on the Antarctic Peninsula (DeLaca and Lipps 1976) and elsewhere around the continent. However, three species of macroalgae can commonly be found in McMurdo Sound, Ross Sea (77.7°S), which is as far south in the world as open ocean ever occurs (Miller and Pearse 1991), and 17 species occur further north in the Ross Sea at Terra Nova Bay (74.8°S) (Cormaci et al. 1992). Of the common macroalgae in McMurdo Sound, the only two nonencrusting species are *Phyllophora antarctica* and *Iridaea cordata* (Miller and Pearse 1991). Amsler et al. (1998) examined the palatability and chemical defenses of *P. antarctica* and *I. cordata* to the very common and abundant sympatric sea urchin *Sterechinus neumayeri*. Because *S. neumayeri* does not reliably consume anything in laboratory aquaria, a phagostimulation assay was developed that measured how long an urchin would hold an algal thallus disk or filter paper disk containing algal extract plus a feeding stimulant at its mouth. Thallus disks of both macroalgal species were rapidly rejected as were filter paper disks containing either lipophilic or hydrophilic extracts of both species, indicating the presence of strong chemical feeding deterrents. However, carbon from these algae does enter the detrital food chain in McMurdo Sound (Norkko et al. 2004).

A majority of the biomass of *P. antarctica* and *I. cordata* in McMurdo Sound is present as drift (Miller and Pearse 1991; Norkko et al. 2004), presumably because of thalli being torn from the substrate by anchor ice or ice scour. Many of the drift plants are maintained in the photic zone because they are preferentially used as covering material by *S. neumayeri* (Amsler et al. 1999). Since the urchins readily cover with other materials when macroalgae are not present (Dayton et al. 1970) and are chemically deterred from eating the algae (Amsler et al. 1998), this preference for using the algae as cover was puzzling. Dayton et al. (1970) reported observations from nature indicating that nonalgal cover materials could be used as a physical defense against large sea anemones such as *Urticinopsis antarctica* and *Isotealia antarctica*, which can be very common and important predators of the urchins. When the sea urchins encountered the anemones, the anemone's tentacles attached to the cover material, which the urchins then released as they escaped. Amsler et al. (1999) tested the ability of both *P. antarctica* and *I. cordata* to defend urchins against *I. antarctica* in controlled laboratory experiments and reported that both macroalgal species were very effective as this form of defense. By extracting secondary metabolites from the macroalgal thalli, we tested the hypothesis that the same chemicals that defend the algae from the urchins in turn help defend the algae-covered urchins from anemones. However, we observed no difference in the escape from anemones of urchins covered with extracted versus unextracted, live macroalgae. Consequently, the preference of sea urchins for macroalgae as cover remains unknown, although it could be related to the lesser mass of macroalgae needed to cover an urchin, compared with shells, pebbles, etc. Nevertheless, the macroalgae benefit from being the preferred sea urchin cover because it results in

a high percentage of the total macroalgal biomass being retained in the photic zone where the algae are still able to photosynthesize (Schwarz et al. 2003) and reproduce (Amsler et al. 1999), and therefore, make considerable contributions to maintaining the macroalgal populations at these sites.

4.4 The Arctic

Much less is known about macroalgal-herbivore relationships or their chemical mediation in the Arctic Ocean. In the Alaskan Arctic, Dunton and Schell (1987) used stable isotope analyses to conclude that kelps are an important carbon source for herbivores such as gastropods and chitons. In Arctic Norway, Sivertsen (1997) examined patterns of sea urchin overgrazing of kelps. A few studies have described animal communities associated with macroalgae in the Arctic (Lippert et al. 2001 and references therein). However, to our knowledge, there is only one published study on the palatability of Arctic macroalgae to sympatric consumers. Wessels et al. (2006) examined the palatability of 19 species of macroalgae from Spitsbergen to 19 species of sympatric, macroalgal-associated invertebrates. Of the 19 invertebrate species, only 2, an amphipod and a sea urchin, consumed macroalgae. The macroalgal species most palatable to these two invertebrate species often differed markedly. For example, kelp stipes and, in one kelp species, also the blades, were low in relative palatability to the amphipods while both stipes and blades of most (but not all) kelp species examined were readily consumed by the sea urchins. However, palatability of some macroalgae was similar to the two animal types, for example, the red alga *Palmaria palmata* was highly palatable to both while the acidic brown alga *Desmarestia virdis* was very low in palatability to both species. Laboratory-based no-choice feeding bioassays correlated well with preferences in field-based multiple-choice assays. In laboratory assays in which each consumer species was offered a choice of fresh algal thallus versus homogenized algal thallus incorporated into an artificial food in order to remove physical defenses, 14 algal species were preferred by at least one of the grazers as homogenate, suggesting a common role for physical defenses. However, this pattern was more the case with amphipods than with sea urchins. Wessels et al. (2006) did not prepare and bioassay chemical extracts, nor did they directly look for the presence of defensive secondary metabolites, yet concluded that their data suggested a likely role of chemical defenses in only 2 of the 19 species examined: the red alga *Ptilota gunneri* and the acidic brown alga *D. virdis*. Should this apparent paucity of chemical defenses in macroalgal chemical defenses prove to be true there and in other parts of the Arctic, this would be an additional marked difference to add to the many that already distinguish Arctic and Antarctic marine communities.

Acknowledgments We are grateful to M. Amsler, K. Iken, and two anonymous reviewers for constructive suggestions on the manuscript and to K. Iken for coordinating the anonymous review of this chapter. Most of the studies on macroalgal chemical ecology described herein were supported by National Science Foundation awards OPP-9526610, OPP-9530735, OPP-9814538,

OPP-9901076, OPP-0125152, and/or OPP-0125181 to the authors. Preparation of this manuscript was supported in part by NSF awards OPP-0442769 and OPP-0442857. These studies would not have been possible without the hard work of numerous members of our past and present field and laboratory teams or without the logistical support of the employees and subcontractors of Raytheon Polar Services Company and Antarctic Support Associates.

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5
Macroalgal and Cyanobacterial Chemical Defenses in Freshwater Communities

F.A. Camacho

5.1 Introduction

Algae and cyanobacteria are important sources of primary production in freshwater systems and they figure prominently in estimates of carbon budgets, dissolved oxygen concentration, and nutrient recycling in those habitats (Howarth et al. 1988; Schippers et al. 2004). In recent years, these groups have garnered much negative attention as a result of their ability to dominate aquatic systems receiving chronic nutrient inputs. Freshwater blooms of these photoautotrophs have been shown to be positively influenced by an array of abiotic factors, including low N:P ratios, low light levels, high pH, and stratification of the water column (Elser 1999; Paerl et al. 2001). Nevertheless, there has been a growing understanding that biologically active secondary metabolites also play a role in mediating the persistence of these outbreaks by deterring herbivores and shifting grazing pressure toward chemically undefended species (Sterner 1989).

The toxins synthesized by freshwater cyanobacteria have been demonstrated to have dramatic effects on terrestrial organisms as well. Livestock deaths associated with contaminated water supplies are common in many countries, and have been recorded for more than a century (Carmichael 1994). In 1996, 76 patients from two renal dialysis clinics in Caruaru, Brazil, died from acute liver failure after water containing putative cyanotoxins was drawn from a nearby reservoir and was used in hemodialysis units (Pouria et al. 1998). Microcystins have been implicated as the most likely causative agents (Carmichael et al. 2001). Because many of these toxic cyanobacterial blooms occur in shallow, well-mixed water bodies, such as reservoirs, the potential for these compounds to enter drinking water supplies has emerged as a major public health concern in the last two decades (WHO 2003). Additionally, the potential exists for these toxins to bioaccumulate and biomagnify in aquatic food webs, providing an indirect route of exposure for humans through consumption of fish and macroinvertebrates (Sasner et al. 1984; Negri and Jones 1995).

F.A. Camacho
Marine Laboratory, University of Guam, UOG Station, Mangilao, GU 96923, USA
e-mail: frank.camacho@guamcc.edu

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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This chapter will focus on the role of secondary metabolites in promoting the growth and persistence of freshwater macroalgae and cyanobacteria and their effect on trophic interactions in aquatic systems. Historically, studies of chemically mediated interactions in freshwater systems have focused on the detection of predators by prey (Wisenden 2000) and subsequent behavior and life-history modifications in response to certain predator cues (e.g., Crowl and Covich 1990; Stibor and Navarra 2000). More recently, aquatic macrophytes have been demonstrated to synthesize compounds that are deterrent to herbivores (reviewed in Newman 1991; Burks and Lodge 2002). In contrast, the biological activity of freshwater cyanobacterial and macroalgal compounds has only rarely been examined in a broader ecological context. It is important to note that, unlike their cyanobacterial and unicellular algal counterparts, few studies have reported isolating biologically active secondary metabolites from freshwater macroalgae. As a result, much of the material presented here is from studies of toxic cyanobacteria. Macroalgal examples will be discussed where appropriate.

5.2 Cyanobacteria and Macroalgae: Evolutionary and Ecological Perspectives

Cyanobacteria are ancestral to the algae and are among the most ancient of life forms, with a fossil record spanning ~3.5 million years (Schopf 2000). Their relative antiquity, ability to conduct oxygenic photosynthesis and nitrogen fixation, and their ubiquity in both terrestrial and aquatic systems illustrate the ecological and economic importance cyanobacteria have had on the evolution of our biosphere. Similar to their eukaryotic algal counterparts, cyanobacteria occupy a diverse array of aquatic habitats, including thermal springs, Antarctic lakes, and man-made reservoirs (Whitton and Potts 2000). In addition, host-cyanobacterial symbioses have evolved in many eukaryotic lineages, including plants and animals (Vagnoli et al. 1992; Meeks 1998; Thacker and Starnes 2003). Morphologically, cyanobacteria may be solitary or colonial. Most colonial forms are held together by extracellular polysaccharide (EPS), typically in the form of mucus. However, the EPS of many filamentous cyanobacteria (e.g., *Phormidium*, *Lyngbya*) forms a robust sheath that helps the filaments to resist mechanical damage, reduce oxygen diffusion (in N-fixing species), and deter potential herbivores (Stal 2000; Camacho and Thacker 2006). Many filamentous species are actually planktonic, with average filament lengths <1 cm (Komárek and Kling 2003). However, some filamentous genera also contain benthic, mat-forming species (e.g., *Oscillatoria* and *Lyngbya*).

The taxonomic diversity of freshwater macroalgae represents a counterpoint to the diversity of these groups in marine systems. Fewer than 3% of the 4,000 species of rhodophytes occupy freshwater habitats, and most occur in lotic, or flowing-water, systems (Sheath 2003). Similarly, only five of the seven species of freshwater brown algae (Phaeophyceae) have been recorded from North America (Wehr 2003). In contrast, more than 75% of the over 4,000 species of aquatic chlorophytes have been isolated from freshwater habitats, including many filamentous and colonial forms (Graham and Wilcox 2000).

5.3 Cyanotoxin Structure and Synthesis

There are two major classes of freshwater cyanobacterial toxins, broadly categorized according to their physiological effects on vertebrates: hepatotoxins and neurotoxins. Although these hydrophilic toxins are highly soluble, they are typically released only upon cell lysis following mechanical damage or cell senescence (Sivonen and Jones 1999).

5.3.1 Hepatotoxins

Hepatotoxins include microcystins, which are cyclic heptapeptides (Fig. 5.1a) and cylindrospermopsin, a sulfated guanidinium alkaloid (Fig. 5.1b). Microcystins bind to certain protein phosphatases responsible for regulating the distribution of cytoskeletal proteins (Zurawell et al. 2005; Leflaive and Ten-Hage 2007). Hepatocytes exposed to microcystins eventually undergo cellular deformation, resulting in intrahepatic bleeding and, ultimately, death (Carmichael 2001; Batista et al. 2003). In contrast, cylindrospermopsin appears to have a different mode of activity, possibly involving inhibition of protein or nucleotide synthesis (Codd et al. 1999; Froscio et al. 2003; Reisner et al. 2004). Nevertheless, microcystins are the most common cyanotoxins isolated from cyanobacterial blooms (Sivonen and Jones 1999).

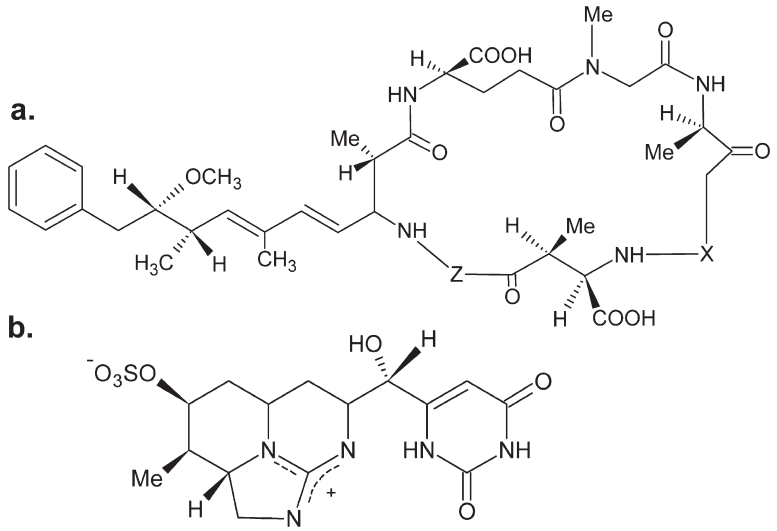

Fig. 5.1 Common cyanobacterial hepatotoxins. (**a**) Generalized structure of microcystin, a cyclic heptapeptide. Note that X and Z are L-amino acids. For example, microcystin-LR possesses lysine and arginine residues at X and Z, respectively. (**b**) Cylindrospermopsin, a hepatotoxic alkaloid from *Cylindrospermopsis raceborskii*

Table 5.1 Toxic freshwater cyanobacteria

Cyanobacterial genera	N-fixation	Toxin class
Anabaena	Yes	Anatoxin-a
		Anatoxin-a(S)
		Microcystins
		Paralytic shellfish toxins
Aphanizomenon	Yes	Anatoxin-a
		Cylindrospermopsin[a]
		Paralytic shellfish toxins
Cylindrospermopsis	Yes	Cylindrospermopsin
		Paralytic shellfish toxins
Lyngbya	Yes	Cylindrospermopsin[b]
		Paralytic shellfish toxins
Microcystis	No	Microcystins
Oscillatoria	Yes	Anatoxin-a
		Anatoxin-a(S)
		Microcystins

Modified from Sivonen and Jones (1999) and Carmichael (2001)
Note that certain genera contain several cyanotoxic species
[a]Preußel et al. (2006)
[b]Includes cylindrospermopsin and deoxy-cylindrospermopsin (Seifert et al. 2007)

Microcystins were first characterized from the scum-forming freshwater cyanobacterium *Microcystis aeruginosa* (Sivonen and Jones 1999). Since then, more than 70 structural variants have been identified from at least ten different aquatic and terrestrial cyanobacterial genera (Zurawell et al. 2005; Carmichael 2001). Among freshwater species, microcystins are synthesized by both unicellular (e.g., *Microcystis*) and filamentous forms (e.g., *Oscillatoria*) and by both N-fixing and non-N-fixing species (Table 5.1). The brackish-water cyanobacterium *Nodularia spumiginea* synthesizes a hepatotoxin (nodularin) that is structurally similar to microcystins (Rinehart et al. 1988). The broad phylogenetic distribution of species capable of synthesizing microcystins and nodularin suggests that these biosynthetic pathways are either relatively ancient (Rantala et al. 2004) or capable of being transferred horizontally between disparate taxa (Moffitt and Neilan 2004).

5.3.2 Neurotoxins

Cyanobacterial neurotoxins are small ringed alkaloids and have dramatic effects on various components of vertebrate neurons. They are all water soluble and are synthesized by several cyanobacterial genera (Table 5.1). The most commonly isolated neurotoxins are the paralytic shellfish toxins, although several other potent neurotoxic alkaloids are synthesized by freshwater cyanobacteria (Table 5.1).

Paralytic Shellfish Toxins

The paralytic shellfish toxins (PSTs; Fig. 5.2a) include saxitoxin (STX) as well as STX analogs such as neo-saxitoxin (neo-STX), gonyautoxin (GTX), and the decarbamoyltoxins (Sivonen and Jones 1999). These molecules are of particular concern in marine systems, where they have been implicated in human deaths following the consumption of contaminated seafood (Van Dolah 2000). The causative agents in those cases are several genera of marine dinoflagellates that are common components of red tides (e.g., *Alexandrium* sp.; Horner et al. 1997; Van Dolah 2000).

PSTs affect vertebrate neurons by blocking sodium channels, preventing the propagation of action potential (Kao and Nishiyama 1965; Lipkin and Fozzard 1994). This inhibition may eventually lead to paralysis and subsequent respiratory failure (Carmichael 1994). Nevertheless, the toxicity of individual PSTs varies remarkably (Onodera et al. 1997b). By far, the most potent PST is saxitoxin, commonly found in *Anabaena* and *Aphanizomenon* (Kuiper-Goodman et al. 1999). In contrast, certain PST isolates from *Lyngbya wollei* (i.e., *L. wollei* toxins) have no discernable toxic effects (as measured with mouse bioassay) and constitute most of the total intracellular toxin concentration (Onodera et al. 1997b).

Contributing to the diversity of PSTs in cyanobacterial blooms is the ability of these molecules to undergo structural changes under different pH and temperature conditions. Negri et al. (1997) reportedly found high (>90%) intracellular concentrations of variants of cyanotoxins from *Anabaena circinalis* in Australia. However, the concentrations of decarbamoyltoxins increased with culture age, apparently as a result of the degradation of the less-toxic cyanotoxins (Negri et al. 1997). *L. wollei* is capable of synthesizing around nine different PSTs, especially decarbamoylgonyautoxins and other sulfated toxins (Onodera et al. 1997b), although it has not been found to produce STX or neo-STX (Carmichael et al. 2001).

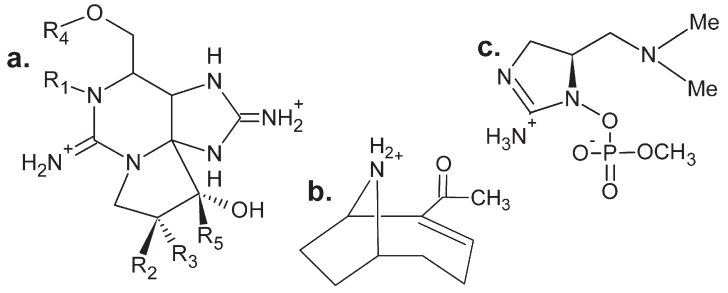

Fig. 5.2 Neurotoxic cyanobacterial secondary metabolites. (**a**) Generalized structure of paralytic shellfish toxins. R indicates a variable functional group and may include hydroxyl ($R_1$and R_5) and sulfate (R_2 and R_3) groups. Note that R_4 may comprise a carbamoyl group (e.g., saxitoxin and neo-saxitoxin), a hydrogen molecule (e.g., decarbamoyltoxin), or an ester (e.g., *Lyngbya wollei* toxins). (**b**) Anatoxin-a, an acetylcholine mimic. (**c**) Anatoxin-a(S), an organophosphate analog of acetylcholinesterase

Other Neurotoxins

The remaining cyanobacterial neurotoxins include the acetylcholine-mimic anatoxin-a (Fig. 5.2b) and the acetylcholinesterase inhibitor anatoxin-a(S) (Fig. 5.2c). Anatoxin-a is a 10-Carbon alkaloid that competitively binds to nicotinic acetylcholine receptors in vertebrate neurons (Aronstam and Witkop 1981; Thomas et al. 1993). Symptoms of anatoxin-a poisoning include convulsions, paralysis, asphyxiation, and, ultimately, death (Kuiper-Goodman et al. 1999; Carmichael 2001). Cyanobacterial genera synthesizing anatoxin-a (and the structurally similar homoanatoxin-a) include *Anabaena*, *Aphanizomenon,* and *Oscillatoria* (Table 5.1; Carmichael 2001). Anatoxin-a(S) is a guanidinium methyl phosphate ester (Carmichael 2001) whose ability to bind acetylcholinesterase in vertebrate neurons resembles certain organophosphate pesticides (Kuiper-Goodman et al. 1999; WHO 2003). As a result, acetylcholine is not efficiently degraded, allowing muscle cells to become overstimulated and fatigued (Zurawell et al. 2005). Death from anatoxin-a(S) intoxication is preceded by intense salivation (the "S" in the toxin name), extreme cramps, and paralysis (WHO 2003). Anatoxin-a(S) has been isolated primarily from *Anabaena flos-aquae* and *A. lemmermanni* (Table 5.1; Onodera et al. 1997; Carmichael 2001).

Other Cyanobacterial Secondary Metabolites

In addition to cyanotoxins, freshwater cyanobacteria are also capable of synthesizing a variety of other secondary metabolites with putative biological activity. The best characterized are the terpenoids geosmin and 2-methyl isoborneol (MIB). Geosmin and MIB are volatile organic compounds that impart an earthy or musty odor to cyanobacterial mats (Izaguirre et al. 1982; Sklenar and Horne 1999). Approximately 50 cyanobacterial species synthesize these compounds (Watson 2003). Ecologically, these substances have been purported to be bactericidal, and may facilitate the growth of sympatric algae (Sklenar and Horne 1999), although the allelopathic nature of these compounds has been debated (Legrand et al. 2003).

5.4 Macroalgal Secondary Metabolites

Despite evidence demonstrating the biological activity of crude macroalgal metabolites (Dodds and Gudder 1992; Gross 2003), only a handful of these compounds have been isolated and characterized from freshwater macroalgae. For example, species of *Chara* (Characeae, Nitellaceae) are capable of synthesizing several sulfur-containing compounds (Gross 2003), including charamin, a bactericidal quaternary ammonium compound isolated from *Chara globularis* (Anthoni et al. 1987). This genus elicits a strong odor resembling hydrogen sulfide (Allen 1882).

The tuft-forming green alga *Cladophora glomerata* synthesizes a wide variety of toxic fatty acids, including capric and palmitoleic acids, which have been demonstrated to be insecticidal and allelopathic (Dodds and Gudder 1992).

5.5 Inducible Synthesis of Secondary Metabolites

The role of inducible defenses has emerged as a cornerstone of chemical ecology in both terrestrial (Karban and Baldwin 1997) and marine systems (e.g., Harvell 1986; Cronin and Hay 1996; see Chap. 7). However, there are few examples of herbivore-induced defenses among freshwater algae and cyanobacteria. Thacker et al. (2005) placed paired cultures of the PST-producing cyanobacterium *L. wollei* in containers separated by mesh and added the freshwater snail *Pleurocera annuliferum* to one of the compartments. After a 16-day period, *L. wollei* cultures that were in direct contact with snails had a higher relative growth rate and a higher concentration of saxitoxins, compared with control mats (Thacker et al. 2005). Similarly, Jang et al. (2003) exposed strains of *Microcystis* to *Daphnia* and observed an increase in microcystin concentration among samples directly exposed to herbivory and to herbivore cues only. However, it is unknown to what extent, if any, nutrient recycling by *Daphnia* may have enhanced toxin synthesis.

To date, no clear evidence of inducible defenses among freshwater macroalgae has been reported, in contrast to their marine algal counterparts. For example, certain species of marine brown algae increase phlorotannin production in response to damage by mesograzers (Amsler and Fairhead 2006; see Chaps. 3 and 7). Whether *Chara* and *Cladophora*, two species of freshwater chlorophytes with putative allelopathic activity, increase allelochemical concentration in response to competitors remains to be seen (see Sect. 5.7.3).

5.6 Effects on Consumers

Most studies on cyanobacterial-invertebrate interactions have focused on the effects of cyanotoxins upon micrograzers, including copepods and cladocerans. Generally, these zooplankton appear capable of discriminating between toxic and nontoxic strains of live cyanobacteria (DeMott and Moxter 1991; DeMott et al. 1991). In cladocerans, exposure to either pure microcystin or saxitoxin leads to an enhanced postabdominal rejection response (Haney et al. 1995) and decreased movement and filtration in order to minimize consumption and filtration of toxic filaments. However, physiological sensitivity can enhance the susceptibility of zooplankton despite selective feeding mechanisms (Gilbert 1990). There is some evidence that micrograzers can evolve adaptations to tolerate consumption of toxic cyanobacteria, and may actually exhibit enhanced growth when fed cyanotoxic cells (e.g., Fulton III and Paerl 1987). Sarnelle and Wilson (2005) reported that *Daphnia*

pulicaria isolated from eutrophic lakes demonstrated positive growth rates when fed a partial diet of *Microcystis*. These results indicate that cyanotoxins may be an important selective force in natural systems and that the resulting micrograzer phenotypes may be better adapted to control cyanobacterial outbreaks within the prey size range of these planktonic herbivores.

Significant concentrations of cyanotoxins have been found to accumulate in the tissues of macroinvertebrates such as mollusks and crustaceans, presenting an indirect route of exposure for invertebrates, fish, and aquatic mammals at higher trophic levels (Negri and Jones 1995). In natural systems, mortality among benthic invertebrate herbivores is probably low because most bloom-forming bacteria are planktonic and only periodically come into contact with the benthos. Nevertheless, Kotak et al. (1996) determined that enhanced mortality of snails at the end of a bloom cycle in Canadian lakes was due to consumption of *Microcystis* cells that had formed a scum on the surface of macrophytes. Oberemm et al. (1999) found that aqueous microcystins, saxitoxins, and anatoxin-a all resulted in developmental delays in fish and salamander embryos. Interestingly, more severe malformations and enhanced mortality were observed when larvae were exposed to crude cyanobacterial extracts than to pure toxins applied at natural concentrations (Oberemm et al. 1999).

There are numerous reports on herbivorous waterfowl and mammals that have died following consumption of cyanobacterial mats (e.g., Krienitz et al. 2003; WHO 2003). Most deaths are probably due to incidental ingestion of contaminated water during drinking or while feeding on aquatic macrophytes.

5.7 Allelopathic Effects

Competition in aquatic communities between algae and cyanobacteria has progressed beyond classic models of resource limitation to include the role of allelopathic chemicals capable of influencing the distribution, abundance, and survivorship of potential phototrophic competitors. Typically, these water-soluble compounds have a negative effect on other competitors, resulting in a decrease in biomass or growth rate of a competing alga (Leflaive and Ten-Hage 2007).

5.7.1 Allelopathy in Cyanobacteria

Many cyanobacterial toxins have been demonstrated to also function as potent allelochemicals. Keating (1977) analyzed the successional sequence of bloom-forming cyanobacteria in a New England pond. Generally, the growth of dominant, successional species was enhanced by filtrates from the preceding dominant cyanobacterium. In contrast, filtrates from succeeding dominants always inhibited growth of the preceding dominant species (Keating 1977). Schagerl et al. (2002) reported that cell-free filtrates from certain toxic cyanobacteria, particularly *Nostoc* and *Anabaena*, consistently reduced microalgal and cyanobacterial growth rates in culture, although the inhibition they observed was generally weak. The activity of

Nostoc was attributed to a putative antibiotic released during the stationary phase of colony growth, suggesting that this may be an inducible allelopathic response to nutrient limitation (Schagerl et al. 2002).

The commercial availability of certain toxin standards has allowed researchers to examine the physiological mechanisms of allelopathy by cyanobacteria. The best known examples are from studies on microcystins, which affect plants and aquatic algae by interfering with protein phosphatases in a manner similar to their effect on vertebrate enzymes (Babica et al. 2006). However, there is evidence that microcystins can also promote the formation of reactive oxygen species (ROS) in photoautotrophs, which can cause extensive damage to cellular membranes and enzymes (Babica et al. 2006; Leflaive and Ten-Hage 2007).

Neurotoxins, such as saxitoxin and anatoxin-a, have been implicated in mediating competitive interactions between toxic cyanobacteria and other photoautotrophs, but few studies have explicitly examined the allelopathic effects of these compounds (e.g., Kearns and Hunter 2001). Although it is reasonable to assume that these compounds bind to algal and cyanobacterial sodium channels in a similar fashion as in vertebrate neurons, support for this hypothesis is currently lacking.

5.7.2 Allelopathic Effects of A. flos-aquae *on a Motile Alga*

One role of cyanobacterial allelochemicals may be to alter the motility and distribution of competing photoautotrophs. In a recent study, Kearns and Hunter (2001) examined the effects of toxic metabolites from the filamentous cyanobacterium *A. flos-aquae* on a unicellular phytoplankton species, *Chlamydomonas reinhardtii*. *A. flos-aquae* synthesizes both microcystins as well as anatoxins, providing the authors with an ecologically relevant opportunity to assess the individual and combinatorial effects of these toxins on an alga.

Cells of *C. reinhardtii* were exposed to cell-free filtrates from *A. flos-aquae*, pure microcystin-LR or anatoxin-a, or combinations of the toxins. Both the position of the cells and the chlorophyll-a concentration of the cultures were observed for 12 days. Exposure to crude extracts as well as to combinations of the toxins significantly decreased chlorophyll levels in the cultures. Furthermore, these compounds were all capable of paralyzing the algae and thus promoted the settlement of *C. reinhardtii* cells. One intriguing aspect of this dynamic interaction is the separate finding that *C. reinhardtii* may actually induce toxin synthesis in *A. flos-aquae* (Kearns and Hunter 2000), essentially signaling its own demise.

5.7.3 Allelopathy in Algae

Among freshwater macroalgae, species of *Chara* have most frequently been shown to have negative chemically mediated effects upon other aquatic photoautotrophs (Gross 2003). These algae often have a strong odor associated with them, resulting

in their nickname "muskweed," and may form dense mats in ponds and lakes with alkaline pH (Lembi 2003). Crude extracts from *Chara* have been tested against both algae and cyanobacteria and have been shown to inhibit growth of these groups (Berger and Schagerl 2003; Gross 2003). It is important to note, however, that other studies have found weak to no allelopathic effects of *Chara* species on unicellular chlorophytes (Mulderij et al. 2003; Lürling et al. 2006), suggesting that the inhibitory effects of *Chara* are taxon-specific.

In addition to *Chara*, several other freshwater macroalgae are capable of mediating allelopathic interactions. Under eutrophic conditions, *Cladophora glomerata* may be a nuisance species, forming dense, seasonal blooms in lakes (Dodds and Gudder 1992). *C. glomerata* can also dominate the surfaces of rocks in flowing streams, where they serve as a secondary substrate for epiphytic microalgae. To test whether *C. glomerata* could prevent overgrowth by periphyton, Dodds (1991) exposed the epiphytic diatom *Nitzchia fonticola* to crude *C. glomerata* extracts in different solvents. Extracts significantly lowered photosynthetic rates in the diatom, although the maximum depression observed was only 10% when compared with those in controls. In contrast, Mohamed (2002) reported that crude extracts from *Spirogyra* actually enhanced *Oscillatoria* growth in Egyptian canals. However, *Spirogyra* may have limited to no effect upon phytoplankton and aquatic macrophytes (Irfanullah and Moss 2005).

5.8 Secondary Metabolites and Trophic Interactions

Cyanobacterial and macroalgal secondary metabolites have the broader potential to alter food web dynamics and community structure through a number of direct and indirect pathways. In particular, herbivores may reduce predation risk by shifting their distribution to habitats avoided by predators (i.e., "enemy free space" *sensu*; Jeffries and Lawton 1984). Empirical studies of seaweed-herbivore associations have revealed that these mesograzers utilize chemically defended and unpalatable macroalgae as a refuge from predation (Hay et al. 1990). In freshwater systems, there is considerable potential for secondary metabolites in algae and cyanobacteria to influence foraging patterns of predators on infloral invertebrates. Benthic invertebrates and even small amphibians frequently use *Cladophora* tufts as a refuge from predation, particularly from bluegill sunfish (Dodds and Gudder 1992). Similarly, certain species of caddisflies (Trichoptera) preferentially build cases within the filaments of red algae in streams (Resh and Houp 1986).

Temporal and spatial shifts in the abundance of chemically undefended algae and cyanobacteria may leave grazers with few dietary options except to consume low-quality, chemically defended food items. While some herbivores may be able to either tolerate or inactivate these toxins (Sarnelle and Wilson 2005), species that lack tolerance may be faced with the dilemma of either remaining on a low-quality patch or migrating to a different patch, thus increasing their risk of predation by visual predators (Krivan and Vrkoc 2000). In systems that experience seasonal

fluctuations in the abundance of toxic cyanobacterial species, there may be a concomitant shift in the taxonomic and numerical abundance of primary consumers with differential sensitivities to cyanotoxins. Hansson et al. (2007) observed the eventual replacement of larger, microcystin-sensitive zooplankton groups (e.g., cladocerans and certain copepods) by smaller, more tolerant strains in both laboratory and field assays. This may ultimately affect the foraging efficiency of predatory fish and macroinvertebrates.

5.9 Bioaccumulation of Metabolites at Higher Trophic Levels

Previous studies have found that cyanotoxic compounds may accumulate in sympatric plants as well as in the tissues of herbivorous fish and invertebrates (reviewed in Zurawell et al. 2005). The accumulation of cyanotoxins at these trophic levels provides a direct path to both aquatic and, potentially, terrestrial consumers (Negri and Jones 1995; Kotak et al. 1996; Giovannardi et al. 1999). However, these compounds are rarely encountered in higher trophic levels in freshwater systems (Kotak et al. 1996; Zurawell et al. 2005). Nevertheless, attempts to minimize cyanotoxins in water bodies for recreational use should remain a major focus of environmental and public health managers, especially in light of the evidence that low doses may still have sublethal effects on the larval development of aquatic vertebrates (Oberemm et al. 1999).

5.10 Summary

Research on the chemical ecology of freshwater cyanobacteria and macroalgae is in its infancy relative to studies from marine systems. However, there is a growing awareness of the importance of chemically mediated interactions in lakes, rivers, and streams (Newman et al. 1991; Burks and Lodge 2002). Freshwater cyanobacterial and macroalgal metabolites have the potential to affect the survivorship, reproduction, and distribution of competing aquatic photoautotrophs and herbivores. These interactions may further influence the foraging patterns of predators, although few explicit tests of these hypotheses have been performed to date.

A holistic understanding of the ecological role of these compounds is still lacking, particularly in the context of food web theory and trophic cascades. There are no studies on freshwater macroalgal or cyanobacterial chemistry that have explicitly analyzed the fitness costs of synthesizing allelopathic or deterrent compounds within a theoretical framework (e.g., optimal defense theory, carbon nutrient balance; see Chap. 7). In addition, future research should attempt to resolve the extent that inducible defenses occur in cyanobacteria and how those compounds can alter foraging behavior in fish and other predators that consume infloral prey items. We also have a woefully limited understanding of other chemically mediated

interactions, such as chemotaxis by freshwater macroalgal spores, algal settlement cues, and abiotic regulation of secondary compounds in macroalgae. Likewise, the extent that herbivores may employ cyanotoxins (e.g., sequestration) as a means of chemical defense is currently unknown. Thus, the interactions reviewed in this chapter highlight only a few of the many potential ecological phenomena that may be affected by freshwater algal and cyanobacterial secondary metabolites.

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6
New Perspectives for Addressing Patterns of Secondary Metabolites in Marine Macroalgae

K.N. Pelletreau and N.M. Targett(✉)

6.1 Interpreting Patterns of Chemical Defense in the Marine Environment

Natural products can structure relationships between organisms, affect resource allocation, influence competition, mediate species distributions, and select for traits leading to the potential diversification of species (e.g. see reviews by McClintock and Baker 2001; Potin et al. 2002; Pohnert 2004; Ianora et al. 2006). Although there have been numerous efforts to determine patterns of metabolite distribution in marine ecosystems, particularly in macroalgae, these efforts have focused primarily on a macroscale: global patterns, patterns within specific habitats (e.g. the intertidal zone), and patterns correlated with changes in biotic and abiotic factors. Work in terrestrial ecosystems has led to the development of ecological models that describe trade-offs, costs, and benefits, and have been applied to marine algal systems (e.g. Cronin 2001; Jormalainen et al. 2003; Honkanen and Jormalainen 2005; Dworjanyn et al. 2006b; Ianora et al. 2006). However, the response of algal secondary metabolites to stimuli is a complex process, and these models do not consistently predict patterns of macroalgal metabolite production.

Understanding this inherent complexity mandates the use of an approach with multiple perspectives that resolves the mechanisms behind regulation, expression, accumulation, localization, and transport of secondary metabolites. Macroscale patterns (e.g. global distribution patterns of secondary metabolites within or across algal taxa, or apparent predispositions within certain algal groups toward particular defense responses) are a direct function of what we are defining as microscale phenomena: molecular and biochemical processes occurring within an alga, the spatial distribution of compounds within the alga, and the varied temporal responses

K.N. Pelletreau
University of Delaware, College of Marine and Earth Studies, Lewes, DE 19958, USA
e-mail: kpellet@udel.edu

N.M. Targett
University of Delaware, College of Marine and Earth Studies, Lewes, DE 19958, USA
e-mail: ntargett@udel.edu

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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exhibited by an alga in the environment. It is the combination of factors at the microscale – within the cell (genetic history and biochemical machinery), within the thallus (differential distribution), and over time (short- and long-term responses) – that ultimately sculpts observed chemical profiles.

This review will primarily focus on "patterns" of chemical defense from the microscale perspective (cellular, subcellular, molecular, and temporal), exploring spatial and temporal patterns of response, as well as the innate molecular information and biochemical mechanisms involved in secondary metabolism that exist across different algal divisions (for additional reviews of various patterns of algal chemical defense, see Hay and Steinberg 1992; Van Alstyne et al. 2001a; Potin et al. 2002; Pohnert 2004; Amsler and Fairhead 2006; Ianora et al. 2006).

6.2 A Brief Review of Macroscale Patterns of Algal Chemical Defenses

Over 16,000 natural products have been characterized from marine organisms (Bhakuni and Rawat 2005), and include isoprenoids (e.g. terpenes, sterols, and quinones), eicosanoids (e.g. polyunsaturated aldehydes, algal hormones), phenolic compounds, polyketides, alkaloids, acetogenins, and compounds of mixed biogenesis (see Chap. 1). Marine natural products have a greater number of halogenated compounds and alkaloids relative to terrestrial systems. Specific metabolite classes may show prevalence within one of the three main macroalgal divisions: phaeophytes synthesize phlorotannins; rhodophytes produce halogenated compounds, primarily isoprenoids and acetogenins; chlorophytes produce an abundance of terpenoid compounds; however, there is also overlap of metabolites between algal divisions. Within each compound class small changes in similar structures (e.g. modified side groups) result in a broad diversity of marine natural products (e.g. Blunt et al. 2006; Moore 2006; Nicholas and Phillips 2006; see Chap. 1). Understanding the differences in ecological function resulting from the structural variations, and the factors that influence these changes in structure are important in considering patterns (both spatial and temporal) of metabolite production in algae.

Secondary metabolites in marine systems are commonly found from the tropics to the poles in varying concentrations (Van Alstyne and Paul 1990; Steinberg and Van Altena 1992; Targett et al. 1992; Amsler et al. 2005; Fairhead et al. 2005, see Chaps. 2–4), suggesting that local factors are as important as any latitude-associated factor in determining metabolite concentrations. This is contrary to terrestrial systems, where vigorous studies have shown that chemical and morphological traits vary with latitude (i.e., higher palatability of temperate plants, lower palatability of tropical plants) (Salgado and Pennings 2005; Pennings et al. 2007). Large-scale geographic surveys of marine secondary metabolites tend to obscure the contribution of local factors, yielding high levels of variability and no obvious patterns (Pavia and Åberg 1996). This variability is likely due to numerous microhabitats with different suites of selective forces leading to diverse and specific macroalgal chemical profiles. For example, the alga *Stypopodium zonale*, a member of the

brown algal family Dictyotaceae, produces diterpenes in both temperate and tropical populations (White and Jacobs 1983; Obrien et al. 1984; Gerwick et al. 1985). The dominant diterpene metabolite produced varies according to geographic location, with atomaric acid in temperate locales and stypoldione in tropical locales (e.g. Pereira et al. 2004). Both compounds showed activity against two different grazers; however, the bioactivity of atomaric acid, a dominant "temperate" diterpene, was more deterrent to the herbivores tested than was the "tropical" stypoldione. In this study, the bioactivity was correlated with the unique chemical structure, which in turn appears dependent on local selection factors. Similarly, minor modifications to side chain groups of furanones resulted in significantly different antifouling activities (Dworjanyn et al. 1999), while phlorotannins exhibit varied bioactivity based upon their chemical structure and molecular size (Steinberg 1988; Boettcher and Targett 1993; Dworjanyn et al. 1999). Thus, it is clear that the efficacy of secondary metabolites in mediating ecological interactions is dependent on their specific chemical structure, which in turn is affected by the forces at work in the local environment. Abiotic and biotic factors that structure microhabitats can select for subtle changes in metabolite structure and quantity, both of which relate to bioactivity.

The relationship between the specific structure and activity of natural products and their ecological function has been established in terrestrial systems. For example, phenylpropanoids may serve different ecological/physiological functions, and their relative abundances vary according to elicitors present and/or environmental conditions; there is no "catch-all" response. Plants will produce anthocyanins in response to freezing, but not to pathogen attack, during which coumarins might be produced in higher quantities (Dixon and Paiva 1995). These compounds, although relatively similar in structure, and derived from branch points of the same metabolic pathway, appear to have highly specific bioactivities. Both the marine and terrestrial studies cited earlier illustrate the need for understanding microscale aspects of secondary metabolites, including chemical structure of the compounds produced in response to specific environmental conditions and the biosynthetic mechanisms involved. Subtle differences in metabolite structure can result in significant changes in bioactivity at the organismal level and beyond.

Inducible and activated defenses complicate the search for universal patterns of secondary metabolites and continue to highlight the importance of localized phenomena. The ability of exogenous cues to induce the production of secondary metabolites was first reported in marine algae by Van Alstyne (1988) when phlorotannin production in *Fucus distichus* was induced by natural and artificial grazing. In brown algae, induced responses in the form of compound production or modified herbivore behavior have since been observed in response to herbivory (e.g. Van Alstyne 1988; Cronin and Hay 1996a; Pavia and Toth 2000; Sotka et al. 2002; Borell et al. 2004; Rohde et al. 2004; Weidner et al. 2004; Macaya et al. 2005; Toth et al. 2005; Molis et al. 2006), artificial grazing and wounding (Van Alstyne 1988; Peckol et al. 1996; Hammerstrom et al. 1998; Lüder and Clayton 2004), waterborne cues (Toth and Pavia 2000; Rohde et al. 2004; Macaya et al. 2005), volatile cues (Arnold et al. 2001; Pelletreau et al., unpublished data), and UV light (Pavia et al. 1997). However, induction of defense is not always detected, even in response to these same triggers (e.g. Pavia and Toth 2000; Toth and Pavia 2002; Dethier et al.

2005; Macaya et al. 2005; Fairhead et al. 2006; Molis et al. 2006). Induction of terpenoids in brown algae has also been reported (Cronin and Hay 1996a); however, this phenomenon has not received as much attention as the induction of phlorotannins.

In red algae induced response to herbivory was first observed in *Pterocladia capillacea* after exposure to grazing by amphipods (Weidner et al. 2004). Observed reduction of grazing on the same tissue in subsequent experiments implied initial grazing caused a chemical change, rendering the alga less palatable. Subsequent work (Ceh et al. 2005) showed that grazed tissue of *Hypnea pannosa* reduced amphipod consumption. For both *P. capillacea* and *H. pannosa*, amphipod herbivory on the induced tissue resumed after 2 weeks. Similarly, Diaz et al. (2006) found that previously grazed red alga *Galaxaura diessingiana* elicited significant reduction in herbivory relative to the nongrazed pieces, yet two additional red algal species, *Hypnea spicifera* and *Gracilaria capensis,* showed no response to grazers. Further work exploring the presence and magnitude of induced responses in rhodophytes is warranted. Induction of secondary metabolites in the chlorophytes has been documented only by Diaz et al. (2006); they found that terpenoid compounds in *Codium platylobium* were induced following the alga's exposure to waterborne cues of grazed conspecifics.

Two recent meta-analysis studies (Toth and Pavia 2007, and Chap. 3) have aided in clarifying patterns of induced defenses in macroalgae. These studies determined factors that do not play a role in inducing herbivore response (i.e., type of elicitor, artificial vs. natural food), highlighted factors that are important (i.e., size of the herbivore used and the timescale of the experiment), and illustrated distinct differences between algal groups in their ability to induce responses as observed through herbivore reactions (Toth and Pavia 2007, Chaps. 3 and 7).

In contrast to inducible defenses, activated defenses are common in chlorophytes (Paul and Van Alstyne 1992; Cetrulo and Hay 2000; Pohnert and Jung 2003), have been documented once in rhodophytes (Van Alstyne et al. 2001b), and are not yet verified in the phaeophytes (see Cetrulo and Hay 2000). Activated defenses occur when mechanical damage (such as grazing) alters the defense compound to produce a more toxic compound (Paul and Van Alstyne 1992). Examples of activated terpenoid compounds include the following: caulerpenyne in *Caulerpa* spp., which converts into oxytoxins; and halimedatetraacetate in *Halimeda* spp., which converts into the more toxic halimedatrial (Cimino et al. 1990; Paul and Van Alstyne 1992; Gavagnin et al. 1994; Jung and Pohnert 2001). The conversion of dimethylsulfoniopropionate (DMSP) to acrylic acid and dimethyl sulfide is another example common in ulvoids (see Chap. 9).

Specificity of molecular bioactivity and differentially induced defenses are only two examples of factors that can confound the interpretation of patterns at the macroscale. As our knowledge of marine systems continues to expand, the relative abundance of secondary metabolites in different geographic locations may be better understood. However, the literature supports the idea that local pressures and habitat, genetic composition, mode of response and metabolism of the algae play a significant role in shaping distribution patterns of secondary metabolites (e.g. Wright

et al. 2000, 2004; Edwards et al. 2006). Large-scale geographic patterns may be hard to deduce in light of the numerous potential microhabitats that likely generate varied chemical compositions within and between algal classes (cf. Pavia and Åberg 1996). Focusing research on metabolite production at a finer resolution and applying innovative molecular and biochemical techniques will better shape our understanding of the essential factors and selective forces involved in macroalgal secondary metabolism.

6.3 Patterns of Secondary Metabolites at the Microscale

6.3.1 Metabolite Distribution Within the Thallus

Studies that address the site of production, the transport, and the deposition of secondary metabolites provide another level of resolution that needs to be considered when evaluating the function of secondary metabolites and their patterns of distribution. Unlike approaches that quantify an alga's total metabolite concentration, studies that localize metabolites contained within algal tissues provide evidence of more subtle changes in metabolite distribution in response to stimuli and may clarify metabolite function.

The data from these studies support the hypothesis that secondary metabolites in marine algae have functions other than, or in addition to, herbivore deterrents. Localizing metabolites within certain regions of the thallus may have a minimal effect on large grazers that will potentially consume the entire algal thallus, and thus do not preferentially select tissue types or cell layers. Surface oriented distribution patterns imply defense against pathogen or bacterial colonization, epiphytism, mesograzers (small immobile herbivores), or protection against abiotic influences.

Although algae are under constant threat of bacterial infection, there are few observations of infections in vivo, which have made macroalgae the focus of intense research regarding antimicrobial or antifungal compounds (Smit 2004). Although it is assumed that macroalgae contain antimicrobial compounds, the nature of these metabolites and their location within an alga remain largely unknown in most algal species. In differentiated terrestrial plants, metabolites can be stored in vesicles, gland cells, cavities, canals, or other specialized structures (Fahn 1988; Duke et al. 1994; Wink 1997), and their distribution and localization within the plant have direct effects on their ecological function (Bernays and Chapman 1994). Although macroaglae may not have differentiated tissues, they do have particular cell types and have shown differential patterns of metabolite distribution within the thallus. To elucidate ecological function of some secondary metabolites, work has turned to determining the composition and concentration of algal surface metabolites, their site of production, and modes of delivery (reviewed by Steinberg et al. 2002 and the text that follows).

In brown algae, phlorotannins are localized in specialized bodies called physodes (Ragan 1976). Shifting the experimental approach, from chemical assays of total phlorotannin concentration to microscopic methods that describe physode transport and establish the timeframes at which phlorotannins accumulate in response to abiotic or biotic stimuli, has provided new insight into the understanding of phlorotannin production and function. It is known that physodes are derived from the endoplasmic reticulum (ER) and Golgi of the cell (Schoenwaelder and Clayton 2000). It appears that physodes are transferred across the cytosol and incorporated into the cell wall, where the phlorotannins are assumed to have a structural role and thus be involved in primary metabolism (Schoenwaelder and Clayton 2000; Arnold and Targett 2003).

Chemical measurements of tannin content in several brown algae have implied an induced response of phlorotannins in response to wounding (e.g. Van Alstyne 1988; Peckol et al. 1996; Hammerstrom et al. 1998). A recent combined microscopy approach was able to detect a rapid and dynamic production of phlorotannins within cells after wounding of proximal tissue (Lüder and Clayton 2004). Phlorotannin-containing physodes accumulated around the site of injury and extended outward. This accumulation was apparent as early as 24 h after wounding and was observed throughout the 9-day experiment (see Fulcher and McCully 1971). The active accumulation and mobilization of the phlorotannin pool were clearly demonstrated using light, fluorescence, and electron microscopy. Although this was considered to be a strong response on the part of the alga, the amount of phenolic accumulation could not have been detected via traditional spectrophotometric methods, even with their increased sensitivity (Stern et al. 1996). In this case, the application of combined microscopy clearly illustrated a sophisticated defense response in brown algae. It is possible that in many cases where phlorotannin induction has not been observed, it indeed occurred, but at levels too low to be detected with current methods.

Physodes accumulate in the outer region of mature brown algae, such as the epidermis and the cortical cell layers, as well as in meristematic or apical cells. Localization in these cell layers implies a defensive role against biotic (herbivores/pathogens) and abiotic (UV light) factors. *Hormosira banksii* develops a protective dark brown layer or "sunburn" which consists of oxidized phenolics leached from dead cells on the algal surface upon exposure to intense light treatment (Schoenwaelder 2002). Shibata et al. (2004) also found that the phlorotannins in the brown algae *Eisenia bicyclis* and *Ecklonia kurome* accumulated in the outer cortical cell layer, and were exuded only upon cell death. Neither phlorotannins, nor their monomeric building block 1,3,5-trihydroxybenzene, was secreted from healthy cells, but other phenolic compounds (2,4-dibromophenol and 2,4,6-tribromophenol) were secreted (Shibata et al. 2006) and exhibited some of the same functionalities as phlorotannins (e.g., UV absorbing capabilities). Studies have shown that phenolic compounds exuded into the surrounding seawater may serve a bioactive role in this capacity, perhaps as surface settlement inhibitors or sunscreens (Ragan and Glombitza 1986; Swanson and Druehl 2002).

The localization of several defensive compounds in red algae has clarified their ecological function, in a manner analogous to studies on terrestrial plants. The red alga *Delisea pulchra* is known for its production of halogenated furanones, which are implicated in a range of activities from herbivore deterrents to settlement inhibitors (Maximilien et al. 1998; Dworjanyn et al. 1999). Dworjanyn et al. (1999) combined microscopy techniques with chemical methods and culture studies to relate furanone function to algal biology. Fluorescence microscopy verified that furanones are stored in the large vacuole of specialized gland cells. The number of gland cells, the furanone concentration at the surface of the thallus, and the total furanone content was highest at the growing tip and lowest toward the base of the alga (Dworjanyn et al. 1999). The concentration of furanones in young actively growing tissue is consistent with the optimal defense theory (ODT; see Chap. 7), and suggests a protective role of furanones in the young tissue. The gradient of furanones from the surface toward the interior of the thallus was likely bactericidal or algicidal, since the concentration of furanones (~100 ng cm^{-2}) was well above a biologically active level (de Nys et al. 1995; Maximilien et al. 1998). And at biologically relevant concentrations, *D. pulchra* extracts, furanone fractions, and pure furanones significantly reduced settlement success by *Ulva* sp., *Ceramium* sp., *Polysiphonia* sp., and *Ectocarpus siliculosus* – common fouling macroalgae (Dworjanyn et al. 2006a). The red alga *Asparagopsis armata* contains more than 100 described halogenated compounds (McConnell and Fenical 1977) and specialized gland cells with tubular connections to a parent cell wall. This connection facilitates transport of halogenated compounds to the algal surface without cell damage (Paul et al. 2006a, b). The dominant halogenated compounds detected in the alga, bromoform and dibromoacetic acid, were detected in surrounding growth media, supporting the theory that these compounds are actively transported to the external region of the alga and secreted, likely as defense compounds.

Combining microscopy and traditional biochemical methods to examine the multiple factors involved in metabolite production and function, i.e., abundance of gland cells, the alga's ability to produce metabolites and form protective transport mechanisms, and the efficacy of specific metabolites against colonization, enabled the generation of a more thorough understanding of the ecological role of halogenated compounds in red algae (Dworjanyn et al. 1999, 2006a; Paul et al. 2006a, b). Lüder and Clayton (2004) observed de novo synthesis of tannin-containing physodes at wound sites, indicating that physodes are not transported from other parts of the alga. Adapting innovative methods to the study of secondary metabolites is a new area of exploration that will allow us to determine whether an alga has the ability to generate defense compounds in situ, or must transport compounds to specific sites in response to environmental stimuli. Understanding these cellular functions, i.e., the life of secondary metabolites within the cell and within the alga and the factors that affect the mobilization and production, will clarify patterns of distribution within the algal thallus, as well as variable patterns between individuals. Hence, subcellular phenomena involving secondary metabolites within algal cells have direct impacts on the observed patterns of distribution at broader perspectives.

6.3.2 Temporal Responses Within Algae: Seconds to Weeks

Timescales of defense responses, such as the variation in time to initiation and/or duration of response, affect patterns of distribution and abundance within an alga. Investigations conducted on a longer scale of days to weeks overlook short-term (<1 day) responses within the alga, such as the oxidative burst, (the rapid production and excretion of reactive oxygen species), signaling cascades, and gene transcription and translation. The synthesis, transport, bioaccumulation, and secretion of metabolites occur on longer timescales. With the exception of activated defenses, which have not been reported in brown algae, responses on various timescales ranging from seconds to weeks appear in all three algal divisions. The apparent pervasiveness of certain responses within algal groups, such as the induction of phlorotannins in the Fucales for several days to weeks, or the oxidative burst in the Laminariales, is most likely due to intensive study on certain phenomena in a few species and may not represent the overall prevalence of these mechanisms within certain algal groups.

Short-term, rapid responses of macroalgae in response to environmental stimuli are now well documented (Table 6.1). It is probable that many of the long-term responses studied to date, such as the accumulation of phlorotannins, are tightly linked to events on these shorter timescales. Oxidative burst appears to be a common physiological response within macroalgae (e.g. Collén and Pedersen 1994; Küpper et al. 2002, 2006; Ross and Van Alstyne 2007, see Chap. 12). For example, brown algae recognize self (alginate) and nonself (lipopolysaccharides) elicitors via a rapid induction of the oxidative burst response (Küpper et al. 2001, 2002, 2006). The efficacy of the oxidative burst against bacterial infection also has been shown in several studies (Küpper et al. 2002, 2006; Potin et al. 2002) and appears to be linked to other short-term responses such as fatty acid oxidation and the triggering of oxylipin cascades, similar to immune responses seen in higher plants (Bolwell et al. 1995; Wojtaszek 1997).

Investigations at these short timescales (within seconds) in marine systems are not numerous primarily because of methodological constraints. However, advancements in methodology may lead to discovery of this defense response in many algal species, where the capacity for rapid self-defense against infection would confer a selective advantage. The oxidative burst has been found in organisms ranging from bacteria to mammals, and its detailed mechanisms (signaling, gene information) have been well defined (e.g. Lamb and Dixon 1997; Pohnert and Boland 2002; Mahalingam and Fedoroff 2003; Halliwell and Gutteridge 2007). This established knowledge of the oxidative burst in other organisms has been directly applied to algae and has led to a rapid and thorough understanding of the mechanisms at work (e.g. Dring 2006; Lesser 2006 and references within). In contrast, some of the long-studied phenomena in algae (e.g. phlorotannin production in Fucales) remain uncharacterized, likely because of lack of the same mechanisms in better studied organisms.

It may be that temporal responses to environmental pressures occur in tandem at both the short and long terms, but further investigations are needed to confirm such

Table 6.1 Types of rapid defenses within the different macroalgal divisions, the relative response time of each mechanism, and the elicitor used in each study

Defensive mode of action	Phylum	Species	Time of response	Defense against	Source
Activated defense	Chlorophyceae	*Halimeda* spp.	Seconds	Wounding	1
		Caulerpa taxifola	Seconds	Wounding	2
		Ulva fenestrate	10 min	Wounding	3
	Rhodophyceae	*Polysiphonia hendryi*	10 min	Wounding	3
Oxidative burst	Phaeophyceae	*Laminaria* spp.	15 min	Oligogluronates	4
		Fucus vesiculosus	10 min	Oligogluronates	4
		Laminaria digitata	30 min	Pathogenic algae	4
		L. digitata	60–90 min	Bacterial LPS	5
	Chlorophyceae	*Dasycladus vermicularis*	35–45 min	Wounding	6
		Acrochaete aperculata	10 min	λ-Carrageenan	7
	Rhodophyceae	*Chondrus crispus*[a]	20 min	Pathogenic algae	7
		Gracilaria conferta	30 min	Pathogenic bacteria	8
		Eucheuma platycladum	10 min	Wounding	9
Fatty acid oxidation and oxylipin pathway[b]	Phaeophyceae	*L. digitata*	30–60 min	Bacterial LPS	5
	Rhodophyceae	*C. crispus*[a]	24 h	Pathogenic algae	10
		C. crispus[a]	24 h	Methyl jasmonate	10
		Gracilaria chilesis	30 min	Wounding	11

LPS lipopolysaccharides
1 Paul and Van Alstyne 1992; *2* Jung and Pohnert 2001; *3* Van Alstyne et al. 2001b; *4* Küpper et al. 2002; *5* Küpper et al. 2006; *6* Ross et al. 2005; *7* Bouarab et al. 1999; *8* Weinberger and Friedlander 2000; *9* Collén and Pederson 1994; *10* Bouarab et al. 2004; *11* Lion et al. 2006
[a]Gametophyte stage
[b]Oxylipin production directly involved in a defense response

interactions. The role of plant-plant signaling and induction of metabolite synthesis may, in fact, be linked by short-term reactions such as the oxidative burst and the oxylipin pathway, as seen in terrestrial plants (Liechti and Farmer 2002; Farmer et al. 2003). These multitemporal responses may aid in explaining how an induced defense can be an effective mechanism for fitness even against large grazers such as sea urchins, which can cause fatal damage to an alga in time periods much shorter than those reported for biosynthesis of metabolites. It warrants consideration that long-term defense responses may be (1) secondary to an initial (yet

undetected) defense response, (2) protection against secondary infection (pathogens) as a result of the grazing, (3) or a result of kin selection in response to a localized change in herbivore pressure. In the many systems where induced responses on timescales of days to weeks have been observed, additional investigation into immediate responses may identify additional protective mechanisms actively at work.

6.4 Advances in the Characterization of Patterns of Chemical Defenses

6.4.1 Phylogeny Meets Ecology

"What drives selection?" remains the underlying question behind the observed distribution patterns of secondary metabolites. In very broad terms there are two driving forces: phylogeny and ecology. Secondary metabolites are derived from a combination of phylogenetic factors, such as the genetic information and biochemical potential (i.e., what enzymes are present) and ecological factors, such as the biotic and abiotic components of an ecosystem. Terrestrial plant research has shown that these factors interact to shape the evolution of secondary metabolite regulation and production. Both phylogeny and ecology are critically important in the characterization of the distribution and function of natural products. Multiple models have emerged for predicting observed phenotypic, genotypic, and geographic differences in plant defenses, including trade-offs and costs of secondary metabolite production (see reviews by Loomis 1953; McKey 1974; Rhoades 1979; Bryant et al. 1983; Tuomi et al. 1988; Herms and Mattson 1992; Cronin and Hay 1996b; Cronin 2001; Strauss et al. 2002; Stamp 2003; see Chap. 7).

Several studies have addressed the popular concept of "trade-offs" in terrestrial plant chemical ecology. Leimu and Koricheva (2006) performed a meta-analysis study on 31 studies covering 17 plant species, to determine the relationship between tolerance traits (i.e., increased photosynthesis) and resistance traits (i.e., increased chemical defense). They concluded, "there is no overall trade-off in plants between tolerance and resistance to herbivores." Agrawal and Fishbein (2006) proposed reinstating the term "syndromes" when discussing plant defenses, thereby steering away from considerations of patterns as distinctly defined trade-offs and embracing a collective outcome of multiple selective pressures, including both phylogeny and ecology. Recent studies in marine systems have attempted to tease apart the roles of phylogeny versus ecology in production of secondary metabolites by investigating the genetic profile (genotype) of algae along with biotic or abiotic factors (Jormalainen et al. 2003; Jormalainen and Honkanen 2004; Wright et al. 2004; Honkanen and Jormalainen 2005; Dworjanyn et al. 2006a). Genetic variability of herbivores and their response to chemical defenses have also been investigated (Sotka 2003). Together, these studies have broadened our understanding of the role

of genetic variation in chemical defenses, better characterized costs associated with defense, and isolated the dominant selective pressures (i.e., phylogeny, herbivory, environment).

It is assumed that selection of chemical defense compounds in marine systems is driven primarily by the presence of herbivores and their selective grazing pressure, as is common in terrestrial systems. However, this assumption has not been rigorously tested in many marine habitats. While numerous studies identify factors that cause variation in the concentration or distribution of algal secondary metabolites, few address the role of genetic composition in the observed variation. Wright et al. (2004) were the first to investigate the relationship between genetic profile of the red alga *D. pulchra* and variability in chemical defense compounds. Specifically, they set out to determine whether the variability in chemical compounds was heritable (i.e., correlated to genotype), and whether this heritability responded to grazing selection pressure. In genetically identical individuals, there was a large variation of furanone concentration (3.8 ± 0.5 to 33.9 ± 2.5 mg/g dry mass). There was a significant relationship between genotype, total furanone concentration, and furanone structure, supporting a genetic basis for the observed variation in secondary metabolites (Wright et al. 2004). However, the calculated heritability values for chemical composition (both total and individual) implied that environment probably played a stronger role in the observed variation.

Jormalainen and Honkanen (2004) compared associations between genotypes and fouling organisms in *Fucus vesiculosus* to address the role of genotype in resistance to fouling, including the relationship of phlorotannin to epiphyte load. Past history of the alga, variable fitness costs, and genotype all played a role in phlorotannin production, but a large amount of variability remained within each of these measures (Jormalainen and Honkanen 2004). On closer examination, the researchers found that genotypes of *F. vesiculosus* taken from two distinct initial populations showed different tendencies toward resistance (i.e., chemical defense) or tolerance of fouling organisms (Honkanen and Jormalainen 2005). Patterns of genetic variation were not consistent across laboratory and field experiments. In the field, fouling varied across individuals, implying different levels of genetic resistance toward fouling throughout the community. However, in laboratory experiments, a different population of plants (different from those used in the field) showed no significant variation toward fouling (Honkanen and Jormalainen 2005). These methods illustrate that trends in fouling resistance are affected by both a genetic component and microhabitat and the observed variability does not appear to be uniform across different populations. Phlorotannin production also varied between different populations, but was not found to be correlated to epiphyte load.

Determining costs of secondary metabolites is inherently difficult as a result of the variable responses, multiple physiological roles of the metabolites, as well as the different types of costs involved in the production of secondary metabolites (production, transport, storage, maintenance) (Strauss et al. 2002). Emerging studies are now taking more of these variables into consideration. For example, work with clonal populations of *D. pulchra* has estimated costs of furanone production in relation to chemical defense and phenotype by investigations of reproductive

success, growth, and furanone content (the common trade-off approach), experimental and field correlations between genetic history, growth and furanone content, growth rate of algae with and without furanone production, and life history and furanone production (Dworjanyn et al. 2006a). In both laboratory and field experiments, growth rate was found to be positively linked with furanone production. The trait was family-dependent, implying a genetic correlation between furanones and growth. From this evidence, one would assume that there is little or no cost of furanone production, as there was no apparent trade-off in fitness correlates. However, when growth rate was compared across populations of furanone-producing and nonproducing populations, there was a significantly greater growth rate in the populations producing no furanones. Additionally, variation in growth rates was dependent on the clonal family that was used, supporting the idea that interindividual variability in chemical production exists.

Incorporating genetic history and the relationship of genetically driven variability of chemical defenses in these pioneering studies supports the importance of understanding genetic underpinnings and their involvement in the selection of traits involved in secondary metabolism. The merging of genotype (molecular information) and ecology (cues that trigger changes in that molecular information) will provide a stable scaffolding upon which to build a better framework for identifying patterns of metabolite production and their role in an alga's ecology. The investigations that incorporate genetic composition in their approach provide additional evidence for the importance of interindividual variability, for the idea that variability of chemical defenses can be genetically as well as environmentally based, and for the notion that populations of the same species may exhibit different patterns of genetic-based variability.

6.4.2 Metabolic Similarities Between Algal Groups

Although studies in marine chemical ecology often piggyback on terrestrial knowledge, in marine algae there is a lack of specific evidence for many of the secondary metabolite biochemical pathways, especially at the gene and enzyme levels. Radioisotopic labeling experiments have proven valuable in establishing the presence or absence of particular pathways in algal groups and the likely steps involved in compound production. Analysis of pathways can also be extremely valuable from an evolutionary perspective, since the same biosynthetic abilities are not observed universally across algal groups (Table 6.2); this is likely due to their unique evolutionary histories. Enzymatic inhibition of metabolic pathways and structural determination of metabolites are two other approaches most often used to determine the presence of secondary metabolic pathways. Molecular characterization of the specific genes encoding enzymes is also beginning to provide additional insight into the distribution patterns of metabolic pathways of secondary metabolism in algae. Such microscale techniques will enable us to define molecular switches (biochemical and environmental regulators of gene expression) and patterns

Table 6.2 Common biosynthetic pathways, their occurrence in cyanobacteria and algae, methods used to detect the pathways, enzymes verified as present, and indication of the presence of genetic information for the enzyme

Pathway	Algal group	Present	Absent	Enzymes verified[a]	Genetic information	Source
Eicosenoid	Cyanobacteria	ENZ/SC	–	LOX	No	1
	Phytoplankton	ENZ/SC	–	LOX	No	1
	Chlorophyta	ENZ/SC	–	LOX	No	1
	Rhodophyta	ENZ/SC	–	LOX	No	1
		MM	–	12-LOX	Yes	2
	Phaeophyta	ENZ/SC	–	LOX	No	3
Terpenoid MVA *(mevalonate-dependent)*	Cyanobacteria	–	X	–	–	–
	Phytoplankton	IL	–	–	No	4
	Chlorophyta	–	X	–	–	–
	Rhodophyta	IL	–	–	No	5
	Phaeophyta	IF	–	–	No	6
Terpenoid MEP *(mevalonate-independent)*	Cyanobacteria	IL	–	–	No	5
	Phytoplankton	IL	–	–	No	4
	Chlorophyta	IL	–	–	No	7
	Rhodophyta	IL	–	–	No	8
	Phaeophyta	–	UNK	–	–	–
Shikimate	Cyanobacteria	MM	–	EPSP synthase	Yes	9
	Phytoplankton	INH	–	EPSP synthase	No	10
		MM	–	Multiple	Yes	11
	Chlorophyta	MM	–	Multiple	Yes	11
	Rhodophyta	ENZ	–	SDH	No	12
		MM	–	Multiple	Yes	11
	Phaeophyta	INH	–	EPSP synthase	No	13
Phenylpropanoid	Cyanobacteria	–	UNK	–	No	–
	Phytoplankton	–	UNK	–	No	–
	Chlorophyta	–	UNK	–	No	–
	Rhodophyta	ENZ	–	PAL	No	12
	Phaeophyta	–	UNK	–	No	–
Polyketide	Cyanobacteria	MM	–	PKS/NRPS	Yes	14
	Phytoplankton	MM	–	PKS	Yes	15
	Chlorophyta	–	UNK	–	No	–
	Rhodophyta	–	UNK	–	No	–
	Phaeophyta	–	UNK	–	No	–

ENZ enzyme assays, *SC* structural composition, *MM* molecular methods, *IL* isotopic labeling, *IF* isotopic fractionation, *INH* inhibition studies, *UNK* unknown, *LOX* lipoxogenase, *EPSP synthase* 5-enolpyruvylshikimate-3-phosphate, *SDH* shikimate dehydrogenase, *PAL* phenylalanine ammonium lyase, *PKS* polyketide synthase, *NRPS* nonribosomal peptide synthase

1 Gerwick 1999; *2* Liu et al. 1994; *3* Boonprab et al. 2003; *4* Cvejic and Rohmer 1999; *5* Disch et al. 1998; *6* Chikaraishi et al. 2006; *7* Schwender et al. 2001; *8* Schwender et al. 1997; *9* Mayes et al. 1993; *10* Shick et al. 1999; *11* Richards et al. 2006; *12* Bouarab et al. 2004; *13* Pelletreau et al., unpublished data; *14* Dittman and Weigand 2006; *15* Rein and Barrone 1999

[a]Empty columns imply no *direct* evidence of these enzymes from these systems

of genetic regulation in marine algae. There are five biochemical pathways involved in the production of the most commonly observed secondary metabolites: the eicosanoid pathway, the isoprenoid pathway(s), the shikimate pathway, the phenylpropanoid pathway, and polyketide pathways.

The Eicosanoid Pathway: The most well characterized pathway for secondary metabolism is the eicosanoid pathway (Gerwick et al. 1993a, b; Gerwick 1999). Eicosanoids are produced by organisms ranging from bacteria to humans, and in terrestrial plants and animals the products of polyunsaturated fatty acid (PUFA) oxidation (oxylipins) are known to be involved in signaling and response to external stimuli. A well-characterized example is the jasmonic acid cascade involved in defense and signaling responses in higher plants (Farmer et al. 1992, 2003; Liechti and Farmer 2002). In algae this pathway is responsible for the formation of numerous oxylipins (potential signals), polyunsaturated aldehydes (potentially defensive), and gamete attractants (hormones) (Pohnert and Boland 2002; Potin et al. 2002; Boonprab et al. 2003; Paffenhofer et al. 2005; Pohnert 2005). The fatty acid precursors, as well as sites of oxidation, vary between algal groups, but eicosanoid metabolism as a whole appears to be well distributed among all algal groups, although certain algae exhibit unique properties. For example, the precursors in animals are C_{20} PUFAs, and in terrestrial plants, C_{18} or C_{16} PUFAs; however, the rhodophyte *Chondrus crispus* was found to metabolize *both* C_{20} and C_{18} PUFAs in response to pathogen attack (Bouarab et al. 2004). This work indicates that both the eicosanoid (C_{20} PUFA) and octadecanoid (C_{18} PUFA) pathways exist in red algae and both are involved in the defense of the *C. crispus* against the obligate algal endophyte *Acrochaete operculata*. This suggests the possibility that in early evolution of eukaryotic organisms, both pathways (eicosanoid and octadecanoid) may have been involved in generating signaling molecules and defense responses.

The Isoprenoid Pathway: Two pathways are known to exist for the synthesis of terpenes and steroids: a mevalonate-dependent pathway (MVA) and a mevalonate-independent pathway (2-*C*-methyl-D-erythritol-4-phosphate pathway; MEP). In animals the mevalonate-dependent pathway is prevalent, whereas both pathways exist in plants. The pathways are localized, with the MVA found in the cytosol and the MEP found in the plastids (Lichtenthaler et al. 1997; Lichtenthaler 1999). In algae, the distribution of these pathways is variable and may provide insight into an alga's evolutionary history. Similar to higher plants, a division between cytosolic and plastid-derived isoprenoids has also been observed in diatoms (Cvejic and Rohmer 2000) and red algae (Schwender et al. 1997; Wanke et al. 2001). This was not the case in cyanobacteria, where the MEP route was responsible for all isoprenoids produced, and in the euglenophytes, where the MVA pathway was solely responsible for production (Disch et al. 1998; Kim et al. 2004). Synthesis of terpenoids in Chlorophyta occurs exclusively via the MEP pathway (Schwender et al. 2001; Pohnert and Jung 2003). There is no division of terpene production between the two pathways, as seen in higher plants, possibly suggesting that the MVA pathway may have evolved multiple times or may have been lost in an early cholorphyte ancestor. Although terpenoid compounds are common in brown algae and have been extensively isolated and characterized from algal tissue, there is little

experimental evidence tracking the biosynthesis of these compounds in the phaeophytes. The similarity between brown and red algae of isotopic fractionation (ε) during sterol production suggests that the brown algal sterols are produced through a mechanism that is similar to that found in red algae (the MVA pathway) (Chikaraishi 2006), but no other experimental evidence exists to date to verify the mechanisms of isoprenoid production in brown algae.

The Shikimate Pathway: The shikimate pathway is responsible for the production of aromatic amino acids, and as such, its presence is necessary in phototrophic organisms. It is also responsible for making phenylalanine, the precursor of many plant secondary metabolites. Presence of the shikimate pathway in macroalgae was not experimentally verified until Richards et al. (2006) identified genes involved in all of the steps of the shikimate pathway in both green and red algae (*Chlamydomonas reinhardtii* and *Cyanidoschyzon merolae* respectively) as well as in the diatom *Thalassiosira pseudonana*. Work with *N*-phosphonomethlyglycine (glyphosate), which directly targets enzymes unique to the shikimate pathway, inhibited the production of mycosporine-like amino acids (MAAs), UV-protecting compounds (with potentially other ecological roles) commonly found among red algae and microalgae (Shick et al. 1999; Shick and Dunlap 2002). Glyphosate also caused a reduction in phlorotannin content and mortality in *F. vesiculosus*, providing anecdotal evidence that the shikimate pathway is present in brown algae (Pelletreau et al., unpublished data) Recently, presence and activity of shikimate dehydrogenase, a regulatory enzyme in the pathway, were measured in the red alga *D. pulchra* (Bouarab et al. 2004). The presence and upregulation of this enzyme in response to exposure to the signaling molecule methyl jasmonate (MeJA) provided the first enzymatic evidence that this pathway may be involved in defense response in *D. pulchra* (Bouarab et al. 2004). The detailed characterization of the shikimate pathway in higher plants and its role in primary and secondary metabolism via the generation of aromatic amino acids (which then leads to many secondary metabolites) render it an excellent candidate for comparing biochemical mechanisms across algal groups.

The Phenylpropanoid Pathway: The phenylpropanoid pathway, ubiquitous in higher plants, uses phenylalanine as a starting unit to generate an expansive range of metabolites. The only evidence to date of a phenylpropanoid pathway in marine algae is the presence and induced activity of the major regulatory enzyme phenylalanine ammonium lyase (PAL) in the red alga *C. crispus* (Bouarab et al. 2004). *C. crispus* exhibited an upregulation of this enzyme in response to oxylipins, resulting in lowered susceptibility to endophytic infection. Hence this pathway may be present in some macroalgae and involved in a similar defense response as is seen in higher plants. Although it would seem most likely that similar enzymes would be found in the chlorophytes because of their evolutionary relationship to higher plants, there is no experimental evidence of phenylpropanoid pathway enzymes in this algal group (Stafford 2000), or in phaeophytes.

The Polyketide Pathway: One metabolic process that has received much attention from natural product chemists is the production of secondary metabolites via polyketide synthase (PKS) enzymes. Polyketide synthases condense small starting

units, often acetate or malonate, into complex and diverse end products. Many of the toxins produced by harmful algal species and cyanobacteria are generated via polyketide synthases (e.g. Shimizu 2003; Dittmann and Wiegand 2006). Dinoflagellates are known to produce some of the largest and most complex polyketides yet identified (Rein and Borrone 1999; Berry et al. 2002; Snyder et al. 2003). These polyketides have been implicated in neurotoxic shellfish poisoning (brevetoxins), ciguatera fish poisoning (ciguatoxins), and diarrhetic shellfish poisoning (okadaic acid). In the macroalgae there has been little to no evidence of such an enzyme-mediated synthesis of secondary metabolites. This is curious for several reasons: (1) PKS are broadly distributed – found in bacteria, phytoplankton, diatoms, fungi, and higher plants; (2) the structure of many algal secondary metabolites implies the involvement of such a pathway; and (3) PKS enzymes are thought to have evolved from fatty acid synthases, which are necessary enzymes in all organisms because of their involvement in membrane production. Further investigation will provide evidence supporting the presence and function of this pathway in macroalgae.

Information regarding the presence of the metabolic pathways, their genetic underpinnings, and their regulation in marine algae has enhanced our understanding of the mechanisms by which certain secondary metabolites can be produced, and clarified our interpretations of distribution and production patterns of secondary metabolites both within and between algal groups. This detailed information strengthens our experimental capabilities, allowing researchers to directly examine responses to cues at the molecular level and enabling studies that address specific cellular responses, on-off switches, and potentially even the discovery of novel metabolites and pathways. A finely resolved understanding of what pathways are involved in metabolite production, what regulates these pathways, and what elicits pathway production enables a holistic understanding of all factors involved in shaping patterns of metabolite distribution.

6.4.3 The Use of Molecular Tools to Characterize Patterns of Gene Response Involved in Macroalgal Defenses

Plant chemical ecologists have embraced the use of molecular tools to characterize plant natural products and interactions between organisms at the cellular and molecular level (Dixon et al. 2002; Dixon 2005a, b; Xie and Dixon 2005; Ouborg and Vriezen 2007). The application of molecular tools to macroalgal chemical ecology has been slow to develop, mostly because of logistical difficulties such as effective nucleic acid extraction from algal tissue that contain high levels of inhibitors and coprecipitates (Phillips et al. 2001; Pearson et al. 2006; Varela-Alvarez et al. 2006), and the effects of these inhibitors on downstream applications. In terrestrial systems, advances in molecular understanding have been predicated on model organisms that are collectively studied by the scientific community (i.e., *Arabadopsis*). In marine algal systems no model organism has yet emerged. Despite

these hindrances, there have been several studies that have incorporated the use of molecular tools to better characterize metabolic processes of chemical defense at the cellular level in response to various cues (discussed below). In response to specific agents (e.g. methyl jasmonate), researchers can observe which of the genes and enzymes involved in secondary metabolism are up- or downregulated. The available information for molecular study in phycology is limited, especially when compared to the knowledge base for plant and fungal systems; however, this is changing rapidly. The presence of this information (expressed sequence tag (EST) data in the NCBI database, genome sequencing) serves as a positive feedback mechanism for future studies by increasing the collective knowledge of algal molecular composition.

To date, the only full size genome sequenced from a nonparasitic protist is of the diatom, *Thalassiosira pseudonana,* (Armbrust et al. 2004). The two other complete algal genomes are highly reduced: the unicellular red alga *Cyanidioschyzon merolae* (Matsuzaki et al. 2004), and the cryptophyte *Guillardia theta* (Douglas et al. 2001). At present, no macroalgal genomes have been fully sequenced, although several have been proposed, including the phaeophyte *Ectocarpus siliculosus* (Peters et al. 2004), and the rhodophyte *Porphyra yezoensis* (Waaland et al. 2004). These algal genomes will be particularly valuable in light of the unique evolutionary history that exists between the major algal divisions, and for their potential to yield insight into the evolution and modification of metabolic pathways and mechanisms in biosynthesis. It is also worthwhile noting that such novel metabolic mechanisms may also lead to new drug discoveries and applications.

Phycological molecular work has focused on phylogeny, using molecular techniques to trace endosymbiont lineages and determine the correct characterization of algal groups and evolutionary processes (e.g. Keeling 2004; Keeling et al. 2005; Patron et al. 2006; Waller et al. 2006; Rogers et al. 2007). One of the first molecular approaches to macroalgal ecological study was to characterize, at the genetic level, the processes occurring during desiccation in the brown alga *F. vesiculosus* (Pearson et al. 2001). Subtractive suppressive hybridization, followed by reverse Northern blot analysis, showed that 60–70% of the randomly selected clones (genes) were differentially expressed in hydrated versus desiccated algae. When these genes were compared to other genes in the NCBI database, the majority showed little or no homology to known genes. This dissimilarity is likely biased by the paucity of information on this algal group in the NCBI database, but may also reflect the fact that Phaeophyta contain distinct genetic patterns and information as a result of its unique evolutionary path. Pearson et al. (2001) were able to identify the upregulation of several photosynthetic genes in response to desiccation. Their work has served as a starting point for investigating genetic response to environmental stress.

More recently, EST libraries from protoplasts and mature specimens of the rhodopyhte *C. crispus* were generated to investigate the genes involved in stress response (which may involve production of secondary metabolites) and cell wall regeneration (Collén et al. 2006a). Eighteen percent of the genes in the stressed library were attributed to cell rescue, defense, and cell death, whereas only 3.5% of

the genes in the unstressed library were allocated to these processes, indicating significant effort into defense and/or protection. A large number of heat shock proteins (HSP), vanadium bromoperoxidase, antioxidant enzymes, and detoxification genes (potentially involved in the breakdown of halogenated compounds) were also identified. Red algae show unique traits in their metabolic pathways and in their genetic composition relative to other algae. Only genetic information can afford us a level of understanding that allows us to question whether we can assume that the defense systems between algal groups will function identically if they have fundamentally different building blocks underlying these responses.

Using the information generated from the EST library, Collén et al. (2006b) investigated the role of volatile MeJA on gene expression of *C. crispus* through microarrays and verification with quantitative real-time PCR, the first application of these methods in red macroalgae. Methyl jasmonate is a widely known signaling molecule in defense response in terrestrial plants, and there is evidence that it (or structurally similar molecules) functions as an inducer of defense response in marine algae (Arnold et al. 2001; Bouarab et al. 2004). *C. crispus* was exposed to MeJA for a 24-h period. DNA samples were taken at regular intervals and the expression patterns were compared. Six percent of the genes examined showed a response to MeJA some time during the course of the experiment (Collén et al. 2006b). Potential stress- and defense-related genes that were upregulated overlapped with some of those described by Collén et al. (2006a). Other genes involved in metabolism and in unknown functions were also upregulated following exposure to MeJA, while several others were downregulated. From this study, the authors concluded that algae undergo a dynamic response upon exposure to MeJA; that MeJA functions as a stress hormone by increasing the expression of stress-related genes; and that based on the genes expressed, the reactive oxygen mechanism may be involved. Using molecular methods, Collén et al. provided strong evidence supporting the role of MeJA in defense response of red algae, implying the presence of a valid signaling system in red algae.

6.5 Conclusion

Research in marine chemical ecology traditionally has focused primarily on the effects of herbivory and abiotic factors on the production of secondary metabolites in macroalgae. Standard techniques measured the total amount of secondary metabolites produced in response to manipulation and attempted to characterize tradeoffs in fitness versus cost. A microscale approach (i.e., subcellular, microscopic and molecular) combined with knowledge of genetic variability and composition can provide more comprehensive insight into the triggers for observed patterns of chemical defense and the up- and downregulation of metabolites. Similarly, understanding the regulatory factors involved in gene expression, the signaling molecules and cascades involved in cellular response, and the total timescale involved in the defense response from perception of cue to production of metabolite are all important

considerations. By incorporating these considerations, we will enhance our present understanding of chemical responses and better interpret observed patterns of metabolites at the cellular, organism, and ecosystem levels.

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7
Macroalgal Models in Testing and Extending Defense Theories

H. Pavia(✉) and G.B. Toth

7.1 Introduction

The ecological roles of secondary metabolites have become one of the most expansive areas of research within plant ecology during the last two to three decades. They have also become one of the most theory-laden fields of plant ecology, if not ecology in general, with a number of general models (theories) seeking to explain and predict patterns of chemical defenses in plants. (We use the terms model or theory rather than hypothesis because we define the latter term in a more restricted way: a testable prediction that is logically derived from an explanatory model/theory.) The discovery and characterization of tens of thousands of natural products in terrestrial plants and marine organisms during the last century called for models that could provide explanations and categorizations to this ample form of biodiversity. Suggestions that the primary function of these so-called secondary metabolites was as a defense against consumers (Dethier 1954; Fraenkel 1959) led ecologists to formulate a series of general defense models, especially during the 1970s and 1980s, to explain the distribution and variation of secondary metabolites. These defense models have allowed natural product chemists and chemical ecologists to put their specific work into a broader context and they have become a significant driving force for further studies of secondary metabolites and their ecological roles. The most influential defense models have been reviewed and discussed repeatedly in terrestrial (e.g., Herms and Mattson 1992; Karban and Baldwin 1997; Stamp 2003) as well as marine (e.g., Hay and Steinberg 1992; Cronin 2001) orientated literature. Defense theories can be categorized in different ways depending on the patterns they are intended to explain, i.e., inter- or intraspecific variation in defenses, or on the type of explanations they offer, e.g., proximate physiological

H. Pavia
Göteborg University, Department of Marine Ecology, Tjärnö Marine Biological Laboratory, SE 452 96 Strömstad, Sweden. e-mail: henrik.pavia@marecol.gu.se

G.B. Toth
Göteborg University, Department of Marine Ecology, Tjärnö Marine Biological Laboratory, SE 452 96 Strömstad, Sweden

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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mechanisms or ultimate evolutionary rationales. In this chapter we focus on a few influential models for intraspecific variation in defenses, because these are the models that, with a few exceptions, have been explicitly tested in studies with macroalgae during the last decade. Models for interspecific variation in plant defenses, e.g., the Resource Availability Model (Coley et al. 1985) and the Plant Apparency Model (Feeny 1976), have not been explicitly addressed in studies with seaweeds during recent years (see Amsler et al. 2005 for an exception), and results from the few earlier studies have already been reviewed elsewhere (Hay and Steinberg 1992; Steinberg 1992; Cronin 2001).

The most commonly tested models for intraspecific variation in macroalgal defenses during the last 10–15 years have been (i) the Optimal Defense Model (ODM) (McKey 1979; Rhoades 1979), with a focus on inducible defenses and intraindividual distribution of defenses, (ii) the Carbon-Nutrient Balance Model (CNBM) (Bryant et al. 1983), and (iii) the Growth-Differentiation Balance Model (GDBM) (Loomis 1932; Herms and Mattson 1992). All these three models have had strong influence on studies of chemical defenses in terrestrial plants, and the publications introducing or extending the models represent some of the most widely cited papers within the field of plant ecology. Nevertheless, there has been considerable confusion about the scopes, assumptions, and predictions of plant defense models. As pointed out by Stamp (2003) in a recent critical review of defense models, a clearer understanding of the basics and logical predictions of defense models would promote more critical experimental tests of these models, preferably with contrasting hypotheses that could potentially separate between the explanatory power of different models.

7.2 Defense Theories

7.2.1 Optimal Defense Model

The ODM is a demand-based model focusing on the plants' need for defenses and on the evolutionary pros and cons of defense allocation per se. It was originally formulated during the 1970s, assuming that herbivory is the primary selective force for the expression of defensive plant traits (McKey 1974; Rhoades 1979). The framework of the ODM can, however, also be used to predict spatial and temporal variations in different types of defenses against other natural threats, such as pathogens and epiphytes. Like other models based on an optimality approach, the ODM assumes that natural selection will tailor the expression of different traits toward the combination that is most favorable in terms of fitness, within the given environmental/physiological and genetic constraints. In short, the ODM states that defense chemicals are produced in direct proportion to the risk and the negative fitness consequences of attacks, and in inverse relationship to the costs of their production (Zangerl and Bazzaz 1992). The assumption that chemical defenses are costly, i.e., that they can be produced at the direct expense of other functions such as growth and reproduction, is an important underlying principle of the ODM, as well as

several other models of the evolution of defensive characters (e.g., Rhoades 1979; Coley et al. 1985; Fagerström et al. 1987; Simms and Rausher 1987). As a consequence, the ODM predicts that there should be selection for optimal allocation of defense chemicals in space and time, e.g., higher concentration in plant parts that are most vulnerable to attacks and that contribute most to plant fitness (Rhoades 1979; Bazzaz et al. 1987). One way to optimize the use of costly defense chemicals is to have an inducible increase in the production of these compounds as a response to attacks (Tallamy and Raupp 1991; Karban and Baldwin 1997). This potentially allows plants to produce large amounts of defense chemicals when they are needed, without having to pay the cost of being defended when enemies are absent.

7.2.2 Carbon-Nutrient Balance Model

In contrast to the ODM, the CNBM is a resource- or supply-based model, stating that the production of defense chemicals is determined by the relative availability of carbon and nutrients (Bryant et al. 1983, 1987, 1989; Reichardt et al. 1991). It is focused on the way in which resource availability affects the phenotypic expression of plant secondary metabolites, and it suggests that when acquired resources exceed the necessary levels for primary metabolism (growth), the excess can be shunted over to production of secondary metabolites. For example, when growth is nitrogen-limited, plants will increase their production of so-called carbon-based secondary metabolites, such as tannins. The CNBM, especially in its recent versions (e.g., Bryant et al. 1989; Tuomi et al. 1990; Reichardt et al. 1991; Herms and Mattson 1992), is in many ways closely related to overflow models of secondary metabolism, where secondary metabolites are seen essentially as waste products (Robinson 1974; Haslam 1985; Waterman and Mole 1989), although the models do not preclude that secondary compounds can have defensive properties. The CNBM has lately been severely criticized, and several authors have advocated a view where "It is time to accept that the CNBM is beyond repair…" (Koricheva 2002), and the CNBM "no longer warrants consideration as a viable hypothesis" (Nitao et al. 2002). These "funeral orations" (Lerdau and Coley 2002) have in turn been met by attempts to emphasize the useful aspects of the CNBM (Lerdau and Coley 2002; Stamp 2003), and by a sharp assertion that much of the critique of the CNBM is due to a fundamental misunderstanding of the model and misstatements of its predictions (Stamp 2003).

7.2.3 Growth-Differentiation Balance Model

Similar to the CNBM, the GDBM has been regarded as a supply-side model, as it emphasizes that the levels of defenses are indirectly determined by the supply of secondary metabolites, rather than actively regulated in order to fulfill defensive demands. The GDBM is based on the premise that there is a physiological trade-off

between growth and so-called differentiation processes, e.g., secondary metabolite production, and it focuses on changes in this trade-off with resource availability and ontogenetic shifts (Herms and Mattson 1992). In a timely manner (cf. Roff 1992; Stearns 1992), Herms and Mattson (1992) used the framework of the GDBM to discuss and predict how physiological constraints of differentiation processes are related to the evolution of different plant life-histories. In conformity with the CNBM, but in a more explicit and general way, the GDBM predicts that any factor that will inhibit growth more than photosynthesis will increase the availability of internal resources for differentiation processes, e.g., chemical defense production. The GDBM is also a more general model than the CNBM in that it can be used to predict intraspecific, including intraindividual, as well as interspecific variation in the production of secondary metabolites (Cronin 2001). In her comprehensive review of defense theories, Stamp (2003) concluded that the GDBM is a more mature model than the CNBM. Rigorous experimental tests of the GDBM are, however, very difficult to design and conduct (Stamp 2004), which is probably the reason why relatively few attempts have been made to address the GDBM in terrestrial (Stamp 2004), as well as marine systems (Cronin 2001).

7.3 The Status of Defense Models in Terrestrial Plant Ecology

The empirical evidence for the ODM (including its corollary inducible defense model), the CNBM, and the GDBM is mixed, and predictions derived from the models, especially the CNBM, have been falsified repeatedly (Herms and Mattson 1992; Berenbaum 1995; Karban and Baldwin 1997; Stamp 2003). Predictions of the models, however, have also gained support in numerous studies, and none of them have ever been claimed to be completely rejected (Berenbaum 1995; Stamp 2003). The continuous coexistence of the models probably to a large extent reflects natural variation in the responses and functions of different taxa and systems, providing many different theories with at least some support. The formulation of general models to explain all existing phenotypic variations in allocation of plant chemical defenses is probably not a realistic expectation. The continuous coexistence may, however, also be explained by a wide-spread confusion about the assumptions and predictions of the models (Pavia et al. 1999; Stamp 2003). Several authors have pointed out that although the ODM, CNBMs and GDBM can generate different predictions, they are not mutually exclusive models (see Stamp 2003 and references therein), and that they should not even be regarded as competing models (e.g., Tuomi 1992). The CNBM can be seen as a mechanistic model providing proximal explanations for the way that resource availability affects phenotypic expression of chemical defenses; the GDBM, as a model focusing on constraints for the evolution and expression of differentiation products; and the ODM is an evolutionary optimality model providing ultimate predictions of variation in defense levels, within given environmental and internal constraints. Still, from an evolutionary perspective, some of the statements made by advocators of source-based models, such as the CNBM, show an almost depreciating attitude toward plant

secondary metabolism, and toward natural selection as a tool to refine the metabolic machinery of plants. For example, it has been argued that "evolution of plant defense may…have proceeded independent of consumer adaptation" (Jones and Firn 1991) and that "plastic responses of plants to resource variation may be a consequence of substrate availability and not a result of regulated and adaptive responses" (Lerdau and Coley 2002). Given that secondary metabolites can have important functions as defensive and protective agents, there are no good reasons to assume that the production of defense chemicals in plants should be less well adapted, e.g., specific in activation and effects, and finely regulated by enzymes, than the secondary metabolism or immune system of animals. It seems far from any logic of evolutionary biology to assume that large phenotypic variation in chemical defenses is essentially a consequence of plants' incapability to regulate their metabolism. Indeed, several studies have shown that there is a high degree of specificity in the elucidation of plant chemical responses, in support of the notion that plants are able to actively regulate their secondary metabolism (see references in Tallamy and Raupp 1991; Karban and Baldwin 1997; Toth and Pavia 2007). Finally, it should be clarified that an adaptive, demand-based approach to plant chemical defense does not rule out that the relative availability of different resources (e.g., light and nutrients), or physiological and genetic constraints, are important in determining production of defense chemicals. All other things being equal, changes in, e.g., the carbon-nutrient ratio will change cost-benefit ratios of defenses and will thus alter the optimal level of investments in chemical defenses.

7.4 Empirical Tests of Defense Theories in Marine Studies

The criteria for inclusion of studies in this summary have been as follows: (1) use of marine macroalgae as model organisms, and (2) explicit tests of any of the described defense theories (ODM, CNBM, or GDBM) either through observations or through manipulative experiments. We have not included studies that only measure variation in secondary metabolites in different seaweed parts or after different treatments, unless they have specifically mentioned any of the models or hypotheses derived from the models in the introductory part of the paper. Therefore, the summary presented here does not represent an exhaustive review of all data from algal studies that are relevant for defense theories and algal chemical defenses.

7.4.1 Optimal Defense Model

Inducible Defense Model

Vascular plants are known to induce a variety of phenotypic changes in response to damage, including changes in secondary metabolite concentrations. Induced plant responses may lead to increased resistance toward future herbivory if changes have

negative effects on the preference and/or performance of herbivores (Karban and Baldwin 1997). Results from studies on damage-induced responses and resistance in marine macroalgae have been summarized in several recent reviews (e.g., Potin et al. 2002; Pohnert 2004; Amsler and Fairhead 2006; Paul et al. 2006; Toth and Pavia 2007). Furthermore, Jormalainen and Honkanen (2007) present a meta-analysis of the literature in Chap. 3 of this book, and therefore, we will not describe each of these studies in detail here, although the literature is summarized in Table 7.1. In general, the number of studies investigating damage-induced responses and resistance in marine macroalgae has increased dramatically during the last 5 years (Table 7.1). The overall results from meta-analyses summarizing this literature show that herbivore-induced resistance is present in seaweeds (Chap. 3; Toth and Pavia 2007), but the response depends on the macroalgal and herbivore species tested and on the timescale of the experiments (Toth and Pavia 2007). Brown and green, but not red, seaweeds induce significant resistance to further herbivory in response to grazing by small crustaceans and gastropods, but not in response to large gastropods and sea urchins (Toth and Pavia 2007). No significant differences in induced resistance were found between different seaweed tissues (meristematic vs. nonmeristematic vegetative), between temperate or tropical seaweeds, or in response to different types of damage (natural or simulated herbivory, or chemical cues; Toth and Pavia 2007).

To our knowledge, no herbivore-induced deterrent metabolites have been identified in green and red seaweeds, and by far the most studied class of secondary metabolites in seaweeds in relation to the IDM (and CNBM, see Table 7.2) is the phlorotannins (polyphenols). Phlorotannins are polymers of phloroglucinol (1,3,5-trihydroxy benzene) and are found only in brown seaweeds, where they have both primary and secondary functions (Ragan and Glombitza 1986). Phlorotannins can be induced in response to damage-related cues in some seaweed species (e.g., Van Alstyne 1988; Yates and Peckol 1993; Hammerstrom et al. 1998; Pavia and Toth 2000a; Arnold et al. 2001; Lüder and Clayton 2004), but not in all (e.g., Steinberg 1994; Toth and Pavia 2002; Hemmi et al. 2004, 2005). Jormalainen and Honkanen (Chap. 3) have analyzed the results from the current literature by using meta-analysis and have found a marginally significant overall effect of damage on induced phlorotannin content in brown seaweeds. However, when the data set was divided into studies using different types of inducing cues, only simulated damage resulted in significantly induced phlorotannin levels (Chap. 3).

In contrast to the research field of damage-induced responses in seaweeds, only a few studies have investigated hypotheses concerning the effects of abiotic threats on the production of secondary metabolites in marine macroalgae by explicitly testing hypotheses derived from the IDM (Pavia et al. 1997; Pavia and Brock 2000; Toth and Pavia 2000b; Fairhead et al. 2006). Because phlorotannins can form complexes with metal ions, they have been proposed to function as protection against toxic metal ions (Ragan and Glombitza 1986). However, the only study that has tested the effect of heavy metal exposure on the production of secondary metabolites in macroalgae did not find a significant effect of increased copper concentrations in the water on the phlorotannin content in the fucoid seaweed *Ascophyllum nodosum*

Table 7.1 Published studies explicitly testing hypotheses derived from the Induced Defense Model, a corollary model to the Optimal Defense Model, concerning variation in the concentration of secondary metabolites or resistance to herbivores in different macroalgal species

Reference	Species	Order	Treatment	Dependent variable
Van Alstyne 1988	*Fucus distichus*	Fucales	Simulated herbivory	Phlorotannins Resistance
Van Alstyne 1989	*F. distichus*	Fucales	Simulated herbivory	Phlorotannins Resistance
Renaud et al. 1990	*Padina gymnospora*	Dictyotales	Herbivory	Resistance
Yates and Peckol 1993	*F. vesiculosus*	Fucales	Simulated herbivory	Phlorotannins
Steinberg 1994	*Ecklonia radiata* *Sargassum vestitum*	Laminariales Fucales	Simulated herbivory	Phlorotannins
Steinberg 1995	*Alaria nana*	Laminariales	Simulated herbivory	Phlorotannins
Cronin and Hay 1996a	*Dictyota menstrualis*	Dictyotales	Herbivory	Diterpenes Resistance
Martinez 1996	*Lessonia nigrescens*	Laminariales	Simulated herbivory	Phlorotannins
Peckol et al. 1996	*F. vesiculosus*	Fucales	Simulated herbivory	Phlorotannins
Pavia et al. 1997	*Ascophyllum nodosum*	Fucales	Simulated herbivory	Phlorotannins
Hammerstrom et al. 1998	*Agarum fimbriatum* *Pleurophycus gardneri* *Laminaria complanata* *La. Groenlandica* *Hedophyllum sessile*	Laminariales	Simulated herbivory	Phlorotannins
Cetrulo and Hay 2000	42 species		Simulated herbivory	Resistance
Pavia and Brock 2000	*As. Nodosum*	Fucales	Herbivory	Phlorotannins
Pavia and Toth 2000a	*As. nodosum*	Fucales	Herbivory Simulated herbivory	Phlorotannins Resistance
Toth and Pavia 2000a	*As. nodosum*	Fucales	Water-borne cues	Phlorotannins Resistance

(continued)

Table 7.1 (continued)

Reference	Species	Order	Treatment	Dependent variable
Toth and Pavia 2000b	*As. nodosum*	Fucales	Copper	Phlorotannins
Arnold et al. 2001	*F. vesiculosus*	Fucales	Methyl jasmonate	Phlorotannins
Sotka et al. 2002	*S. filipendula*	Fucales	Herbivory	Resistance
			Water-borne cues	
Taylor et al. 2002	*S. filipendula*	Fucales	Herbivory	Resistance
Toth and Pavia 2002	*As. nodosum*	Fucales	Herbivory	Phlorotannins
			Simulated herbivory	
Jormalainen et al. 2003	*F. vesiculosus*	Fucales	Herbivory	Phlorotannins
Borell et al. 2004	*As. nodosum*	Fucales	Herbivory	Phlorotannins
			Simulated herbivory	Resistance
Hemmi et al. 2004	*F. vesiculosus*	Fucales	Simulated herbivory	Phlorotannins
				Resistance
Lüder and Clayton 2004	*E. radiata*	Laminariales	Simulated herbivory	Phlorotannins
Rohde et al. 2004	*F. vesiculosus*	Fucales	Herbivory	Resistance
			Water-borne cues	
			Simulated herbivory	
Weidner et al. 2004	9 species		Herbivory	Resistance
			Water-borne cues	
Ceh et al. 2005	*Hypnea pannosa*	Gigartinales	Herbivory	Resistance
	S. asperifolium	Fucales		
	Cystoceria myrica			
Dethier et al. 2005	*F. gardneri*	Fucales	Herbivory	Phlorotannins
Hemmi et al. 2005	*F. vesiculosus*	Fucales	Herbivory	Phlorotannins
Macaya et al. 2005	*Glossophora kunthii*	Dictyotales	Herbivory	Resistance
	Macrocystis integrifolia	Laminariales	Water-borne cues	
Molis et al. 2005	*E. cava*	Laminariales	Herbivory	Resistance

			Water-borne cues	
Rothäusler et al. 2005	*Glossophora kunthii* *Le. nigrescense* *Chondracanthus chamissoi* *Grateloupia doryphora*	Dictyotales Laminariales Gigartinales Ceramiales	Herbivory	Resistance
Toth et al. 2005	*As. nodosum*	Fucales	Herbivory	Phlorotannins Resistance
Coleman et al. 2006	*As. nodosum*	Fucales	Herbivory Amylase	Phlorotannins Resistance
Diaz et al. 2006	*Codium extricatum* *Co. platylobium* *Galaxaura diessingiana* *Gracilaria capensis* *Hy. spicifera*	Bryopsidales Nemaliales Gracilariales Gigartinales	Herbivory Water-borne cues	Resistance
Fairhead et al. 2006	*Desmarestia menziesii*	Desmarestiales	Herbivory Simulated herbivory	Phlorotannins Resistance
Rothäusler and Thiel 2006	*Le. nigrescens* *M. integrifolia*	Laminariales	Herbivory	Resistance
Svensson et al. 2007	*As. nodosum*	Fucales	Herbivory	Phlorotannins
Toth 2007	12 species		Herbivory Water-borne cues	Resistance
Toth et al. 2007	*As. nodosum*	Fucales	Herbivory	Phlorotannins Resistance

Table 7.2 Published studies explicitly testing hypotheses derived from the Optimal Defense Model concerning intraplant variation in either the concentration of secondary metabolites or resistance in different macroalgal species

Reference	Species	Order	Tissue types (assigned fitness value)	Fitness estimate	Dependent variable
Steinberg 1984	*Alaria marginata*	Laminariales	Reproductive (high) Nonmeristematic vegetative (low)	No	Phlorotannins Resistance
Paul and Fenical 1986	40 species	Caulerpales	Blades (high) Stipes (high) Holdfasts (low)	No	Terpenoids
Tuomi et al. 1989	*Fucus vesiculosus*	Fucales	Reproductive (high) Nonmeristematic vegetative (low)	No	Phlorotannins Toughness
Tugwell and Branch 1992	*Ecklonia maxima* *Laminaria pallida* *Macrocystis angustifolia*	Laminariales	Holdfast (high) Stipe (high) Reproductive sori (high) Infertile nonmeristematic vegetative fronds (low)	No	Phlorotannins
Cronin and Hay 1996c	*Dictyota ciliolata*	Dictyotales	Young (high) Old (low)	No	Lipophilic extracts Resistance
Van Alstyne et al. 1999	21 species	Laminariales Fucales	Meristem (high) Reproductive (high) Blade (low)	No	Phlorotannins
Honkanen et al. 2002	*F. vesiculosus*	Fucales	Apical (high) Basal (low)	No	Phlorotannins
Pavia et al. 2002	*Ascophyllum nodosum*	Fucales	Basal (high) Apical (intermediate) Reproductive (low)	Yes	Phlorotannins Resistance
Sotka et al. 2002	*Sargassum filipendula*	Fucales	Blades (low) Stipes (high)	No	Resistance

Taylor et al. 2002	*S. filipendula*	Fucales	Bottom stipe (high) Top stipe (intermediate-high) Bottom blade (low) Top blade (intermediate-low)	Yes	Resistance Induction
Toth and Pavia 2002	*L. hyperborea*	Laminariales	New meristem (high) Old meristem (low) New blade (low) Old blade (low)	No	Phlorotannins
Fairhead et al. 2005a	*Desmarestia anceps* *D. menziesii*	Desmarestiales	Primary stem (high) Holdfast (high) Lateral branches (low)	No	Chemical defenses Physical defenses Resistance
Fairhead et al. 2005b	*D. anceps* *D. menziesii*	Desmarestiales	Primary stem (high) Holdfast (high) Lateral branches (low)	No	Phlorotannins
Toth et al. 2005	*As. nodosum*	Fucales	Basal (high) Apical (low)	No	Phlorotannins Resistance

(Toth and Pavia 2000b). Furthermore, both phlorotannins and mycosporine-like amino acids (MAAs) have been proposed to function in the protection against harmful UV radiation, because they absorb light in the UV range (e.g., Ragan and Glombitza 1986; Pavia et al. 1997; Amsler and Fairhead 2006). Pavia et al. (1997) showed that the phlorotannin content in *A. nodosum* increased when seaweeds were exposed to increased levels of UV-B radiation in a field experiment, and that phlorotannins contribute significantly to the UV absorption of algal extracts. In addition, when UV-B radiation was filtered out, phlorotannin content in *A. nodosum* decreased significantly when compared with that in controls (Pavia and Brock 2000). However, no change in phlorotannin content of *A. nodosum* was found when UV-B levels were increased in a laboratory experiment (Pavia and Brock 2000), but this experiment was performed during a different season, when initial phlorotannin levels were significantly higher compared with those during the study by Pavia et al. (1997). No effect of UV-B radiation was found on the phlorotannin content of the Antarctic brown algal species *Desmarestia anceps* and *D. menziesii* (Fairhead et al. 2006). Since the first experimental test of the causal relationship between UV radiation and phlorotannin levels by Pavia et al. (1997), several other studies have investigated the effect of increased UV radiation on the production of phlorotannins (e.g., Swanson and Druehl 2002; Henry and Van Alstyne 2004; Roleda et al. 2006), as well as MAAs (e.g., Karsten et al. 1998, 1999; Franklin et al. 1999, 2001; Hoyer et al. 2002; Kräbs et al. 2002; Peinado et al. 2004), in macroalgae. However, because these studies did not explicitly address the IDM in the introduction, we have chosen not to discuss them in this chapter (but see Chap. 13).

Intraindividual Variation

Intraplant variation in herbivore resistance, or chemical and physical defenses have been described in a large number of macroalgal species (e.g., Hay 1996; Van Alstyne et al. 2001a). Many of these studies are purely descriptive or explorative and do not explicitly test hypotheses derived from the ODM (i.e., there is no mention of the ODM or no specific hypotheses formulated in the introduction), and they have therefore not been included in this review. Studies predicting different levels of chemical or physical resistance in different macroalgal parts (e.g., meristems, reproductive tissues, holdfasts, primary stems, and other nonmeristematic vegetative tissues) have been included (Table 7.2). In all but two studies (Pavia et al. 2002; Taylor et al. 2002), however, differences in fitness value between different plant parts were based on general reasoning (e.g., meristems and reproductive tissues are more valuable, compared to nonmeristematic vegetative tissue) rather than specific and objective measurements and rankings. Furthermore, a few macroalgal studies have tested ODM-derived predictions of intraplant variation in defenses based on assumed differences in attack risk (e.g., Paul and Van Alstyne 1988). Seaweeds are different from terrestrial plants because they do not have underground structures, and all plant parts should be equally accessible to herbivores. Differences in attack risk of seaweed tissues have nevertheless been correlated to their susceptibility by

measuring the presence or amount of grazing damage (e.g., Paul and Van Alstyne 1988). However, the presence of damage is a result of the interaction between the risk of herbivore attack and the deterrent effect of chemical and structural defenses (i.e., if a plant part is well defended, the herbivores will not eat that part even if it is accessible to them; cf. Toth et al. 2005), and, therefore, herbivore damage may not be an evolutionarily relevant measure of attack risk.

Most studies investigating intraplant variation in secondary metabolites in relation to hypotheses derived from the ODM have measured phlorotannin content in different brown algal species (Table 7.2). In one of the early and influential studies, tissue levels of phlorotannins were found to be higher in the more valuable reproductive sporophylls than in the nonmeristematic vegetative parts of the kelp *Alaria marginata*, (Steinberg 1984). Furthermore, the high phlorotannin levels were correlated to a decreased herbivore pressure by the herbivorous gastropod *Tegula funebralis*, both in laboratory and field experiments, showing that reproductive parts of *A. marginata* were more resistant to grazing than were nonmeristematic vegetative parts (Steinberg 1984), in support of the ODM. In contrast, the phenolic (phlorotannin) content tended to be higher in nonmeristematic vegetative than in reproductive parts of the fucoid *Fucus vesiculosus* in the northern Baltic Sea (Tuomi et al. 1989). However, the phlorotannin content in different parts of this species was negatively correlated to toughness, and the authors concluded that although reproductive parts had low levels of potential chemical defenses, they were well defended mechanically (Tuomi et al. 1989). Moreover, higher phlorotannin content was found in the basal parts than in the apical meristem of *F. vesiculosus*, and the authors argued that basal parts might be more valuable than apical meristems because the seaweeds risk loosing more biomass when grazers feed on the basal parts (Honkanen et al. 2002).

Tugwell and Branch (1992) compared the phlorotannin levels in different tissues (holdfast, stipe, meristems, reproductive sori, and infertile nonmeristematic vegetative fronds) of three kelp species (*Ecklonia maxima, Laminaria pallida*, and *Macrocystis angustifolia*) on the west coast of the Cape Peninsula of South Africa. They found higher levels of phlorotannins in the holdfast, stipe, and meristems than in nonmeristematic vegetative tissue of all three species. Phlorotannin levels were also high in the reproductive tissues of *E. maxima* and *M. angustifolia*, but low in *L. pallida*. Furthermore, when high levels of phlorotannins were detected, they were located in the outer meristoderm rather than in the cortex and medulla (Tugwell and Branch 1992). In a large screening investigation using 21 fucoid and kelp seaweed species from the Northeastern Pacific, Van Alstyne et al. (1999) found significant intraplant variation in phlorotannin content in ten species. Of these species, higher levels of phlorotannins were found in the meristematic than in nonmeristematic vegetative tissues for both kelps and fucoids. Furthermore, phlorotannin content was higher in reproductive tissues than in nonmeristematic vegetative tissues for kelps, but lower for fucoids (Van Alstyne et al. 1999).

Apart from phlorotannins, variation in herbivore resistance (measured in consumption bioassays) among different algal parts has also been investigated in a few studies (Table 7.1). Cronin and Hay (1996b) investigated consumption by two

herbivores (*A. longimana* and the sea urchin *Arabica punctulata*) on young (apical) and old (middle) parts of the brown seaweed *Dictyota ciliolata*. They hypothesized that apical parts should have a higher fitness value than middle parts because the apical parts contain the meristems in this species. However, both the herbivore species preferred the young apical parts and this preference was correlated both to a lesser toughness and to a lower content of chemical defenses in apical than in basal parts (Cronin and Hay 1996b). Therefore, the authors concluded that their results were not in accordance with the predictions of the ODM (Cronin and Hay 1996b). In contrast, Fairhead et al. (2005a) hypothesized that the primary stem had the highest fitness value in two Antarctic brown seaweeds (*Desmarestia anceps* and *D. menziesii*), followed by the holdfast and the lateral branches (which contain both meristems and reproductive tissues). In accordance with these predictions, the primary stem of *D. anceps* was most strongly defended both chemically and physically through a higher concentration of secondary compounds and greater toughness. The holdfast was strongly physically defended, while the lateral branches were only moderately chemically defended (Fairhead et al. 2005a). The patterns of chemical and physical defenses between different tissue types in *D. menziesii* were less clear. However, the authors concluded that this was in accordance with the ODM, because tissue differentiation is also less pronounced in *D. menziesii* than in *D. anceps* (Fairhead et al. 2005a). The phlorotannin content in different parts of *D. anceps* and *D. menziesii* did not follow predictions of the ODM (Fairhead et al. 2005b). *D. anceps* had slightly lower phlorotannin content in the primary stem than in holdfasts and lateral branches, while there was no significant difference in the phlorotannin content of different *D. menziesii* parts (Fairhead et al. 2005b).

All of the studies mentioned earlier have assumed, rather than measured, the fitness value of different algal parts. Pavia and coworkers (2002) used demographic elasticity (proportional sensitivity) analysis to estimate the fitness value of different plant parts (meristems, stipes, and reproductive tissue), representing different life history processes (growth, stasis, and fertility), of *A. nodosum*. They found that stipes had the highest fitness value in *A. nodosum*, followed by mersitems and reproductive tissue. The fitness values of different *A. nodosum* tissues were strongly positively correlated to both the phlorotannin content and the resistance to the herbivorous gastropod *Littorina obtusata* (Pavia et al. 2002). In a later study, it was shown that induced increase in phlorotannin content and resistance, measured as negative effects on herbivore performance, is also correlated to the fitness value of *A. nodosum*, with significantly higher induced responses in basal tissues than in apical meristems (Toth et al. 2005). Meristematic top blades contributed more to seaweed growth than did bottom blades in the fucoid *Sargassum filipendula* (Taylor et al. 2002), indicating that the top blades have higher fitness value than bottom blades. Furthermore, bottom stipes were assumed to have a fitness value higher than that in top stipes because they attach the rest of the biomass to the substratum. The amphipod *Ampitoe longimana* consumed more of top blades and stipes than bottom blades and stipes. However, this was not correlated to chemical defenses or the nutritional content of the different parts. Rather, bottom blades are the toughest and appeared to be mechanically defended. In contrast to *A. nodosum*

(Toth et al. 2005), induced resistance was found only in the top blades of *S. filipendula* (Taylor et al. 2002). The authors concluded that valuable basal stipes are mechanically and constitutively defended, while the valuable meristematic tissues have inducible chemical defenses (Taylor et al. 2002).

7.4.2 Carbon-Nutrient Balance Model

This section contains results from studies testing hypotheses derived from the CNBM concerning the effects of resource availability (nutrients and light) on the secondary chemistry of macroalgae. We excluded studies investigating effects of resources on macroalgal resistance to herbivores through changes in palatability in bioassays (e.g., Weidner et al. 2004), because the palatability of macroalgae to herbivores is affected both by secondary chemistry and by nutritional content (e.g., Cruz-Rivera and Hay 2003). Furthermore, because the CNBM does not explicitly assume that the secondary chemicals have a defensive function, the predicted changes in secondary chemistry may not always be correlated to changes in palatability, and vice versa.

Most of the included studies have investigated the effect of nutrients on the phlorotannin content of fucoid (especially *F. vesiculosus*) seaweeds either through observational correlations or through manipulative experiments (Table 7.3, but see Puglisi and Paul 1997) with varying results. Ilvessalo and Tuomi (1989) found that the phlorotannin content in three different populations of *F. vesiculosus* from the northern Baltic Sea was negatively correlated to the nitrogen content in the seaweed thallus. These results were corroborated by Yates and Peckol (1993), Peckol et al. (1996), and Pavia and Toth (2000b) in different *F. vesiculosus* populations from the east coast of the United States and the west coast of Sweden. In contrast, there was no significant correlation between the carbon-nitrogen ratio and phlorotannin content, and no significant difference in phlorotannin content of *F. vesiculosus* collected at different distances from eutrophicating fish farms in the northern Baltic Sea (Honkanen et al. 2002; Hemmi et al. 2005). In addition, either no correlations or positive correlations between tissue phlorotannin and nitrogen content were found for *A. nodosum* on the Swedish west coast (Pavia et al. 1999; Pavia and Toth 2000b) and on the Isle of Man in the Irish Sea (except for one site where a weak negative correlation was found; Pavia et al. 1999). In conclusion, previous correlative studies testing hypotheses derived from the CNBM have found large intra- and interspecific variation in the relationship between the nitrogen and phlorotannin content in fucoid seaweeds. Furthermore, no significant correlation between monoterpene concentrations and tissue nitrogen and phosphorous was found for the red seaweed *Portieria hornemannii* in Guam (Puglisi and Paul 1997).

Several studies have tested hypotheses derived from the CNBM by manipulating the availability of resources (nutrients or light) either in laboratory or in field experiments (Table 7.3). The phlorotannin content in *F. vesiculosus* at sites with naturally low nitrogen levels was decreased when seaweeds were fertilized in the

Table 7.3 Published studies explicitly testing hypotheses derived from the Carbon-Nutrient Balance Model concerning variation in the concentration of secondary metabolites in different macroalgal species

Reference	Species	Order	Treatment	Dependent variable
Ilvessalo and Tuomi 1989	*Fucus vesiculosus*	Fucales	Observation	Phlorotannins
Yates and Peckol 1993	*F. vesiculosus*	Fucales	N, P	Phlorotannins
Arnold et al. 1995	*Lobophora variegata*	Dictyotales	N	Phlorotannins
Cronin and Hay 1996c	*Dictypta ciliolata*	Dictyotales	N	Terpenoids
	Sargassum filipendula	Fucales	Light	Phlorotannins
Peckol et al. 1996	*F. vesiculosus*	Fucales	N	Phlorotannins
Puglisi and Paul 1997	*Portieria hornemannii*	Gigartinales	N, P, K	Monoterpenes
Pavia et al. 1999	*Ascophyllum nodosum*	Fucales	Observation	Phlorotannins
Van Alstyne and Pelletreau 2000	*F. gardneri*	Fucales	N, P, Fe	Phlorotannins
Pavia and Brock 2000	*Ascophyllum nodosum*	Fucales	N	Phlorotannins
Pavia and Toth 2000b	*A. nodosum*	Fucales	Observation	Phlorotannins
	F. vesiculosus		Light	
Hemmi and Jormalainen 2002	*F. vesiculosus*	Fucales	N, P	Phlorotannins
Honkanen et al. 2002	*F. vesiculosus*	Fucales	Observation	Phlorotannins
Jormalainen et al. 2003	*F. vesiculosus*	Fucales	N, P	Phlorotannins
Hemmi et al. 2004	*F. vesiculosus*	Fucales	N, P, K	Phlorotannins
Hemmi et al. 2005	*F. vesiculosus*	Fucales	N, P	Phlorotannins
Koivikko et al. 2005	*F. vesiculosus*	Fucales	N, P, K	Phlorotannins
Svensson et al. 2007	*A. nodosum*	Fucales	N, P, K	Phlorotannins
Toth et al. 2007	*A. nodosum*	Fucales	N, P, K	Phlorotannins

field, but no such response was found for seaweeds growing at a high nitrogen site (Yates and Peckol 1993; Peckol et al. 1996). Furthermore, nutrient enhancement either reduced (Jormalainen et al. 2003; Hemmi et al. 2004) or had no significant effect (Pavia and Toth 2000b; Hemmi and Jormalainen 2002; Hemmi et al. 2005) on the phlorotannin content of *F. vesiculosus* in laboratory experiments. Juvenile *F. gardneri* exposed to nitrogen and phosphorous decreased their phlorotannin content when compared with control cultures (Van Alstyne and Pelletreau 2000). The phlorotannin content in the tropical brown algae *Lobophora variegata* was significantly lower in nutrient-enriched algal cultures (Arnold et al. 1995). However, no significant effect of increased nutrient levels on the phlorotannin content was found in *S. muticum* in the field (Cronin and Hay 1996c) or *A. nodosum* in laboratory experiments (Pavia and Brock 2000; Pavia and Toth 2000b; Svensson et al. 2007) or in the field (Toth et al. 2007). In conclusion, nutrient addition

appears to either decrease or have no effect on the phlorotannin content of brown seaweeds. Furthermore, nutrient enrichment increased triglyceride concentrations, but did not affect concentrations of ochtodene (a cyclic monoterpene) in *P. hornemannii* (Puglisi and Paul 1997), or the concentration of diterpenes in *Dictyota ciliolata* (Cronin and Hay 1996c).

Effects of experimental nutrient enrichment can be confounded with decreased light (i.e., carbon fixation) availability if enhanced nutrient levels increase the amount of epiphytes on the seaweed thallus (e.g., Cronin and Hay 1996c; Jormalainen et al. 2003). Jormalainen et al. (2003) argued that the decreased phlorotannin content found in nutrient-enriched *F. vesiculosus* was due to light limitation caused by an increased amount of periphyton rather than due to a shift in resource allocation from phlorotannin production to growth, because nutrient-enriched seaweeds with periphyton grew significantly less than enriched seaweeds where periphyton was removed. The hypothesis that light availability affects the phlorotannin content in seaweeds was explicitly tested by exposing *F. vesiculosus* and *A. nodosum* from sun-exposed and shaded sites either to ambient sunlight or to shading in a 2-week laboratory experiment (Pavia and Toth 2000b). *F. vesiculosus*, but not *A. nodosum*, exposed to ambient sunlight had a significantly higher phlorotannin content than did plants that were shaded. On the other hand, *A. nodosum* collected in sun-exposed sites showed higher phlorotannin levels compared with plants collected in shaded sites (Pavia and Toth 2000b). Light also had a significant positive effect on the phlorotannin content in *S. filipendula* that were grown under ambient light or shading in natural populations (Cronin and Hay 1996c). However, increased light availability decreased the concentration of diterpenes in *D. ciliolata* (Cronin and Hay 1996c). Furthermore, the ochtodene concentration in shaded *P. hornemannii* decreased significantly, compared with initial field controls (Puglisi and Paul 1997). Finally, it should be noted that studies on the effect of natural light, as a resource, on the secondary metabolism of photosynthetic organisms, might confound increased resource availability with an increased demand for protection against UV radiation (see Pavia et al. 1999).

7.4.3 Growth-Differentiation Balance Model

Although there are numerous previous studies that have compared the secondary metabolite content or herbivore resistance in meristematic/juvenile or nonmeristematic vegetative/adult macroalgal tissues under different nutrient environments (e.g., Paul and Fenical 1986; Hay et al. 1988; Paul and Van Alstyne 1988; Denton et al. 1990; Van Alstyne et al. 2001b; Pavia et al. 2003; Stiger et al. 2004; Connan et al. 2004, 2006; Plouguerné et al. 2006, and references in Tables 7.2 and 7.3), we know of only two studies that explicitly formulate and test hypotheses derived from the GDBM (Cronin and Hay 1996b; Van Alstyne et al. 1999). On the basis of the criteria described in the introduction to this chapter, we chose to include only these two studies in this review. Cronin and Hay (1996b) found that the young apical

meristems of *Dictyota ciliolata* were more palatable to two herbivore species (the crustacean *Ampithoe longimana* and the sea urchin *Arabica punctulata*), compared with older parts of the seaweed thallus. Furthermore, lipophilic extracts from old thallus parts were more deterrent than extracts from young apices, and the concentration of two diterpene alcohols (pachydictyol A and dictyol B) was higher in the old parts (Cronin and Hay 1996b) in support of the GDBM. However, in contrast to the hypotheses derived from the GDBM, Van Alstyne et al. (1999) found that both meristems and reproductive tissues of several kelp species had higher phlorotannin levels than did nonmeristematic vegetative tissues. Furthermore, meristems had higher, and the reproductive tissues lower, phlorotannin content when compared with nonmeristematic vegetative tissues in rockweeds (Van Alstyne et al. 1999).

7.4.4 Tests of Hypotheses from More Than One Model

Testing contrasting hypotheses from more than one model in the same experiment/observation is a powerful tool to compare the predictive value of different models. If the same prediction (hypothesis) can be derived from more than one model, there is no logical way to separate between models. For example, the assumption that chemical defenses are costly to produce, which is an important logical building stone of the ODM and other demand-based models, is usually evaluated by testing the prediction that there should be a trade-off between defense levels and growth rate. However, as pointed by Pavia et al. (1999), a negative relationship between growth and concentration of so-called carbon-based secondary metabolites is predicted also by the CNBM, which assumes that growth has higher priority than secondary metabolism and that little or no direct costs are incurred by the production of carbon-based defense chemicals (Bryant et al. 1983; Herms and Mattson 1992; Karban and Baldwin 1997). Consequently, other logically derived predictions are needed to separate between these two models. If growth is nutrient limited, the CNBM predicts that there will be a negative relationship between the availability of the limiting nutrient and secondary metabolite concentration. On the other hand, if secondary metabolite production occurs at the direct expense of growth, as assumed by the ODM, the trade-off between carbon-based secondary metabolite production and growth should be stronger under more nutrient-rich conditions (Simms 1992). These contrasting predictions were tested by Pavia et al. (1999), who found a significant negative relationship between phlorotannin content and growth in *A. nodosum* populations, and that the trade-off was more pronounced in populations with relatively high tissue nitrogen levels. Furthermore, the relationship between tissue nitrogen and phlorotannin content was weak and variable. Consequently, the authors concluded that this specific system provided more support for the ODM, than for the CNBM (Pavia et al. 1999).

Several studies have simultaneously tested the IDM and the CNBM, although these two models are not mutually exclusive, i.e., the predictions are usually not

contrasting (but see Pavia et al. 1999). Peckol et al. (1996) were the first to study the interactive effect of nutrient availability and herbivory on the production of phlorotannins in *F. vesiculosus* on the east coast of the United States. They found a large temporal and spatial variability in the algal response to herbivore and nutrient treatments, and concluded that phlorotannin production is controlled by a complex interaction of environmental, developmental, and defense-related factors (Peckol et al. 1996). In contrast to this suggestion, no significant interactive effect of simulated or natural herbivory and nutrient availability on the phlorotannin production was found for *A. nodosum* from the Swedish west coast (Pavia and Brock 2000; Toth et al. 2007), or for Baltic Sea *F. vesiculosus* (Hemmi et al. 2004, 2005). Svensson et al. (2007), however, found a significant herbivore-induced phlorotannin content in *A. nodosum* under ambient, but not under nutrient-enriched laboratory, conditions. The tissue nitrogen content of the enriched *A. nodosum* plants in the experiment by Pavia and Brock (2000) was somewhat lower than that in the nutrient-enriched plants in Svensson et al. (2007), which may explain the discrepancy between the results. Furthermore, Pavia and Brock (2000) used dissolved ammonium nitrate rather than NPK fertilizer to increase nutrient levels, indicating that nutrients other than nitrogen may be responsible for the significant interaction between herbivory and nutrient enrichment in Svensson et al. (2007).

Jormalainen et al. (2003) found a decrease in the growth and phlorotannin content, and an increase in the amount of epibiota on the seaweed thallus, when *F. vesiculosus* was exposed to high nutrient levels. Removing epibiota from the seaweed thallus resulted in increased growth, but no significant change in the phlorotannin content of the seaweeds. Exposing the seaweeds to gastropods that do not graze directly on *F. vesiculosus*, but remove epibiota and hyaline hairs (which are involved in nutrient uptake of the seaweeds), had no significant effect on the growth, but increased the phlorotannin content, of the seaweeds (Jormalainen et al. 2003). The authors argued that the observed changes in growth and phlorotannin content in *F. vesiculosus* in response to the different treatments supported the CNBM rather than the ODM, and that the induced phlorotannin content is not an adaptive response to snail grazing, but rather a consequence of resource manipulation by the snails. This is an interesting alternative hypothesis; however, the critical treatments and/or experiments to test this hypothesis (e.g., artificial removal of hyaline hairs, measurements of nutrient uptake by the seaweeds, and effects of induced responses on grazer preference/performance) were not performed. Furthermore, the gastropods did graze on *F. vesiculosus*, because the authors showed that snail grazing resulted in shorter hyaline hairs, which should have negative fitness consequences for the alga if it results in a reduced ability of nutrient uptake. Moreover, if induced seaweed responses result in increased resistance to further grazing, they should be considered adaptive responses irrespective of the underlying mechanism (e.g., reduced growth or an increased production of secondary metabolites). Therefore, questioning the effectiveness of induced defenses on the basis of the experiments included in the study by Jormalainen et al. (2003) is premature, although their results do support the predictions derived from the CNBM.

Contrasting predictions derived from the ODM and GDBM have been tested in two previous studies (Cronin and Hay 1996c; Van Alstyne et al. 1999) with different results. Cronin and Hay (1996c) found support for the predictions they derived from the GDBM, because they found lower defense levels in apical parts of *Dictyota ciliolata*. Van Alstyne et al. (1999), on the other hand, concluded that they found more support for the ODM in their screening investigation of 21 kelp and rockweeds, where the highest levels of phlorotannins were found in the nonmeristematic vegetative tissues, which often anchor the algae to the substratum and therefore are crucial for the survival of the plant.

7.5 Summary and Conclusions

In accordance with empirical tests in terrestrial systems, hypotheses concerning variation in macroalgal chemical defenses derived from the ODM, CNBM, and GDBM have both been supported and rejected in several studies. The literature on the IDM has been summarized and analyzed with meta-analyses (Toth and Pavia 2007, Chap. 3), but the number of studies or model species used to test hypotheses derived from the CNBM and GDBM is still too few to draw any firm conclusions from the present data set. To date, the IDM has received most support, while the results from studies investigating intraplant variation in macroalgal defenses within the context of the ODM are more difficult to interpret, mainly because of the lack of objective estimates of fitness values and attack risks among different tissues. Furthermore, predictions from the CNBM have almost exclusively studied variation in phlorotannins, with variable results. Explanations for these variable results could be as follows: variation in the levels of available nutrients or light; the effect of nutrients on phlorotannins varies between different seaweed species; or phlorotannins are problematic metabolites to use for tests of the CNBM because they may also have primary functions. The experiments manipulating light (e.g., Cronin and Hay 1996c; Pavia and Toth 2000b) show some support for the CNBM, but the effect of light could be confounded with induced responses to increases in UV light. Only two studies (Cronin and Hay 1996b; Van Alstyne et al. 1999) have tested predictions from the GDBM, with mixed results. Moreover, we know of no previous studies that have formulated hypotheses, based on the GDBM, concerning how the pattern of secondary metabolites should vary between algae with different life histories. Overall, few algal studies have formulated specific a priori hypotheses that are distinctive and contrasting between different models. We suggest that future research within algal chemical ecology would benefit from (i) the development of more integrative models and critical tests of carefully derived a priori hypotheses that are distinctive for different explanatory models; (ii) taking more advantage of the “uniqueness” of macroalgal systems, e.g., macroalgae are phylogenetically distinct test systems consisting of nonvascular plants with inferior source-sink connections, and a generally intense and less human-eroded herbivore pressure, compared with terrestrial systems; and (iii) a stronger focus on a few

model systems where it is possible to develop knowledge and new techniques concerning genetic and biochemical mechanisms behind algal chemical defenses (cf. Kessler and Baldwin 2002).

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8
Ecological and Physiological Roles of Dimethylsulfoniopropionate and Its Products in Marine Macroalgae

K.L. Van Alstyne

8.1 Introduction

Dimethylsulfoniopropionate (DMSP) is among the most common natural products in marine organisms. It is found in microalgae (Lovelock et al. 1972; Kiene et al. 1996; Malin and Kirst 1997), macroalgae (Challenger and Simpson 1948; Reed 1983; Bischoff et al. 1994; Karsten et al. 1990, 1994; Van Alstyne et al. 2001b), invertebrates (Iida and Tokunaga 1986; Hill et al. 1995, 2000; Broadbent et al. 2002; Broadbent and Jones 2004; Jones and Trevena 2005; Van Alstyne et al. 2006b), and fish (Ackman and Hingley 1968; Ackman et al. 1972; Levasseur et al. 1994), as well as in many salt marsh (Dacey et al. 1987; Pakulski and Kiene 1992; Otte and Morris 1994) and terrestrial plants (Otte et al. 2004), often in high concentrations relative to other secondary metabolites. It is so common that one of the breakdown products of DMSP cleavage, dimethylsulfide (DMS), has been attributed with being responsible for the "odor of the sea" (White 1982); however, more concentrated DMS can have a less pleasant odor that is commonly associated with old or rotting shellfish and fish (Motohiro 1962; Brooke et al. 1968; Ackman et al. 1972; Levasseur et al. 1994).

DMSP is a compound that cannot easily be characterized as either a primary or a secondary metabolite because it has primary functions in some organisms and secondary functions in others (Stefels 2000; Steinke et al. 2002; Yoch 2002); in many, if not most of the organisms that produce it, it probably has both. In addition to their physiological and ecological roles, DMSP and DMS are important to global biogeochemical cycling and possibly climate (Malin et al. 1992; Liss et al. 1994; Malin and Kirst 1997; Yoch 2002).

The purpose of this chapter is to review the role of DMSP and related compounds in marine algae, with an emphasis on benthic macroalgae. The first section of this review will focus on DMSP. The second section will focus on its cleavage and the roles of its cleavage or breakdown products DMS, acrylate, and acrylic acid

K.L. Van Alstyne
Shannon Point Marine Center, Western Washington University, 1900 Shannon Point Road, Anacortes, WA 98221, USA. e-mail: kathy.vanalstyne@wwu.edu

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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on processes ranging from chemical interactions within cells to effects on large-scale climatic processes. For recent reviews providing additional depth of coverage on DMSP in microalgae, the reader is referred to Kiene et al. (1996), Liss et al. (1994), Malin and Kirst (1997), Malin et al. (1992), Simó (2001), Steinke et al. (2002), and Yoch (2002).

8.2 Dimethylsulfoniopropionate

DMS was first described from the red macroalgae *Polysiphonia fastigiata* and *P. nigrescens* by Haas (1935). Over a decade later, Challenger and Simpson (1948) isolated DMSP as the source of DMS from *P. fastigiata* and suggested that an enzyme was involved in DMS generation. That enzyme, which is now called DMSP lyase, was described from *P. lanosa* several years later (Cantoni and Anderson 1956). Since then, DMSP has been found in many marine algal taxa, including chlorophytes, rhodophytes, prymnesiophytes, dinophytes, diatoms, prasinophytes, and chrysophytes (Malin and Kirst 1997).

8.2.1 DMSP Synthesis

DMSP is a tertiary sulphonium compound that is synthesized from methionine (Fig. 8.1). The synthetic pathways from methionine to DMSP in terrestrial flowering plants (Hanson et al. 1994; James et al. 1995), salt marsh plants (Kocsis et al. 1998; Kocsis and Hanson 2000), and green algae (Gage et al. 1997) differ, suggesting that the ability to produce DMSP has evolved multiple times (Stefels 2000). In the flowering plant *Wollastonia biflora*, the conversion of methionine to S-methylmethionine (SMM) occurs via an enzyme located in the cytosol (Trossat et al. 1996). SMM is then decarboxylated and deaminated to form DMSP-aldehyde (DMSP-ald), which is subsequently oxidized to DMSP by an enzyme in the chloroplast (Trossat et al. 1996). In the salt marsh vascular plant *Spartina alterniflora*, the synthetic pathway is similar except that SMM is first converted to DMSP-amine and then to DMSP-ald (Kocsis et al. 1998). In the chlorophyte *Ulva*[1] *intestinalis*, the synthesis of DMSP from methionine proceeds via the intermediates 4-methylthio-2-oxobutyrate (MTOB), 4-methylthio-2-hydroxybutyrate (MTHB), and 4-dimethylsulfio-2-hydroxybutyrate (DMSHB) (Gage et al. 1997).

Photosynthesis is required for methionine production, leading to the possibility that DMSP synthesis may also be constrained by the availability of light (Kirst 1996; Stefels 2000). This is supported by the results of experimental studies of light limitation. When the Antarctic macroalgae *Ulothrix implexa*, *Ulothrix subflaccida*, *Ulva*

[1] The genus *Enteromorpha* was recently synonymized with the genus *Ulva* (Hayden et al. 2003). Throughout this review, species that were formerly called *Enteromorpha* will be referred to as *Ulva*, regardless of how they were named in the original reference.

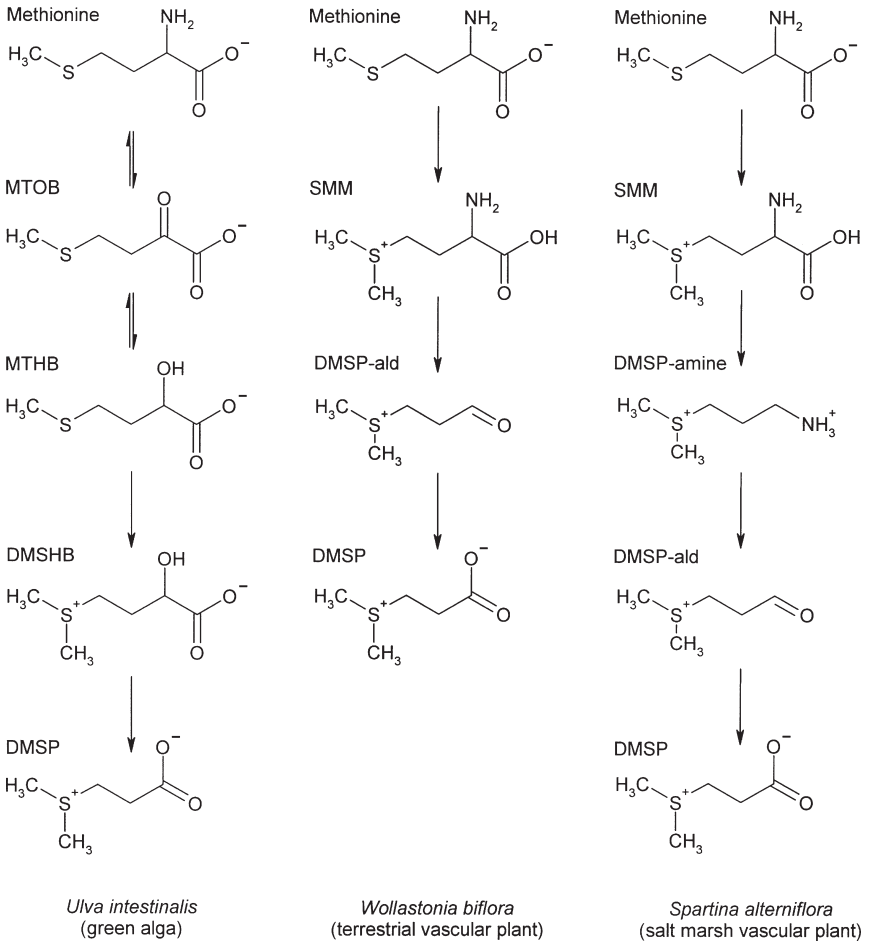

Fig. 8.1 Proposed synthetic pathways for dimethylsulfoniopropionate from methionine for three algal and plant species. *DMSHB* 4-dimethylsulfio-2-hydroxy-butyrate, *MTHB* 4-methylthio-2-hydroxybutyrate, *MTOB* 4-methylthio-2-oxobutyrate, *SMM* S-methylmethionine

bulbosa, *Urospora penicilliformis*, and *Acrosiphonia arcta*, the Chilean alga *Ulva rigida*, and the German alga *Blidingia minima* were grown at irradiances ranging from 2 to 55 µmol m^{-2} s^{-1} in the laboratory, DMSP concentrations were consistently higher in algae grown in the higher light intensities (Karsten et al. 1990, 1991a, 1992). However, when *Ulva lactuca* was exposed to a range of higher light intensities in outdoor sea-tables there was no change in DMSP concentrations (Van Alstyne et al. 2007). The lowest light treatment in this experiment (which had a noon photosynthetically active radiation value of 411 µmol m^{-2} s^{-1}) should have been sufficient to saturate photosynthesis in *U. lactuca* (Nelson et al., submitted).

Seawater chemical components other than salinity (see below) have not been shown to impact tissue DMSP concentrations in macroalgae, although data on the subject are

limited. Seawater nitrogen concentrations did not affect DMSP concentrations in *Ulva lactuca* in experimental laboratory manipulations (Van Alstyne et al. 2007). There have been no studies of sulfur limitation on DMSP production in marine macroalgae; however, sulfate concentrations above 2.5% of those naturally occurring in seawater are sufficient to prevent sulfur from being a limiting factor in DMSP production in the coccolithophorid *Hymenomonas carterae* (Vairavamurthy et al. 1985).

8.2.2 The Distribution of DMSP in Marine Macroalgae

DMSP is found in many marine algal phyla; however, within phyla, some genera, families, or orders contain it in high (10's to 100's of mmol g^{-1} dry mass [DM]) concentrations, whereas within others (including some groups that are closely related) it occurs in low concentrations (<10 mmol g^{-1} DM) or is undetectable (reviewed by Van Alstyne and Puglisi 2007). In macroscopic green algae in the Order Ulvales, DMSP has been found in high concentrations in all but the tropical species *Ulva conglobata* (Bischoff et al. 1994). In the green algal order Caulerpales, DMSP can be present in high concentrations in *Codium* spp.; however, it is undetectable or in low concentrations in other genera. It is notable that this order contains many common tropical genera such as *Avrainvillea*, *Caulerpa*, *Halimeda*, *Penicillus*, *Rhipocephalus*, and *Udotea* (Graham and Wilcox 1999). In other green algal orders, DMSP concentrations tend to be low or undetectable, with occasional exceptions. In the red and brown macroalgae, DMSP occurs in low concentrations or is undetectable in all but two related red algal genera, *Polysiphonia* and *Halopytis* (Reed et al. 1983; Karsten et al. 1994; Van Alstyne et al. 2001b; Van Alstyne and Puglisi 2007).

The tendency for DMSP concentrations to be high in the Ulvales and low in most of the tropical Caulerpales produces a latitudinal cline in DMSP concentrations in the northern hemisphere (Van Alstyne and Puglisi 2007), with concentrations increasing with increasing latitude. This is contrary to the pattern observed for many marine (Bolser and Hay 1996) and terrestrial (Coley and Aide 1991) secondary metabolites, but is similar to the pattern observed for brown algal phlorotannins in the northern Pacific (Steinberg 1986, 1989, 1992; Van Alstyne and Paul 1990; Steinberg et al. 1995; Van Alstyne et al. 2001a). Latitudinal clines have also been reported at lower taxonomic scales. In northern hemisphere *Ulva* species, there is a significant increase in DMSP concentrations with increasing latitude (Van Alstyne and Puglisi 2007). DMSP concentrations also increased from southern sites to northern sites over a scale of 70 km in *Ulva lactuca* that was sampled at six locations in Washington, USA (Van Alstyne et al. 2007). Interpopulation differences in DMSP concentrations among sites have also been described (Reed 1983; Bischoff et al. 1994; Van Alstyne et al. 2001b, 2007); these generally span a single order of magnitude or less (Van Alstyne and Puglisi 2007).

There are only a few examples of intraindividual and temporal variation in DMSP concentrations in marine macroalgae. In *Ulva lactuca*, tissues at the base of

the thallus have significantly higher DMSP concentrations than do tissues in the middle of the thallus or at the edges (Van Alstyne et al. 2007). Several species of marine macroalgae have been shown to have different DMSP concentrations at different times of the year (Reed 1983; Van Alstyne et al. 2001b), and DMSP concentrations can change in response to experimental manipulations in daylength and light intensities in the laboratory (Karsten et al. 1990); however, at this point data from field-collected algae are too limited to determine whether seasonal patterns in tissue DMSP concentrations exist.

8.2.3 Physiological and Ecological Functions of DMSP

Osmotic Acclimation

DMSP is perhaps best known for its role in osmotic acclimation, where it functions as a compatible solute, i.e., an osmolyte that can accumulate in cells without disrupting metabolic processes or causing other damage to the cell (Kirst 1996; Welsh 2000). Osmotic acclimation in algae is accomplished by many molecules other than DMSP, including inorganic ions (primarily K^+, Na^+, Cl^-, and SO_4^{2-}), polyols, sugars, and quaternary ammonium compounds (Kirst 1989). Changes in DMSP in response to salinity changes are less likely to disrupt metabolic processes than do changes in the concentrations of inorganic ions. The use of DMSP as an osmolyte also has an advantage over the use of ammonium compounds, since DMSP does not contain nitrogen (Kirst 1996), which can be a limiting nutrient (Valiela 1995). The high levels of DMSP in some algae have also been suggested to act as an initial buffer against sudden increases in salinity (Kirst 1996).

When DMSP is used as an osmolyte, it tends to respond on a long-term basis rather than a short-term one. When *Ulva intestinalis* was exposed to increased salinity, it increased sucrose and proline concentrations, but not DMSP over 48 h (Edwards et al. 1987). Over 5 weeks, DMSP concentrations increased, proline concentrations remained high, and sucrose concentrations declined, but large DMSP and proline increases were only evident when algae were grown in highly hyperosmotic media (200 and 300% seawater; Edwards et al. 1988). Similar increases in response to increasing salinities occurred in several Antarctic macroalgae (Karsten et al. 1991b, 1992), but only when the algae were maintained in the light (Karsten et al. 1992).

DMSP may not be involved in osmotic acclimation in all algae. When *U. lactuca* (formerly *U. fenestrata*) was grown in media with salinities that ranged from 5 to 95 psu, DMSP concentrations tended to drop slightly in algae exposed to the highest salinities for 24 h (Van Alstyne et al. 2003). When exposed to the same range of salinities for 4 weeks, DMSP concentrations increased by about 23% in algae exposed to the more hypersaline conditions and decreased by about 12% in algae exposed to the more hyposaline media (Van Alstyne et al. 2003). The small magnitude of the effect despite large changes in salinities suggests that DMSP was not the primary osmolyte being used by these algae.

Cryoprotection

DMSP has been suggested to act as a cryoprotectant by stabilizing proteins and by lowering the freezing point depression of cells. Concentrations of DMSP do change in response to changes in temperature. Antarctic green algae (*Ulothrix implexa*, *Ulothrix subflaccida*, *Ulva bulbosa*, and *Acrosiphonia arcta*) that were maintained at 0°C for 72 h had DMSP concentrations that were 2–5 times as high as those in algae maintained at 10°C (Karsten et al. 1992, 1996).

DMSP tends to stabilize enzymes at low temperatures, but has mixed effects at higher temperatures. In vitro additions of 300 mM DMSP to solutions containing phosophofructokinase reduced the loss of enzyme activity that occurred over time at 6°C (Nishiguchi and Somero 1992). At 2°C, the activity of malate dehydrogenase (MDH) extracted from *Acrosiphonia arcta* was also enhanced relative to controls by the addition of DMSP at concentrations of 19–300 mM, but MDH activity decreased relative to controls at 30°C (Karsten et al. 1996). DMSP stabilized lactate dehydrogenase at 50°C when present at 200 mM, but had little effect on enzyme activity at 300 mM (Karsten et al. 1996). It also destabilized glutamate dehydrogenase at 37°C at concentrations of 0.1–0.3 M (Karsten et al. 1996).

An inherent property of osmolytes is that they lower the freezing point depressions of cells (Kirst 1996). Therefore, DMSP may also be acting as a cryoprotectant in algae exposed to freezing temperatures. However, Kirst (1996) argues that cellular concentrations of DMSP are too low to produce a significant freezing point depression.

Methyl Donation

Several papers have commented that DMSP may function as a methyl donor (Challenger and Simpson 1948; Dubnoff and Borsook 1948; Visscher et al. 1994; Marc et al. 1995; Kirst 1996), but the importance of this process in marine macroalgae is not known. Marine bacteria can demethylate DMSP to methanethiol via 3-methiolpropionate or following the cleavage of DMSP into DMS and acrylate (Kiene and Taylor 1988; Taylor and Gilchrist 1991; González et al. 1999); however, the fate of the methyl groups was not determined in these studies.

8.3 The DMSP Cleavage Reaction and Its Products

8.3.1 The DMSP Cleavage Reaction

Although DMSP has a number of reported physiological and ecological functions, its primary function in many species may be the generation of DMS, acrylate, and acrylic acid (Fig. 8.2). The enzyme mediating the cleavage reaction is generically termed DMSP lyase; however, it is possible that DMSP lyase is not a single enzyme,

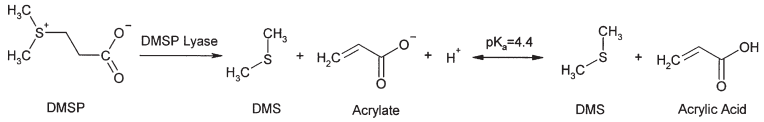

Fig. 8.2 The dimethylsulfoniopropionate (DMSP) cleavage reaction. *DMS* dimethylsulfide

as its properties differ among taxonomic groups. For example, DMSP lyase activity levels differ greatly both within and among algal species. Within the genus *Ulva*, lyase activity levels of crude extracts can range from approximately 0.1 to 100 nmol min^{-1} (mg protein)$^{-1}$ (Steinke et al. 1996). DMSP lyase activity also shows a similarly broad range of activity levels among strains of the phytoplanktonic prymnesiophyte *Emiliania huxleyi*. In this alga, there is no relationship between lyase activity levels and internal DMSP concentrations among strains (Steinke et al. 1998). Another property of the enzyme, the pH optimum, also shows considerable variability. Maximal lyase activities occurred at pH values of 6.2–6.4 in *Ulva clathrata* (Steinke and Kirst 1996) and at a pH of 8 in *Ulva curvata* (De Souza and Yoch 1996). In the phytoplanktonic prymnesiophyte *Phaeocystis* sp., the lyase enzyme has its maximal activity at a pH of 10.5, the highest pH at which it was measured (Stefels and Dijkhuizen 1996). Given that DMSP is likely to have multiple evolutionary origins and that the properties of the lyase enzyme vary among groups, it is likely that the enzyme(s) that cleave DMSP have also evolved more than once. It should be noted that an alternative pathway for the catabolism of DMSP into DMS in bacteria, including some bacteria associated with higher plants, was recently described by Todd et al. (2007).

8.3.2 The Functional Significance of the DMSP Cleavage Reaction

Most of the species that produce DMSP can also cleave it, suggesting that DMSP is produced in order to be able to rapidly generate its breakdown products or to use the cleavage reaction as a means of regulating intracellular concentrations of DMSP, carbon, or sulfur (Stefels 2000). The cleavage products of DMSP have been hypothesized to function as antioxidants (Sunda et al. 2002; Ross and Van Alstyne 2007), antibiotics (Sieburth 1960, 1961), and herbivore feeding deterrents (Wolfe et al. 1997; Van Alstyne et al. 2001b; Van Alstyne and Houser 2003). A more detailed description of each of these functions is described in what follows.

Elimination of Excess Energy and Sulfur

It is thought that productivity in many photosynthetic organisms is limited by nutrient availability rather than light or carbon availability and that plants and algae can generate excess energy in high light conditions. It is also possible for plants to

accumulate excess sulfur as a result of excessive uptake or protein degradation. Stefels (2000) has proposed that the production and subsequent loss of DMSP via its cleavage into DMS and acrylate could be a mechanism for eliminating excess sulfur and energy, particularly under conditions of nitrogen limitation. She also suggests that the cleavage reaction is a mechanism for regulating intracellular DMSP concentrations, which would be necessary during salinity changes if DMSP is being used as an osmolyte.

Production of Antioxidants

Many physiological stresses as well as physical damage and infection can result in the production of reactive oxygen species (ROS) (Lamb and Dixon 1997; Apel and Hirt 2004). ROS, which include hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-), singlet oxygen ($^1O_2^-$), and hydroxyl radicals (OH•), are extremely reactive and can damage lipids, proteins, and nucleic acids (Halliwell and Gutteridge 1989). Most organisms have a suite of enzymatic and nonenzymatic antioxidants to scavenge ROS, but cellular damage can result if the rate of ROS production exceeds the ability of the organism to scavenge them.

Sunda et al. (2002) proposed that the cleavage of DMSP may be a mechanism to generate ROS-scavenging antioxidants when algae are physiologically stressed. Although DMSP can scavenge OH•, acrylate and DMS are 20–60 times more reactive toward it (Sunda et al. 2002). When exposed to conditions known to generate oxidative stresses, such as carbon dioxide (CO_2) and iron limitation, the diatom *Thalassiosira pseudonana* upregulated DMSP production, resulting in 20–60-fold higher DMSP concentrations relative to controls. These increases were accompanied by increases in the antioxidant enzyme ascorbic peroxidase. CO_2 limitation and exposure to UV radiation, which can generate ROS, also caused an increase in DMSP concentrations in *Emiliania huxleyi* (Sunda et al. 2002).

Macroalgae also produce ROS in response to physiological stresses and injury (Collén and Pedersén 1996; Davison and Pearson 1996; Collén and Davison 1999a–c, 2001; Dummermuth et al. 2003; Ross et al. 2005; Ross and Van Alstyne 2007). Although macroalgae use antioxidant enzymes (e.g., ascorbic peroxidase, catalase, glutathione reductase, and superoxide dismutase) to scavenge ROS, some can also use DMSP cleavage as a source of antioxidants. *Ulva lactuca* generates H_2O_2 when exposed to reduced salinity and desiccation (Ross and Van Alstyne 2007). *U. lactuca* from a subtidal population, which would be less likely to be acclimated to ROS-generating stresses, generates more H_2O_2 than do algae from an intertidal population. When exposed to exogenous H_2O_2, both intertidal and subtidal *U. lactuca* lowered tissue DMSP concentrations and generated DMS within 3 h of exposure, indicating that DMSP was being cleaved in response to the H_2O_2; however, it took higher amounts of H_2O_2 to cause significant DMSP losses in intertidal individuals (Ross and Van Alstyne 2007).

Production of Antibiotics

The antibacterial activity of marine phytoplankton concentrates in Antarctic algal blooms can be correlated with densities of the DMSP-producing alga *Phaeocystis*. Sieburth (1960) isolated the compound responsible for this activity and determined that it was acrylic acid, one of the products of DMSP cleavage. He subsequently showed that acrylic acid could inhibit the growth of many bacterial species (Sieburth 1960). The acrylic acid generated by *Phaeocystis* blooms was hypothesized to have other toxic effects, which included inhibiting the growth of diatoms and affecting the survival of herring fry. However, another study showed that acrylic acid does not inhibit the growth of bacteria associated with *Phaeocystis* blooms at acrylic acid concentrations that are typically found in blooms off the Netherlands (Noordkamp et al. 2000). Slezak and coworkers (1994) also found that acrylic acid concentrations in marine waters were too low to inhibit bacterial production and suggested that bacterial inhibition might only occur in situations in which DMSP-producing phytoplankton are tightly aggregated, such as in marine snow. Furthermore, the growth of many bacterial species is stimulated by acrylic acid (Noordkamp et al. 2000), and many can use DMSP as a carbon and sulfur source (Kiene et al. 2000).

One place where acrylic acid may become concentrated enough to have antibiotic effects is the guts of the herbivores that consume algae containing DMSP. In laboratory feeding experiments, sea urchins (*Strongylocentrotus droebachiensis*) were found to generate DMS for 18 h after the removal of algal foods that contained DMSP, but measurable amounts of DMS were not generated when urchins were fed algal species that do not produce DMSP (Van Alstyne and Houser 2003). This suggests that DMSP cleavage may be taking place in the guts of the animals following consumption, which could result in a highly localized concentration of acrylic acid. Diet-derived acrylic acid has been suggested to be responsible for the near absence of bacteria in the anterior gastrointestinal tracts of pygoscelid penguins feeding on euphausids in areas where the phytoplankton is dominated by *Phaeocystis* (Sieburth 1961). In the guts of tropical fishes, acrylic acid has been found in very low concentrations (10–300 nmol g^{-1} fresh mass) and is not thought to have an inhibitory effect on the animals' gut microflora (Dacey et al. 1994).

Production of Antiherbivore Compounds

Oceanographers have been aware of a link between atmospheric DMS concentrations and grazing for decades. The impact of zooplankton grazing on DMS production was first examined by Dacey and Wakeham (1986), who found that grazing by copepods (*Labidocera aestiva* and *Centropages hamatus*) on the dinoflagellate *Gymnodinium nelsoni* resulted in the release of DMS, which helped explain why atmospheric DMS concentrations and phytoplankton biomasses were often not

tightly correlated. Only in the past decade has the cleavage of DMSP during grazing been suggested to have a defensive function. Although DMSP cleavage was first proposed as a microalgal defense (Wolfe et al. 1997), it appears to be a more effective defense in macroalgae than in microalgae (Van Alstyne et al. 2001b; Van Alstyne and Houser 2003).

Evidence for a Macroalgal Activated Defense

When macroalgal DMSP functions in herbivore deterrence, it does so via an activated defense mechanism (*sensu* Paul and Van Alstyne 1992). In an activated defense, the defensive compounds are stored in a nontoxic or a less toxic form and are then rapidly converted to more potent compounds in response to herbivore damage. This is analogous to turning on a light switch if more light is needed in a dark room. The advantage of this system for the alga is that the defenses are stored in a form that is less toxic to the alga, yet are readily available if needed. The disadvantage is that the enzymes needed to catalyze the conversion must be stored and maintained. Since amino acids in enzymes contain nitrogen, the maintenance of a pool of enzymes requires that algae tie up nitrogen, which is often a limiting nutrient (Valiela 1995), making it unavailable for other uses. Activated defenses differ from induced defenses, a defense system in which the synthesis of the defensive compounds is upregulated in response to herbivore grazing. The induction of defenses is analogous to adding lamps (that are permanently switched on) to obtain more light in a dark room. It is possible that the components of an activated defense system could be induced by herbivore grazing, but this has not yet been demonstrated to occur in marine macroalgae.

Several lines of evidence support the existence of a DMSP-based activated defense against sea urchin grazing in northeastern Pacific ulvoid algae:

(1) In these algae, the precursor in the defense system, DMSP, is present in high concentrations relative to other types of chemical defenses (except phlorotannins). DMSP concentrations in ulvoid algae from the region vary between species (Van Alstyne et al. 2001b) and sites (Van Alstyne et al. 2001b), but generally range from 25 to 125 μmol g^{-1} fresh mass (FM) or 0.3–1.7% of the alga's FM (Van Alstyne et al. 2001b, 2007).
(2) DMSP lyase activity has been shown to be present in one of the most common ulvoid algae in the region, *Ulva lactuca*, a species that contains relatively high amounts of DMSP (Van Alstyne et al. 2001b).
(3) DMS is released when ulvoid algae are grazed upon by herbivores. Experiments were conducted in which green sea urchins (*S. droebachiensis*) were placed in sealed gas-tight containers and were offered pieces of algae to consume (Van Alstyne and Houser 2003). During the experiment, the headspace gas inside the containers was sampled every 3 h. After 6 h, the remaining algae were removed, the urchins were returned to the containers, and the concentration of DMS in the headspace was monitored for another 66 h. DMS was present only in the headspace gas of containers that held urchins and algal species that contain DMSP

(*Ulva lactuca*, *Ulva linza*, and *Polysiphonia hendryi*). In containers that held urchins and algae that do not produce DMSP (*Saccharina latissima* [formerly *Laminaria saccharina*], *Nereocystis luetkeana*, and *Mazzaella splendens*), no DMS was found in the headspace gas.

(4) DMS and acrylic acid are more potent herbivore deterrents than DMSP. When DMSP was incorporated into agar and kelp-based artificial foods at a range of concentrations that encompassed those found naturally in ulvoid green algae, it either stimulated or had no effect on feeding by green (*S. droebachiensis*) or purple sea urchins (*Strongylocentrotus purpuratus*) from Washington and Oregon, respectively (Van Alstyne et al. 2001b). However, when DMS and acrylic acid were incorporated into similar diets, they reduced the feeding rates of both urchins (Van Alstyne et al. 2001b; Van Alstyne and Houser 2003).

Taken together, these data show that both the precursor (DMSP) and the activating enzyme (DMSP lyase) are present in the algae; one of the products of the cleavage reaction (DMS) is released during grazing; and, the products of the reaction (DMS and acrylic acid) deter sea urchin feeding while the precursor (DMSP) does not, providing strong support for the hypothesis that DMSP functions as the precursor in an activated antiherbivore defense toward northeastern Pacific green and purple sea urchins. Experiments to determine whether increased DMSP concentrations in *Ulva lactuca* could be stimulated by snail (*Lacuna vincta*) feeding failed to show a response, suggesting that the alga does not induce DMSP production under these conditions (Van Alstyne et al. 2007).

DMSP was originally proposed as the precursor to an activated defense in phytoplankton (Wolfe et al. 1997); however, because of subsequent experimental results, this hypothesis has lost much of its support for planktonic grazers. Wolfe and coworkers (1997) originally proposed this hypothesis based upon experimental results that showed that the protist grazer *Oxyrrhis marina* grazed more rapidly on strains of *Emiliania huxleyi* that contained lower DMSP lyase activity levels and produced lower amounts of DMS during grazing. The DMS and acrylate generated by the cleavage of DMSP were hypothesized to be responsible for reducing the rates of feeding by the microzooplankton. This hypothesis was called into question by a series of follow-up experiments in which it was found that the addition of exogenous DMS and acrylate had no effect on the feeding rates on *E. huxleyi* by four species of protist grazers (Strom et al. 2003). However, when DMSP was added to the seawater containing the algae and grazers, grazing rates by all four grazers were reduced. Strom and coworkers (2003) postulated that DMSP produced by microalgae may serve as a "warning" signal to grazers, alerting them to the presence of acrylate-producing DMSP or other toxic compounds contained within the algae.

Herbivore Specificity

The food preferences of macroinvertebrate herbivores for seaweeds containing high DMSP concentrations vary enormously among herbivore species. When temperate green and purple sea urchins (*Strongylocentrotus droebachiensis* and *S. purpuratus*)

were offered choices of macroalgae in multiple-choice feeding-preference assays in the laboratory, kelps were usually consumed at a greater rate than ulvoid green algae (Van Alstyne and Houser 2003; Van Alstyne et al. 2006a), which contain high concentrations of DMSP (Van Alstyne et al. 2001b). It is notable that consumption rates of ulvoid macroalgae were comparable to feeding rates on *Alaria marginata* (Van Alstyne and Houser 2003; Van Alstyne et al. 2006a), a kelp that often produces high concentrations of phlorotannins (Steinberg 1984; Van Alstyne et al. 1999). Floridian sea urchins (*Echinometra lucunter*) also exhibited a low preference for *Ulva lactuca* relative to five other macroalgal species in laboratory feeding assays, but this preference was not a result of the DMSP in the plants (Erickson et al. 2006).

In contrast, there are other grazers that preferentially consume algae that produce relatively high amounts of DMSP. *Ulva lactuca* and *Ulva linza*, which contain DMSP in millimolar concentrations, were the two most preferred foods of the isopod *Idotea wosnesenskii* and the snail *Littorina sitkana* when the animals were offered choices of seven macroalgal species in laboratory preference experiments (Van Alstyne et al. 2006a). *Ulvaria obscura*, another ulvoid macroalga that produces high concentrations of DMSP, was eaten at much lower rates by these herbivores, but this was most likely because it contains dopamine, a potent antiherbivore compound (Van Alstyne et al. 2006a).

The range of responses by herbivores to algae containing DMSP can reflect the ability of DMS and acrylic acid to deter feeding, but they do not always do so. For example, the tropical sea urchin *Echinometra lucunter* was not deterred by acrylic acid added to artificial foods in concentrations of 1.38 and 2.76% DM or by DMS added at concentrations ranging from 1.4 to 5.5% DM. On the other hand, the littorinid snails *Littorina sitkana*, which prefer to consume high-DMSP ulvoid green algae over many other macrophytes, activate the cleavage of DMSP and are deterred by acrylic acid when it is added at natural concentrations to artificial foods (Van Alstyne et al., unpublished). *L. sitkana* may consume ulvoid algae because they also contain relatively high amounts of nitrogen, which is thought to be a limiting nutrient for many herbivores (Mattson 1980).

Most of the studies that have examined the effects of macroalgal DMSP and related compounds have been conducted by offering herbivores a choice of two agar-based foods that are identical except that one contains the compound being tested (e.g., Van Alstyne et al. 2001b; Van Alstyne and Houser 2003; Erickson et al. 2006). Diffusion-based losses of these volatile and water-soluble compounds do occur during the feeding experiments (Van Alstyne and Houser 2003; Erickson et al. 2006); however, it is still possible to use choice experiments to test herbivore preferences between control foods lacking added substances and foods created with DMSP, DMS, acrylate, or acrylic acid, as long as the experiments are conducted rapidly (within minutes to hours, depending on the size, composition, and surface-to-volume ratio of the food), the loss rates of compounds from the foods are monitored, and the effects of the loss of compounds during the feeding trials are considered when interpreting the results of the experiments. The latter point is especially important when interpreting a nonsignificant result, since a lack of preference could result from the loss of the compound being tested from the food rather than the lack of an effect of the compound on herbivore feeding.

Production of Predator Signaling Compounds

In addition to their use as direct antiherbivore defenses, volatile plant secondary metabolites can be used by plants as an indirect defense (Baldwin and Preston 1999; Kessler and Baldwin 2002; Dicke et al. 2003). A variety of terrestrial plant volatiles are used by predators as signals that alert them to the presence of herbivores consuming the plants (Pare and Tumlinson 1999; Dicke and van Loon 2000; Walling 2000; Gatehouse 2002). It is still debated as to whether the plants emitting these volatile compounds are "talking," that is, sending out a signal to neighbors or predators (Baldwin and Schultz 1983), or whether neighbors and/or predators are "eavesdropping," that is, sensing volatile compounds that are released by attacked plants either incidentally or as a means of inducing a systemic antiherbivore response within the attacked individual (Baldwin and Preston 1999; Kessler and Baldwin 2002; Dicke et al. 2003).

The evidence for the use of volatile compounds as signals to predators in the marine environment is much more limited than for terrestrial systems; however, since DMS is volatile, capable of crossing the sea-air boundary, and has a strong odor, it is a likely candidate for a signaling molecule for both aquatic and airborne predators. Indeed, one of the best examples of predators using "plant" volatiles in tritrophic interactions involves the use of DMS as a signaling molecule. In sub-Antarctic oceanic waters, DMS is used by predatory seabirds that must often travel long distances from their nesting sites to locate prey (Nevitt et al. 1995; Nevitt 1999, 2000, 2006; Nevitt and Haberman 2003; Nevitt and Bonadonna 2005a, b). The prey of these birds tends to be concentrated in patches that are small (tens to hundreds of km^2), ephemeral, distributed over thousands of square kilometers of open ocean (Nevitt 1999; Nevitt and Bonadonna 2005a), and characterized by an abundance of consumers that include herbivorous zooplankton, krill, and fish. Grazing by zooplankton on algae in these patches, particularly on *Phaeocystis pouchetii,* can generate locally high concentrations of DMS in the air over and surrounding the patches (Nevitt et al. 1995; Nevitt 2000; Simó 2001). Some procellariiform seabirds can use the odor of DMS associated with productive oceanic patches to orient toward them, but this ability is species-specific (Nevitt et al. 1995). Wilson's storm petrels were attracted to experimentally generated oil slicks composed of cooking oil and DMS, but cape petrels and albatrosses were observed in similar numbers at oil slicks with and without DMS (Nevitt et al. 1995). Harbor seals are also able to detect DMS at concentrations that would occur in highly productive oceanic waters and may also be using the compound to orient toward foraging grounds (Kowalewsky et al. 2006). Whether predators of macroalgal herbivores use DMS in similar ways is not known.

DMS, Sulfur Cycling, and Climate

The fate of DMSP generated by macroalgae is not well known; however, the role of microalgal DMSP in oceanic and atmospheric sulfur cycles has been well studied. Macroalgal DMSP likely undergoes many of the same processes as microalgal

DMSP. DMSP and related sulfur compounds generated by planktonic microalgae are involved in a complex series of interactions and reactions that occur in seawater and the atmosphere (Malin et al. 1992; Liss et al. 1994; Malin 1996; Malin and Kirst 1997; Steinke et al. 2002; Yoch 2002). In the oceans, DMS can originate from DMSP that is cleaved during grazing, be directly released by healthy algal cells, or be released when cells senesce or are lysed by viruses (Fig. 8.3).

DMS in the oceans can cross the air-sea boundary and move into the atmosphere (Malin et al. 1992; Malin 1996; Liss et al. 1997; Malin and Kirst 1997; Simó 2001;

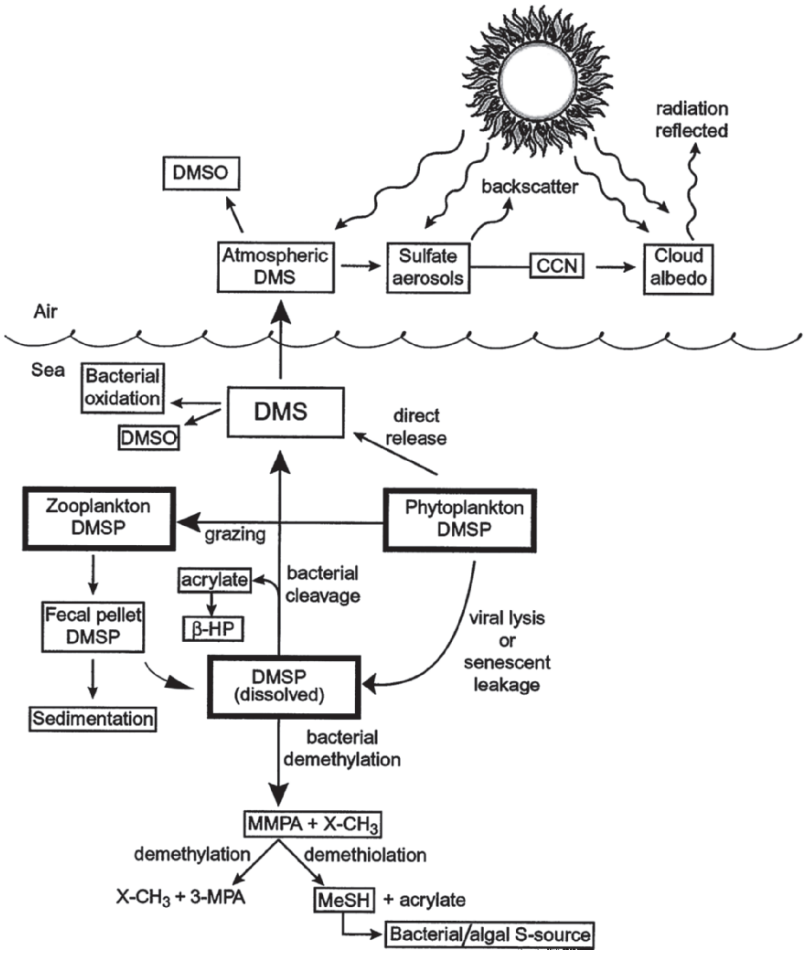

Fig. 8.3 Dimethylsulfoniopropionate and dimethyl sulfide cycling in the open ocean. *DMSO* dimethylsulfoxide, *CCN* cloud condensation nuclei, *MMPA* 3-methylpropionate, *β-HP* β-hydroxypropionate, *3-MPA* 3-mercaptopropionate, *MeSH* methanethiol, *X-CH$_3$* unidentified molecule with a terminal methyl group. (Reprinted from Yoch 2002, with permission from D. Yoch and the American Society for Microbiology)

Yoch 2002), be chemically or photochemically oxidized into dimethylsulfoxide (DMSO) (Hatton et al. 1996, 2004; Simó et al. 2000), or be consumed by bacteria (Kiene and Bates 1990) (Fig. 8.3). Lovelock and coworkers (1972) first recognized that DMS rather than hydrogen sulfide (H_2S) was the primary natural vehicle for the movement of sulfur between the oceans and the atmosphere. DMS is volatile and generally supersaturated in the oceans (Brasseur et al. 1999). Fluxes of DMS across the ocean surface into the atmosphere are estimated to be 13–37 Tg of sulfur annually (Kettle and Andreae 2000) and vary both latitudinally and seasonally. They tend to be highest at low latitudes and during the summer, particularly at higher latitudes (Bates et al. 1992; Liss et al. 1997). Although there is still a large degree of uncertainty regarding the amounts of organosulfur compounds generated from different sources, it is estimated that the oceans are the source of >90% of the biogenic sulfur entering the atmosphere (Malin 1996), the remainder being generated by freshwater swamps, soils, and terrestrial plants (Yoch 2002). Oceanic fluxes of DMS are estimated to constitute at least half of the biogenic sulfur entering the atmosphere globally (reviewed by Yoch 2002). Natural sources of sulfur constitute less than one quarter of the total sulfur flux into the atmosphere (Brasseur et al. 1999); the remainder is anthropogenically generated and is primarily from fossil fuel combustion (Liss et al. 1997).

Once DMS enters the atmosphere, it can break down rapidly and form a variety of oxidation products, which include nonsea salt sulfate, methane sulfonate, DMSO, dimethylsulfone, and sulfur dioxide (Charlson et al. 1987; Malin et al. 1992; Brasseur et al. 1999). The oxidation products of DMS are hypothesized to affect local climate both through absorbing and scattering solar radiation and by acting as cloud condensation nuclei (Fig. 8.3) (Bates et al. 1987; Charlson et al. 1987; Malin et al. 1992; Malin 1996; Malin and Kirst 1997; Yoch 2002). Cloud condensation nuclei (CCN) promote the formation of liquid water droplets, which ultimately lead to the formation of clouds. Charlson and coworkers (1987) hypothesized that the increased albedo resulting from clouds that formed as a result of algal-derived DMS would cause a decrease in temperature and solar radiation at the sea surface, which would then reduce phytoplankton growth and lower DMS production, creating a negative feedback loop (Fig. 8.3).

Could DMS originating from macroalgal DMSP have similar effects on sulfur cycling and local climate? Decaying macroalgae on beaches are reported to have a characteristic sulfur stench that is usually attributed to H_2S (Hanks 1966), and extremely high concentrations of DMS have been reported from sediment porewaters that were associated with ulvoid algal mats (Sørensen 1988). However, despite the presence of sulfur compounds associated with decaying algal mats, Malin (1997) states that macroalgal input to the sulfur cycle is small relative to inputs from phytoplankton. The contribution of DMS to the total CCN pool in coastal areas may also be minor because of the relatively large anthropogenic inputs in these areas. Nonetheless, it should be noted that the lack of data on release rates of DMS into the atmosphere from macroalgal sources makes statements on macroalgal contributions to sulfur cycling and climate largely speculative.

8.4 Summary

DMSP is a multifunctional compound that is widely distributed among marine microalgal and macroalgal species. Based on the existence of at least three different synthetic pathways, it is likely that the ability to produce DMSP has evolved multiple times. The compound also has multiple reported functions, which include serving as an osmolyte, a cryoprotectant, and a methyl donor. However, the most ecologically important function may be to serve as a precursor to the production of DMS, acrylate, and acrylic acid via enzymatic cleavage by DMSP lyase. These breakdown products of DMSP have been suggested to function in the elimination of excess energy and sulfur from algae, deterring feeding by herbivores, reducing the growth of microbes, and scavenging ROS. In addition, DMS may be used by some predators as a signaling molecule to locate feeding herbivores. DMS released by marine microalgae is an important source of biogenic sulfur in the atmosphere, particularly above pristine open ocean waters, and may affect climate via its effects on cloud formation. The impacts of DMSP generated by macroalgae on sulfur cycling and cloud formation are not known, but are assumed to be minor.

Lokvam and coworkers (2006) have proposed that secondary metabolites can be derived from primary metabolites that are hyperproduced and take on additional functions. DMSP and the compounds derived from its cleavage may be an example of such a process. DMSP and its breakdown products clearly have primary functions in many species and secondary functions in some. Even the cleavage reaction appears to have evolved to serve primary functions, such as regulating the amounts of DMSP in cells during osmotic acclimation (Stefels 2000) or generating antioxidants (Sunda et al. 2002). As is true for many marine secondary metabolites (Van Alstyne et al. 2001a), the efficacy of acrylic acid in deterring sea urchin grazing is concentration-dependent (Van Alstyne et al. 2001b), although the relationship between DMS and deterrence is less clear, as all concentrations tested produced approximately the same level of deterrence in laboratory assays (Van Alstyne and Houser 2003). This suggests that algae already producing large amounts of DMSP, perhaps in response to environments that experience high degrees of oxidative stresses or frequent salinity changes, may have secondarily evolved the ability to use the use the breakdown products of DMSP cleavage for additional functions such as herbivore deterrence.

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9
Influence of Algal Secondary Metabolites on Plankton Community Structure

G. Pohnert

9.1 Introduction

Plankton chemical ecologists face numerous challenges in understanding the roles of chemical signalling and defence. They have to deal with a community of diverse species living in high dilutions in the nearly homogenous environments of oceans and lakes. During the annual cycle the community structure changes dramatically, but local influences, such as gradients in light, nutrients, and temperature, also can influence species composition. Despite the fact that the open water is not well-structured on local scales and offers no spatial niches, the plankton is extremely species-rich and never reaches equilibrium (Scheffer et al. 2003).

If only the dominant species are observed, the general prerequisite for an algal bloom or a mass occurrence of specific herbivores is that the growth rates must exceed the sum of all loss processes. But even in this relatively simple case the underlying principles and the required conditions are not fully understood. The traditional concept of plankton ecology relies on spatially and temporally fluctuating resources that determine growth and loss (Arrigo 2005). But in the past few decades we have come to realize that in addition to resources, such as light and nutrients for algae or food quantity and food quality for zooplankton, other factors play major roles as well. In the 1990s the concept arose that toxins and infochemicals (i.e., metabolites that convey information on the interaction between two individuals in their natural environment by evoking a behavioural or physiological response in the receiver (Dicke and Sabelis 1988)) can play a major role in shaping the community structure of plankton. Since then, considerable research efforts have been undertaken to investigate the underlying modes of action of these compounds. Because of the overall importance of ocean and lake ecosystems, this field has attracted much interest, and several excellent reviews have been published during the past few years. In this contribution I do not aim to add one more review on a

G. Pohnert
Department of Bioorganic Analytics, Institute for Inorganic and Analytical Chemistry, Friedrich-Schiller-Universität, Lessing Strasse 8, 07743 Jena, Germany. e-mail: Georg.Pohnert@uni-jena.de

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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specific aspect of this research field but rather provide starting points for further reading by exclusively surveying the existing review articles related to this topic.

9.2 Early Studies

Early studies of plankton secondary metabolites often focused on toxins from harmful algal blooms that exhibit remarkable activity on higher consumers such as invertebrates and mammals (Daranas et al. 2001; Cembella 2003; Sellner et al. 2003; Shimizu 2003). Motivated by human health threats and massive fish kills, investigations of the mode of action of these metabolites were mainly focused on higher aquatic organisms (Landsberg 2002; Dittmann and Wiegand 2006) and the impact on human health (e.g., Friedman and Levin 2005). Aspects of chemical ecology (see references in Prince et al. 2006; Cembella 2003) or general implications for phytoplankton blooms (Smayda 1997) were addressed rather late.

Other early studies focused on pheromones, such as those of the pelagic life stages of brown algae (Maier and Müller 1986; Pohnert and Boland 2002) or of the green alga *Volvox* (Hallmann et al. 1998) (see Chap. 14). In addition, kairomones that trigger life history changes, morphological changes, or behavioural responses in fresh water plankton were recognized early as active components in plankton communication (Lampert and Sommer 1997). In 1996, Hay wrote a concept paper with the title "Marine chemical ecology – what is known what is next?" (Hay 1996), which indicated that until then the field of plankton chemical ecology was mainly focused on the investigation of few specific toxins and little information was available on their natural function. Only a few years later, the same author was able to devote an entire review to the community and ecosystem level consequences of chemical cues in the plankton (Hay and Kubanek 2002), providing the first comprehensive overview of this topic. In this review evidence was first assembled indicating that chemical cues might have indirect and cascading effects on the ecology and evolution of entire communities and ecosystems. But at this time studies on chemically mediated planktonic interactions had rarely progressed to the point where the responsible metabolites were unambiguously identified. Nevertheless, it was already clear that chemical cues are widespread among planktonic organisms.

A one-page essay in which it is suggested that evolution in plankton is driven by "a watery arms race" has attracted much attention (Smetacek 2001). Smetacek discusses the concept that defence of phytoplankton by mechanical protection, increased cell size, formation of spines, or production of noxious chemicals leads to the adaptation of zooplankton to these measures. This process resulted in the shape and properties of phyto- and zooplankton that we observe today. In the years following that essay several candidate molecules were discovered and intensely studied. The concept that chemical signals and defence metabolites are important factors in plankton ecology has now become widely accepted.

9.3 General Considerations

Shurin et al. (2006) analysed real differences between aquatic and terrestrial food webs in a review entitled "All wet or dried up?" and came to the conclusion that there are systematic dissimilarities in energy flow and biomass partitioning in both environments. They further argue that variable selective forces drive differences in plant allocation patterns in aquatic and terrestrial environments. These general considerations have to be taken into account when chemical communication or defence in the plankton are investigated. But physical constraints of the medium also strongly influence chemical communication and defence in the plankton. Zimmer and Butman (2000) discuss the role of fluid dynamics for chemical signalling in the marine environment. In this review the impact of signal diffusion and active transportation in chemical interactions on the small and large scale is illustrated using several examples and models. Interactive effects of hydrodynamic and chemical factors are discussed as well. Weissburg (2000) uses a more theoretical approach to treat the role of hydrodynamics in aquatic chemical signalling, focusing on behavioural implications of fluid flow regimes. That behavioural responses of zooplankton are under the control of viscous forces is nicely illustrated by Yen (2000), Yen et al. (1998), and also commented on in a short essay by Smetacek (2002).

In an important concept article, Wolfe (2000) outlined constraints, mechanisms, and impacts of chemical defence in plankton. In a systematic way he discussed signalling and defence in the microbial biosphere by considering physical and biological limitations. Receptor-mediated interactions and behavioural responses are discussed and put into the context of prey finding in the extremely dilute suspension of plankton. The most important contribution of Wolfe's paper is the systematic answer to the question "How might a single cell defend itself against predation?" Several strategies that may have adaptive advantages to microbial chemical defence are introduced. These include excretion of signals, compartmentation of highly active toxins, activated reactions that are only initiated upon mechanical stimulation of plankton cells, and multifunctional systems. Progress in the field of activated defences was reviewed later by Pohnert (2004), who also covered induced defences, where the biosynthesis of defensive metabolites is initiated only upon reception of chemical signals that indicate high risk of predation or high pathogen pressure.

Some of the proposed strategies would require the sacrifice of the toxin-producing organism itself, since a single cell with a stored toxin or cells that rely on a wound-activated defence mechanism have to be taken up by herbivores for the defence to be effective. This would imply a community-based defence of bloom-forming algae, where part of the population is ingested, thereby protecting the conspecifics (Wolfe 2000). The concept of group selection and kin selection was suggested to be efficient for phytoplankton populations that are composed of asexually dividing cells. Blooms of such algae under the conditions of rapid growth and low mixing were considered to consist mainly of clonal cells. But recent evidence from microsatellite studies indicates that genetic diversity remains high during blooms, with

many different clonal lines making up the population (DeLong 2005; DeLong and Karl 2005). The emerging implications of these findings for plankton (chemical) ecology are briefly discussed in Franklin et al. (2006) and Pohnert et al. (2007). The general view of the ocean microbial diversity gains momentum also through the use of genetic and genomic methods (DeLong and Karl 2005). This field of research now gives a more comprehensive view of uncultivated microbial species, gene and biochemical pathway distributions, naturally occurring genomic variability, and evolutionary aspects (Azam and Worden 2004; Doney et al. 2004; Falkowski et al. 2004; DeLong 2005).

Several reviews on marine chemical ecology and marine secondary metabolites cover aspects of the plankton and only a selection of recent contributions are cited here (Paul and Puglisi 2004; Ianora et al. 2006; Paul et al. 2006).

9.4 Specific Aspects

An important contribution by Cembella (2003) deals with the chemical ecology of eukaryotic microalgae in marine ecosystems, Tillmann (2004) offers a comprehensive view of the interactions between planktonic microalgae and protozoan grazers, and Engel et al. (2002) focus on the chemical ecology of marine microbial defence. The chemical ecology of invertebrate meroplankton and holoplankton, i.e., organisms that spend some component of their life history or their entire life cycle in plankton, has been reviewed by McClintock et al. (2001). This contribution concentrates primarily on the feeding-deterrent properties of marine invertebrate eggs and larvae but also deals with rather general laboratory and field investigations in plankton chemical ecology.

"The enemy of my enemy is my friend" was the punch line of a contribution by Hay et al. (2004) on mutualisms and aquatic community structure, which also included a short chapter on pelagic organisms. The important topic of allelopathy of aquatic autotrophs is covered by three major reviews (Gross 2003; Legrand et al. 2003; Leflaive and Ten-Hage 2007). Franklin et al. (2006) discuss the role of programmed cell death in phytoplankton ecology. Burks and Lodge (2002) and Van Donk (2006) provide very useful reviews specifically concerning fresh water ecosystems.

Morphological, behavioural and life-history responses to the chemical presence of kairomones from potential predators are comprehensively surveyed by Lass and Spaak (2003). Phenotypic plasticity that has been intensely studied in freshwater ecosystems is reviewed by Miner et al. (2005) and van Holthoon et al. (2003). Behavioural responses to kairomones are treated by von Elert and Pohnert (2000).

The biosynthesis of marine natural products by microorganisms has, e.g., been reviewed by Moore (1999, 2005) and Shimizu (1993, 1996, 2003). These contributions provide excellent starting points for further reading. The implications of structuring infochemicals for ecological informatics are discussed by Vos et al. (2006). Problems in the methodology used in the assessment of toxicity or rather general during bioassays are addressed in two reviews (Caldwell et al. 2004; Hay and Kubanek 2002).

It is also worthwhile to recommend the "horizons" series in the *Journal of Plankton Research*. This offers a platform for the discussion of new concepts and critical ideas also related to plankton chemical communication (e.g., Irigoien 2004, 2006; Anderson 2005; Flynn 2005; Irigoien et al. 2005; Mitra and Flynn 2005; Le Quere 2006; Droop 2007).

9.5 Single Metabolites

Several single metabolites or groups of structurally similar compounds have attracted much interest by the research community. The metabolites of pelagic organisms that have been discussed most intensely with respect to ecological roles are without doubt dimethylsulphide and its biosynthetic precursor dimethylsulphoniopropionate. Several reviews deal with the ecological roles of these climate-relevant chemicals that are produced on a large scale by marine plankton (see, e.g., Wolfe 2000; Steinke et al. 2002; Pohnert et al. 2007) and an entire chapter of this book is devoted to this topic (see Chap. 8). Another group of bioactive metabolites that has attracted much attention are polyunsaturated aldehydes that are released upon damage of diatoms. These aldehydes can reduce the egg hatching success and impair larval development of herbivorous zooplankton but might also play a role in cell-cell communication and defence against other phyla. A general review on this topic was published by Pohnert (2005), a concept paper by Paffenhofer et al. (2005), a review focusing on the biosynthesis by Fontana (2007), a contribution highlighting similarities of diatom oxylipins and brown algal pheromones by Pohnert and Boland (2002), and a discussion of problems connected to the assignment of toxicity of these metabolites by Caldwell et al. (2004).

Watson discusses the biological activity of algal odour compounds that are the primary cause of foul source-water odour and have been treated by industry as metabolic waste with focus on their removal (Watson 2003). That these compounds can also have multiple functions in the intact ecosystem is elaborated in this contribution.

In the field of fresh water plankton chemical ecology microcystins from cyanobacteria have stimulated much research and discussion. A critical reflection on the ecological function of these non-ribosomal peptides has recently been published (Babica et al. 2006). Surveys of methods for the quantification of these peptides (McElhiney and Lawton 2005; Msagati et al. 2006) and on the effects on fish (Malbrouck and Kestemont 2006) can be found as well.

9.6 Conclusions

Even if this selection of review articles is not comprehensive, it well reflects the current activities in the field of plankton ecology. Exciting new progress can be expected during the next few years, especially because of the advanced application of new

genetic methods and because of the increasing investigation of the microbial loop which represents a diverse community with nearly unexplored dynamics and communication. Despite the intense research effort, the number of active chemical compounds that are fully characterized is comparably small. Here, advances in high throughput analytical chemistry might offer new approaches enabling a more comprehensive profiling of metabolites from plankton. Even though we are currently accumulating knowledge about the chemical interactions in plankton, we are still in an explorative phase and the first findings will have to be confirmed by carefully designed bioassays reflecting the situation in the natural environment and supported by modelling studies and by the investigation of the fine-scale community structure of the plankton.

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10
Herbivore Offense in the Sea: The Detoxification and Transport of Secondary Metabolites

E.E. Sotka(✉) and K.E. Whalen

10.1 Introduction

The past 25 years of research on algal-herbivore interactions have witnessed remarkable advances in our understanding of algal defenses and the chemical and morphological mechanisms that algae use to protect themselves from being consumed by their herbivores (Hay and Fenical 1988; Paul 1992; Steinberg 1992; Paul et al. 2001; Paul and Puglisi 2004; Paul et al. 2006; see Chaps. 2–6). In particular, we have a tremendous amount of information on the diversity, concentrations, and distributions of algal secondary metabolites within and across seaweed individuals, and in many cases, which of these algal compounds deter particular herbivores (Cronin 2001; Van Alstyne et al. 2001; Amsler and Fairhead 2006).

In contrast to algal defenses, we have far less information on what could be termed "herbivore offense" (Rhoades 1985; Karban and Agrawal 2002); that is, the traits that allow herbivores to increase their feeding rates on algae when these uses benefit the herbivores. A list of "offensive" traits of marine herbivores includes (1) feeding preferences for algae that provide better nutritional quality (e.g., Pennings et al. 1993; Choat and Clements 1998; Jormalainen et al. 2001; Cruz-Rivera and Hay 2003), or that allow refuge from enemies (Hay et al. 1987) or abiotic agents (Sotka 2007), among other selective advantages, (2) physiological adaptations that allow the herbivore to tolerate algal metabolites and structural elements (Targett and Arnold 2001), (3) morphological adaptations that increase feeding efficiency, such as the gut and jaw architectures among herbivorous fishes (Choat 1991; Horn 1992) or the radulae of molluscan grazers (Steneck and Watling 1982; Padilla 1985), (4) sequestration of secondary metabolites to protect herbivores from their own predators (e.g., Paul 1988; Pennings and Paul 1993; Rogers et al. 1995), and (5) gregarious feeding, which can make algae more palatable to herbivore

E.E. Sotka
Grice Marine Laboratory and the Department of Biology, College of Charleston, 205 Fort Johnson Road, Charleston, SC 29412, USA. e-mail: SotkaE@cofc.edu

K.E. Whalen
Biology Department, Woods Hole Oceanographic Institution, MS-32, 45 Water Street, Woods Hole, MA 02543, USA. e-mail: kwhalen@whoi.edu

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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individuals (Trowbridge 1991b). Offensive traits are determined from the herbivores' point of view and represent their evolutionary solutions to the challenges of feeding on structurally and chemically defended algae. The bias toward understanding plant defenses rather than herbivore responses is not limited to marine systems (Rhoades 1985; Karban and Agrawal 2002). Both marine and terrestrial systems will undoubtedly benefit from a focus on the costs and limitations of offensive traits and "help put the herbivore back in plant-herbivore interactions and coevolution" (Karban and Agrawal 2002: p. 641).

Arguably, the offensive traits for which we have the least amount of information are physiological and biochemical mechanisms that allow marine herbivores to tolerate (i.e., consume, manipulate, and/or detoxify) lipophilic compounds produced by seaweeds (macroalgae). Most of the secondary metabolites produced by algae are lipophilic in nature (Paul 1992; but see Steinberg 1992; Van Alstyne et al. 2001), yet the proximate mechanisms that underlie feeding tolerance for these lipophilic compounds are poorly described for almost all marine herbivores (Hay 1996; Paul et al. 2001). We have more information on the herbivore tolerance of water-soluble metabolites such as polyphenols. According to a recent and authoritative review (Targett and Arnold 2001), much of the tremendous variation across herbivores in the antidigestive effects of polyphenols can be explained by the "chemical (pH, redox condition, cation concentration, surfactants, etc.) and biological (microbes) characteristics of their guts" (see also Horn 1992; Horn and Ojeda 1999). This emerging framework represents a remarkable achievement in the study of marine algal-herbivore interactions, but its usefulness in understanding physiological and biochemical tolerance of lipophilic compounds is probably minimal.

In this chapter, we focus on the evolution of resistance for lipophilic chemistry produced by marine algae. First, we introduce the enzymatic architecture that likely mediates tolerance for lipophilic compounds among marine herbivores. Some of these proteins mediate the biotransformation of dietary compounds, while others aid in their efflux from the gut. Although all marine herbivores have these enzymatic mechanisms encoded in their DNA, our understanding of their influence in determining dietary tolerance and preference is currently limited to a very small number of species. Second, we highlight areas where differences among herbivores in tolerance for lipophilic compounds may be mediated by differences in the underlying enzymatic metabolism. We anticipate that these enzyme-compound interactions will help explain a portion of the diversity of herbivore response, and thus, form the basis of a mechanistic understanding of herbivore-algal interactions.

10.2 Proximate Mechanisms of Herbivore Tolerance

10.2.1 Defining "Tolerance"

When confronted with chemically rich seaweeds in their local communities, some herbivores have evolved a means to detect and avoid these foods. These feeding avoidance behaviors are probably mediated by taste, rather than smell, because

metabolites are commonly sequestered within algal membrane bound vesicles and often display poor volatility (Paul 1992). However, when herbivores consume chemically rich seaweeds, either by choice or because few other foods are available, their ability to obtain nutrients and concordantly maximize their fitness may ultimately depend on their ability to metabolize secondary compounds.

In practical terms, marine biologists have measured tolerance for secondary metabolites by assessing feeding behaviors (i.e., feeding rates and preferences), physiological traits (e.g., assimilation efficiency), or fitness (e.g., survivorship, growth, and fecundity). In some groups, these estimates of herbivore tolerance seem tightly correlated. For example, feeding preferences of herbivorous marine amphipods are "positively correlated, to varying strengths, with juvenile performance" when all relevant studies were considered (Taylor and Brown 2006). In other groups, the effects of secondary metabolites on feeding rates or assimilation efficiency may not represent a "good" proxy for herbivore fitness. For example, the effects of a chemically rich diatom diet on herbivorous copepod fitness were generally not manifested in their feeding and growth rates, but as a loss of egg viability and overall fecundity (see review by Ianora et al. 2003). In general, however, these correlations are poorly known for most herbivores (Hay 1996; Cruz-Rivera and Hay 2001; Duffy and Hay 2001; Paul et al. 2001).

If feeding behaviors, assimilation efficiency, or fitness are reasonable proxies for tolerance of secondary metabolites, then it is clear that herbivore individuals, populations, and species profoundly vary in their tolerance (Paul et al. 2001; Stachowicz 2001; Targett and Arnold 2001). Such differential tolerance was recognized early within the emerging field of marine plant-herbivore interactions (Hay 1992; Hay and Steinberg 1992; Paul 1992), much to the dismay of these scholars. The "same metabolite may show considerable difference in its effects even on closely related species of herbivores" (Paul 1992).

We recognize that the overwhelming variation in tolerance among herbivores is a blessing and a curse. On the one hand, there is ample evolutionary material that biologists can exploit to understand patterns of detoxification. At the same time, it is conceivable that "each and every one of these cases is likely to have unique features" as Brattsten (1992) warned when considering insect detoxification. While generalizations should be pursued with caution, evidence for the importance of key detoxification enzymes in protecting terrestrial herbivores against plant allelochemicals is mounting (Yu 1996; Feyereisen 1999; Sorensen et al. 2006; Li et al. 2007) and intriguing parallels to the biochemical resistance mechanisms within marine consumers are beginning to emerge.

10.2.2 Mechanisms of Detoxification and Transport

The detoxification and excretion of xenobiotics (i.e., foreign compounds, including diet-derived allelochemicals) involve a suite of highly complex processes that allow an organism to respond to its internal and external chemical environments. Such metabolic resistance "involves the biochemical transformation

of a substance, ultimately reducing its capacity to interact with a target molecule" (Li et al. 2007). In general, this biotransformation is accomplished by a limited number of enzymes with broad substrate specificities that convert nonpolar xenobiotics into highly water-soluble, less toxic products and eliminate them from the cell and body (Fig. 10.1). In contrast to most housekeeping genes, genes involved in xenobiotic metabolism are strongly inducible by substrates upon which they act (Remmer and Merker 1963; Terriere 1984; Bock et al. 1990; Cole et al. 1992).

Xenobiotic metabolism has classically been divided into three phases: phase I (functionalization), phase II (conjugation), and phase III (transport/excretion) reactions. Phase I enzymes catalyze the addition of a polar functional group into lipophilic substrates, while phase II enzymes use these groups as a "handle" for conjugation with moieties such as glutathione, glucuronic acid, or glycine (Gonzalez and Nebert 1990; Sheehan et al. 2001). Finally, unmodified allelochemicals and their phase I and II metabolites can be excreted from the cell to the extracellular space or compartmentalized into subcellular organelles (i.e., peroxisomes or vacuoles) (Oude Elferink et al. 1993; Van Luyn et al. 1998), by phase III transmembrane ATP-dependent efflux pumps (Bard 2000; Flugge and van Meer 2006; Sorensen and Dearing 2006). The predominant enzymes involved in allelochemical metabolism and transport include cytochrome P450s (phase I), glutathione S-transferases (phase II), and ATP Binding Cassette (ABC) transporters (phase III). In what

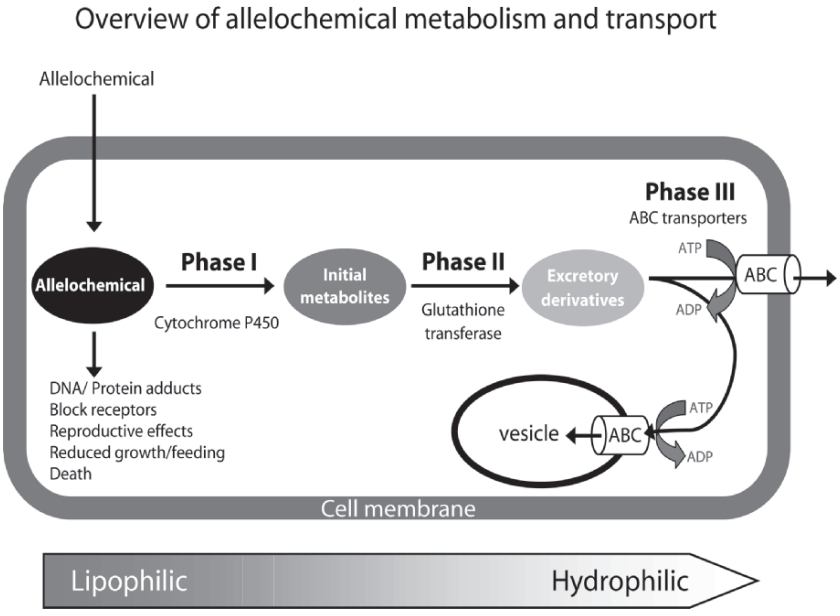

Fig. 10.1 An overview of the three phases of allelochemical metabolism and transport

follows, we briefly outline these enzymatic reactions, their roles in detoxification and/or transport, and their genetic diversity.

We propose that these enzymes play a significant role in the detoxification of algal secondary metabolites by marine herbivores for two primary reasons. First, the ubiquity and diversification of these enzymes in metazoans suggest that these biotransformation and transport proteins represent some of the oldest, largest, and most versatile gene superfamilies (Gonzalez and Nebert 1990; Higgins 1992; Nelson 1999; Sheehan et al. 2001) whose substrates include structurally diverse, lipophilic compounds. Second, and more importantly, knowledge of the participation of these enzymes in dietary allelochemical metabolism has been well described among insect and mammalian herbivores that regularly consume and tolerate vascular plant metabolites (Yu 1992; Feyereisen 1999; Scott and Wen 2001; Foley and Moore 2005; Sorensen et al. 2006). Macroalgae produce similar classes of secondary metabolites as their terrestrial plant counterparts, including terpenes, acetogenins, aromatic compounds, amino-acid derivatives, and polyphenolics (Hay and Steinberg 1992, Hay and Fenical 1988; Paul 1992), although profound differences in chemical structure are known (macroalgae incorporate halogens, produce very few N-based alkaloids, and tend to produce higher molecular weight terpenes (C_{20}) rather than monoterpenes (C_{10}) found routinely among terrestrial plants; see Chap. 1). Thus, the similarities among marine and terrestrial plant chemistry could conceivably select for convergence in the detoxification/transport mechanisms among marine and terrestrial herbivores.

Phase I – Cytochrome P450

The most familiar phase I reactions involve the addition of a polar functional group (e.g., hydroxyl) into a compound to increase its hydrophilicity and facilitate excretion. These reactions are accomplished primarily by a superfamily of inducible, heme-thiolate enzymes collectively termed cytochrome P450 monooxygenases (CYPs) (Parkinson 2001) found in terrestrial and aquatic organisms ranging from bacteria to vertebrates (Omura 1999). In general, the cytochrome P450 enzyme can insert one oxygen atom from molecular oxygen (O_2) into the substrate as it is held at the active site (Omura 1999). This interaction of a cytochrome P450 with a substrate can be envisioned as "a trap that lures a suitably lipophilic molecule into a greasy pocket and then slams an activated oxygen into it" (Brattsten 1992). It is this loose lipid interaction in the active site that allows a single P450 protein to metabolize and detoxify a myriad of lipid-soluble substances (i.e., drugs, environmental pollutants, pesticides, steroid hormones, pheromones, procarcinogens, microbial compounds, and plant allelochemicals) (Coon et al. 1992; Nelson et al. 1996; Rewitz et al. 2006), which also induce the expression of P450s. In addition to hydroxylation reactions, cytochrome P450s can catalyze several other reactions, including dehalogenations, heteroatom (O-, S-, and N-) dealkylations, deaminations, and reductions (Gonzalez and Nebert 1990; Parkinson 2001). Taken together, the broad substrate specificity, catalytic versatility, and diversity of forms make the

P450 gene superfamily an effective means of protection against a variety of dietary compounds (Scott and Wen 2001).

The nomenclature of P450 genes is based on their evolutionary relatedness. Gene families share >40% identity and are assigned numbers (e.g., CYP1); subfamilies share >55% identity and are assigned letters, and numbers specify individual genes (e.g., CYP1A1) (Nelson et al. 1993). A comparison of their molecular evolution within the eukaryotes found that the conserved regions occur around the central heme group, necessary for catalytic activity, while polymorphic regions occur at sites of substrate binding and catalysis (Graham and Peterson 1999; Werck-Reichhart and Feyereisen 2000). Each functional P450 gene appears to encode a unique enzyme (Nelson et al. 1993; Omura 1999), and as of April 2007, more than 2,300 metazoan CYP sequences representing 101 families have been identified (http://drnelson.utmem.edu/CytochromeP450.html).

For many invertebrates, the true number of P450 genes is unknown, and description of enzymatic activities has generally been limited to anthropogenic compounds (e.g., man-made compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pesticides (reviewed in Snyder 2000; Rewitz et al. 2006)). Sequencing of insect genomes has revealed extensive proliferation and divergence of P450 genes in both *Drosophila melanogaster* and *Anopheles gambiae* (Tijet et al. 2001; Ranson et al. 2002). The extreme diversification of insect P450 forms and the acquisition of new gene functions are believed to have occurred via gene duplication and subsequent divergence events most likely as a "response to evolutionary pressure emanating from a highly changeable external environment" (Berenbaum 2002). Four hundred million years ago, as insects evolved to consume terrestrial plants, and plants counteracted by producing defensive compounds to ward off herbivores, new P450s were recruited to detoxify novel plant chemical defenses, resulting in the progressive diversification of some P450 families we see today (Berenbaum 1983; Gonzalez and Nebert 1990; Scott and Wen 2001; Cornell and Hawkins 2003).

We have gained tremendous insight into the evolutionary diversification of P450s by characterizing the function and expression of insect CYPs in response to specific dietary compounds (Berenbaum 1983, 1991; Cohen et al. 1992; Schuler 1996; Feyereisen 1999; Petersen et al. 2001). For example, studies of the swallowtail butterflies (genus *Papilio*) provided the first evidence that herbivore feeding specialization within a genus was associated with the evolution and transcriptional regulation of P450 genes capable of detoxifying the toxic furanocoumarins produced by wild parsnips (reviewed in Schuler 1996). *Papilio polyxenes* (a specialist species that feeds solely on furanocoumarin-containing plants) and two generalist papilionids, *P. glaucus* (feeds occasionally on furanocourmarin-containing plants) and *P. canadensis* (rarely encounters furanocoumarins), express CYP6B genes with specific reactivities reflecting the degree to which furanocourmarins are present in their diets (reviewed in Schuler 1996; Li et al. 2001, 2003). In addition, four times the number of furanocoumarin-metabolizing CYP6B genes have been isolated from generalist *Papilio* species than from the specialist *P. polyxenes,* suggesting that the wider the diet breadth, the greater the requirement for a diversity of detoxification

genes to cope with the range of dietary allelochemicals (Li et al. 2007). Because specialists consistently feed on host plants containing furanocoumarins, it is not surprising that their CYP6B genes would be constitutively expressed with somewhat minimal adjustability, while in generalists transcript expression is barely detectable but highly inducible upon exposure to furanocoumarins. Molecular modeling studies comparing CYP6B1 from the specialist *P. polyxenes* and CYP6B8 from the generalist lepidopteran *Helicoverpa zea*, revealed that the generalist protein had an overall greater flexibility, a more elastic catalytic pocket, and an additional substrate access channel than did CYP6B1 (Li et al. 2004). Subsequent recombinant expression of both enzymes confirmed that the generalist CYP6B8 can metabolize a greater range of structurally diverse plant allelochemicals, albeit at a lower catalytic efficiency, than can the specialist CYP6B1. To the generalist, the cost of increased flexibility, resulting in a less efficient metabolism of furanocoumarins, is balanced by the acceptance of a wider variety of allelochemicals at the catalytic site and the ability to better contend with the unpredictability of plant defenses (Li et al. 2004).

Phase II – Glutathione S-Transferase

Phase I enzymes, consisting largely of oxidative reactions with cytochrome P450s, increase the hydrophilicity of lipid-soluble xenobiotics. In contrast, phase II enzymes are primarily involved in conjugative reactions with cofactors that react with functional groups either present on xenobiotics or introduced/exposed during phase I biotransformation. These phase II conjugative reactions usually result in larger increases in xenobiotic hydrophilicity, further promoting the excretion of conjugates from the cell (Fig. 10.1). A full review of phase II reactions can be found in other texts (e.g., Parkinson 2001). Here, we will concentrate on one particular superfamily of phase II enzymes, the glutathione S-transferases (GSTs), shown to be involved in allelochemical metabolism.

Glutathione S-transferases aid in the excretion process by catalyzing the nucleophilic attack of reduced glutathione (GSH) into hydrophobic compounds that contain an electrophilic carbon, nitrogen, or sulphur atom (Hayes et al. 2005). Seven classes of cytosolic GSTs (alpha, mu, pi, theta, sigma, zeta, and omega) occur widely in metazoans and can be distinguished based on their protein sequence homology (Sheehan et al. 2001). These GST classes exhibit differing but often overlapping substrate specificities for both endogenous and exogenous compounds, including chemotherapeutic agents, insecticides, carcinogens, environmental pollutants, oxidative stress by-products, and natural products (Sheehan et al. 2001; Enayati et al. 2005; Hayes et al. 2005; Li et al. 2007). Each functional GST is composed of two subunits, either identical (homodimeric) or nonidentical (heterodimeric), with each monomer having at least two ligand binding sites: one site highly specific for glutathione and another more variable site that can accommodate a diversity of electrophilic substrates (Doyen et al. 2005; Ortelli et al. 2003). In addition, the detection of alternative spliced gene products and the formation of

heterodimers in this protein family allow for the development of novel GST enzymes, which presumably increases the pool of potential substrates (Sheehan et al. 2001).

The involvement of GSTs in the detoxification of dietary secondary metabolites in terrestrial herbivores has strong empirical support (Yu 1982, 1989; Brattsten 1992; Li et al. 2007). Many plant allelochemicals and marine natural products contain the appropriate functional groups that can be directly conjugated with GSH (Schlenk and Buhler 1988; Brattsten 1992). Analogous to P450 systems, GST-mediated resistance to dietary allelochemicals may occur via gene diversification, gene inducibility, or both. As an example of the former, the degree of polyphagy in five lepidopteran species was positively correlated with the number of purified GST isoforms (Yu 1989). However, it appears more likely that adaptation to host plants is mediated by induction of GST proteins rather than diversification. Various species of insects exhibit a 2- to 40-fold induction of specific GSTs upon changes in host plant diet or when exposed to plant compounds in artificial diets (Yu 1982, 1984; Feyereisen 1999; Snyder 2000). Allelochemicals such as xanthotoxin, indole derivatives, flavones, and allyl glucosinolates that induce cytochrome P450s in insects often also induce GST activity (Yu 1983, 1984, 1986, 1992), but this is not always the case (Yu 1982). Additionally, GSTs isolated from polyphagous insects were able to metabolize a greater diversity of compounds in comparison to less polyphagous and specialist insect herbivores (Wadleigh and Yu 1987, 1988; Yu and Abo-Elghar 2000). Interestingly, some allelochemicals act as GST inhibitors (Lee 1991; Yu 2002) or transcriptional repressors (Ahmad and Pardini 1990), which may indicate a counterdefense strategy mounted by plants to block GST-mediated metabolism of co-occurring allelochemicals by insects (Li et al. 2007).

Phase III – ABC Transporters

In order for allelochemicals to enter the body of a herbivore, absorption must occur across the gut lining. Curtailing the initial absorption of dietary allelochemicals may be a herbivore's "first line of defense" against plant toxins. Studies have citied the lack of absorption or metabolism of lipophilic plant secondary metabolites (i.e., terpenes), conducive to phase I or II detoxification, in the gut of terrestrial herbivores; rather these compounds are excreted unchanged in the feces (Marsh et al. 2006b). While physical barriers or surfactants have been used to explain this limited adsorption in both marine and terrestrial herbivores (Lehane 1997; Barbehenn and Martin 1998; Barbehenn 2001; for review of marine herbivores, see Targett and Arnold 2001), active efflux of plant allelochemicals out of enterocytes into the gut lumen has received limited attention until now.

Originally discovered for their role in chemotherapeutic drug resistance in tumor cells, the ABC superfamily of genes encode membrane proteins that regulate the absorption, distribution, and excretion of a variety of natural and synthetic chemicals by facilitating their unidirectional transport across cell membranes in an ATP-dependent (i.e., energy-requiring) process (Fig. 10.1). Despite the popularity of these efflux transporters, commonly referred to as multidrug transporters, in human

pharmacological studies (Liang and Aszalos 2006), it has only recently been recognized that many of these substrates are products of chemical warfare and competition between and among animals and plants (Smital et al. 2004; Sorensen et al. 2006). Some have speculated that these competitive interactions may have been the evolutionary driving force needed to stimulate the development of these efflux transporters, and the transport of anthropogenic contaminants seen today is merely a result of the broad substrate specificity of these proteins (Smital et al. 2004). Although the original role of multixenobiotic transporter (MXTs) proteins remains unclear, the expression of these transporters in noncancerous tissues and their distribution in marine metazoans suggest a protective role against environmental xenobiotics, including marine natural products (Epel 1998; Bard 2000; Eufemia et al. 2002; Smital et al. 2004).

ABC transporters are a large superfamily of proteins responsible for trafficking molecules across cell membranes (Litman et al. 2001). The location of ABC transporters in tissues exposed to, or critical for, the metabolism of xenobiotics (i.e., liver, kidney, gills, gut, hepatopancreas, intestine, blood-brain barrier) supports their function as toxin efflux pumps (Smital et al. 2004; Leslie et al. 2005). In vertebrates, ABC transporters are classified into seven subfamilies designated ABC-A through G based on gene structure, domain order, and sequence homology (Dean and Annilo 2005). A subset of these subfamilies, ABC-B, ABC-C, and ABC-G, includes proteins that mediate toxin efflux. Collectively, all three subfamilies of efflux transporters are "capable of transporting a vast and chemically diverse array of toxicants, including bulky lipophilic cationic, anionic, and neutrally charged drugs and toxins" as well as phase II conjugates that "encompass dietary and environmental carcinogens, pesticides, metals, and lipid peroxidation products" (Leslie et al. 2005).

Plant secondary metabolites have long been known to be transported by mammalian ABC transporters. Vincristine, an indole alkaloid from herbaceous plants, paclitaxel (a.k.a. taxol), a diterpene derivative from coniferous trees, and digitoxin, a sterol glycoside from foxglove, are preferred substrates of some transporters (Yazaki 2006). Recently, several marine natural products from algae (okadaic acid, calyculin A, caulerpin), tunicates (patellamide D, lamelarins), sea hares (dolastatins), and gorgonians (polyoxygenated steroids) have also been identified as putative substrates for ABC-B proteins (Suganuma et al. 1988; Chambers et al. 1993; Williams and Jacobs 1993; Aherne et al. 1996; Quesada et al. 1996; Schroder et al. 1998; Tanaka et al. 2002). We anticipate that additional marine natural products will be identified as substrates for ABC transporters, and ecologically relevant studies exploring the relationship between MXT gene expression, allelochemical transport, and herbivore resistance should be pursued.

Examples from Marine Consumers

A relatively small number of investigators have focused on the role of detoxification and transport enzymes in the feeding ecology of marine consumers. Here, we outline three examples of this small, but growing body of work. The first example

focuses on the responses of herbivorous molluscs to lanosol, a moderately hydrophobic brominated phenol that was shown to deter Japanese abalone *Haliotis discus*, but not the sea urchin *Strongylocentrotus intermedius* (Kurata et al. 1997). This and other phenols can be found in relatively high concentrations (1–2% by dry weight) on red seaweeds such as *Odonthalia dentata*. When lanosol was injected into an Oregon population of the gumboot chiton *Cryptochiton stelleri* at doses of 2.5 mg/kg and greater, expression of a CYP3A-like protein in the digestive gland increased by 45% relative to the control (DeBusk et al. 2000). Additionally, lanosol exhibited noncompetitive inhibition toward both GSH and the universal GST substrate 1-chloro-2,4-dinitrobenzene (CDNB), suggesting nonenzymatic binding of lanosol to both GSH and the active site sulfhydral groups of GST, thereby blocking CDNB access to the active site (DeBusk et al. 2000) Nonenzymatic binding and subsequent sequestration of secondary metabolites by phase II enzymes, such as GST alpha (formally known as ligandin), would prevent cytotoxic compounds from interacting with their targets (Litwack et al. 1971; Hayes et al. 2005). Interestingly, the authors also find population-level differences in the CYP and GST activity, and suggested (without elaboration) that these profiles could be driven by seasonal or site differences in the algal assemblage.

In a separate study, Kuhajek and Schlenk (2003) compared the P450 and GST activity within the digestive gland tissues of two southern California molluscs, the chiton *Katharina tunicata* and the abalone *Haliotis rufescens*. The authors indicate that *K. tunicata* feeds regularly on filamentous red seaweeds, some of which are chemically defended with lanosol, while the abalone *H. rufescens*, is thought to be as deterred by lanosol as was its Japanese congener *H. discus* (Kurata et al. 1997). Animals were injected for 3 days with 10 mg lanosol/kg or distilled water before digestive glands were dissected and stored for subsequent analysis. Overall, the basal levels of P450 and GST were higher in *H. rufescens*. P450 activity was not induced by exposure to lanosol in either herbivore; however, GST activity was induced in *K. tunicata* when exposed to lanosol, as was found for *C. stelleri*. It is difficult to infer more from these studies, in part, because few empirical data support the notion that these animals consume or avoid lanosol in their natural habitats.

The second example comes from the genome sequencing of the temperate sea urchin *Strongylocentrotus purpuratus* (Sodergren et al. 2006). Its analysis provides us a glimpse into the potential importance of phase I, II, and III enzymes in the ecology of these keystone herbivores. *S. purpuratus* is a generalist consumer, known to consume browns, reds, and greens from nearshore northeastern Pacific habitats. Many of these macrophytes contain polyphenolic-based defenses (Steinberg 1992) and generally lack known lipophilic deterrents, although this has not been adequately tested. Surprisingly, there is a tremendous diversity of detoxification genes in this sea urchin, suggesting that this herbivore has the capacity to metabolize a broad suite of diverse compounds. Sea urchins contain 120 CYP genes mostly in gene families 1–3: 11 CYP1-like, 73 CYP2-like, and 17 CYP3-like genes, which are the major families involved in xenobiotic metabolism in humans (Pascussi et al. 2003). In comparison with human (57 CYPs), pufferfish (58 CYPs), and tunicate (86 CYPs) genomes, many CYP gene families have

Table 10.1 Phase I, II, and III gene diversity in the sea urchin *Strongylocentrotus purpuratus* and humans

Classification	Gene	Urchin	Human
Phase I (oxidative)			
Cytochrome P450s	CYP1-like	11	3
	CYP2-like	73	16
	CYP3-like	10	4
	Total	120	57
Phase II (conjugating)			
Glutathione S-transferase	GST alpha	9	5
	GST pi	1	1
	GST sigma	17	1
	GST theta	6	2
	GST zeta	1	1
	GST omega	4	2
	GST mu	0	5
	Total	38	17
Phase III (transport)			
ABC transporters	ABC-B	12	11
	ABC-C	30	12
	ABC-G	4	5
	Total	65	48

Modified from Goldstone et al. (2006). Hayes et al. (2005) and Dean and Annilo (2005) were used as reference
CYP: cytochrome P450; *GST:* glutathione S-transferase; *ABC:* ATP Binding Cassette

undergone diversification and expansion in the sea urchin (Goldstone et al. 2006). The same gene diversification and expansion is also true for both phase II conjugating enzymes and phase III transporters (Table 10.1). With the aid of microarrays, the dietary regulation of the entire complement of allelochemically responsive genes in the sea urchin can be investigated for the first time and candidate genes can be identified for further biochemical characterization studies.

Finally, one of the clearest examples of a link between detoxification genes and the feeding ecology of a marine consumer comes from the tropical gastropod *Cyphoma gibbosum*, which is a generalist predator of chemically defended, tropical gorgonians. Vrolijk and Targett (1992) measured P450 and GST enzymatic activities from the digestive gland of field-collected *C. gibbosum* feeding on four species of gorgonians. While cytochrome P450 specific content was low and marginally quantifiable, subsequent studies have identified P450 genes whose expression is regulated in a diet-dependent manner (Whalen et al., unpublished data). Interestingly, GST activity was constitutively highly expressed in *C. gibbosum* feeding on all four diets and was among the highest ever reported from a molluscan digestive gland, rivaling values reported from herbivorous insects that feed on chemically defended plants (Brattsten 1992; Vrolijk and Targett 1992). Since this study, proteomic techniques have identified several distinct GSTs, whose activity can be inhibited by

lipophilic gorgonian extracts, suggesting that gorgonian natural products may be substrates for these enzymes (Whalen et al., unpublished data).

10.3 Detoxification and Macroalgal-Herbivore Interactions

As we look to the future, applying a molecular approach to detoxification mechanisms should provide tremendous insight into several vexing issues in the ecology and evolution of marine macroalgal-herbivore interactions. This approach moves beyond simply correlating diet choice with secondary metabolite profiles, and represents the next challenge for marine chemical ecologists: translating population- and species-level variation in herbivore allelochemical tolerance to responses on the biochemical level. Below are sets of ecological and evolutionary questions that would benefit from an understanding of detoxification mechanisms.

10.3.1 Do Detoxification Rates Limit Feeding Rates of Large Grazers?

Large, generalist marine grazers such as fishes and urchins attempt to choose foods that maximize nutritional input (e.g., protein, lipids, and carbohydrate) (Mattson 1980; Choat and Clements 1998) and minimize intake of secondary metabolites (Hay 1991). The untested assumption underlying these optimal foraging decisions is that detoxification and excretion rates are a constraint on toxin intake and thus drive feeding choice (Freeland and Janzen 1974). However, we have virtually no information on such constraints in marine herbivores, because it requires an understanding of the metabolic fate of secondary metabolites.

Patterns emerging from studies of terrestrial herbivores should guide the study of detoxification mechanisms by marine fishes and urchins. For example, it is now clear that the daily maximal detoxification rate appears to reduce feeding rates on individual compounds by several generalist herbivores (Dearing et al. 2005), as predicted by the "detoxification limitation hypothesis" (Freeland and Janzen 1974; Freeland 1991). Brushtail possums (*Trichosurus vulpecula*) consumed more overall biomass when offered two diets containing secondary metabolites (e.g., monoterpenes vs. benzoate) detoxified by different pathways (phase I and phase II), than when offered a single plant secondary metabolite or two diets with competing detoxification pathways (Marsh et al. 2006b). Thus, varying the consumption of chemically defended diets not only prevents the detoxification enzymes from becoming swamped, but enables animals to learn about the consequences of ingesting specific diets, consume more plant material, and induce or maintain critical detoxification enzymes (Marsh et al. 2006a). Perhaps herbivores can sense mounting saturation of enzymes and adjust their feeding behavior accordingly (Provenza et al. 2003). In contrast, the feeding rates of specialist mammals appear less constrained

by detoxification rates because they process specific metabolites more effectively than do generalists (Dearing et al. 2005; Foley and Moore 2005). This phenomenon creates a trade-off, however: the specific phase I pathways used by specialists have finer substrate specificity but are less able to efficiently process novel toxins (Boyle and McClean 2004; Sorensen et al. 2005). Similar evidence for this trade-off has emerged from insect P450 studies (Berenbaum and Zangerl 1999; Li et al. 2004). Much of the terrestrial work focuses on terpenes, because they "appear to be low-potency toxins, especially for specialized herbivores, occur widely, and comprise part of the natural diet of many herbivores" (Foley and Moore 2005: p. 431). In mammalian herbivores, terpenes are detoxified by a suite of rapidly inducible CYPs in families 2 and 3 (Pass et al. 2001, 2002; Pass and McLean 2002; Duisken et al. 2005).

10.3.2 Are Tropical Herbivores More Tolerant of Lipophilic Metabolites Than Are Temperate Herbivores?

Two central predictions of a diffuse coevolutionary arms race (cf. Vermeij 1994) among herbivore and algal species are that (1) algae would evolve greater chemical defenses against herbivores to minimize grazing damage and (2) that herbivores evolve greater feeding tolerance for those metabolites. As we have pointed out, we generally have far more information on the first prediction (algal defenses) than on the second (herbivore offenses). This disparity is particularly evident within tropical habitats, in which diffuse coevolution is likely.

In general, tropical seaweeds have considerably greater concentrations and diversity of lipophilic secondary metabolites than do temperate seaweeds (Paul 1992; Bolser and Hay 1996; Hay 1997; Van Alstyne et al. 2001). The stronger deterrence of tropical seaweeds and their lipophilic metabolites likely represents a macroevolutionary response by tropical seaweeds to an intensification of herbivory (Steinberg 1992; Hay 1997; Paul et al. 2001; Van Alstyne et al. 2001). That is to say, there are greater abundances and a higher diversity of herbivorous fishes in the tropics relative to temperate regions (Lubchenco and Gaines 1981; Birkeland 1997; Floeter et al. 2004; see Chap. 2).

However, a poorly tested assumption that underlies the latitudinal trend in seaweed defenses is the notion that tropical herbivores have a greater feeding tolerance for chemically defended tropical seaweeds than do temperate herbivores. To our knowledge, there is only one marine study that has documented a significantly greater feeding tolerance for lipophilic compounds by tropical, compared with temperate, herbivores. Cronin et al. (1997) found that diterpenoid metabolites from the tropical Pacific seaweed *Dictyota acutiloba* deterred a temperate North Carolina fish (the pinfish *Lagodon rhomboides*) and sea urchin (*Arbacia punctulata*) species at a concentrations lower than those required to deter two parrotfishes, two surgeonfishes, and an urchin (*Diadema savignyi*) from tropical reefs of Guam. A second, but nonsignificant test (Bolser and Hay 1996) compared the feeding responses of a

tropical urchin (*Lytechinus variegatus* collected from Key Largo, Florida) and a temperate urchin (*Arbacia punctulata* from Bogue Sound, North Carolina) to tropical versus temperate congeneric seaweeds. A reanalysis of their data (their Fig. 4) indicates that in seven of nine sets of assays, *Lytechinus* consumed proportionately more of the tropical seaweed than did *Arbacia*. This was not a significant difference (Paired *t*-test, df = 8, $p = 0.153$), probably because of low statistical power of their sample size.

Thus, we require more direct tests of whether tropical marine herbivores have a higher feeding tolerance for secondary metabolites than do temperate herbivores. In addition, differential resistance to chemical defenses among species or among populations (see Sect. 10.4) could be explained by either quantitative differences in the expression of detoxification proteins (i.e., gene induction), or sequence variation among detoxification genes and/or alleles. At either level, there should be strong selection on tropical generalist herbivores to have a greater detoxification versatility (i.e., detoxify a broader suite of allelochemicals) than would temperate generalists, because temperate herbivores rarely consume macroalgae rich in lipophilic chemistry.

10.3.3 Is Host Breadth Mediated by Tolerance of Lipophilic Metabolites?

The forces that sculpt the evolution of specialization among marine herbivores have been explored in terrestrial systems for decades but only recently with marine systems (Hay and Fenical 1988; Trowbridge 1991a; Hay 1992; Sotka et al. 1999; Poore et al. 2000; Krug 2001; Trowbridge and Todd 2001; Cruz-Rivera and Paul 2006). As is the case with host vascular plants and herbivorous insects (Bernays and Chapman 1994), host seaweeds likely exert strong selection on the feeding choice, life history, morphology, and physiology of smaller, "insect-like" (cf. Hay et al. 1987) invertebrates termed *mesograzers* (Steneck and Watling 1982; Duffy and Hay 1991; Jensen 1997; Poore and Steinberg 2001; Cruz-Rivera and Hay 2003; Sotka 2005; Taylor and Steinberg 2005). The mechanism of selection varies with the system in question, but generally, small herbivores specialize on particular host seaweeds because the seaweed minimizes loss to abiotic forces, or maximizes growth or mate encounter rates, among other possibilities.

For two decades, the role of lipophilic chemistry in the evolution of mesograzer host range was heavily influenced by the "enemy-free space" hypothesis by Hay et al. (1987). This theory predicts that small herbivores consume and inhabit seaweeds that are chemically defended against larger, more mobile herbivores such as fishes and urchins. Support for these patterns can be found in several contexts: from temperate North Carolina (e.g., Duffy and Hay 1991, 1994) and New Zealand (Taylor and Steinberg 2005), and the tropical Caribbean (e.g., Hay et al. 1990) and Guam (e.g., Paul 1988; Cruz-Rivera and Paul 2006), and the Antarctic (Huang et al. 2007). In each case, small herbivores tend to be more tolerant of chemically rich seaweeds relative to larger consumers. This is not always the case, and in fact the

opposite pattern may also be true: large grazers may readily consume chemistry produced by tissues (e.g., algal spores) that deter small mesograzers, perhaps because these small tissues are not susceptible to larger grazers (Hay et al. 1998).

There is an alternative, and less explored role of lipophilic chemistry in the evolution of host diets. Among generalist herbivores (small or large), one evolutionary advantage of consuming chemically rich prey is that it increases the available resource base. To test this, Poore et al. (unpublished data) compared the host ranges of herbivores that consumed seaweeds that produced lipophilic secondary metabolites (e.g., diterpenes, acetogenins) versus the host range of herbivores that did not consume these chemically rich seaweeds. The authors focused on the three groups of herbivores for which patterns of host breadth have been systematically assessed: ascoglossan slugs, ampithoid amphipods, and fishes.

Ascoglossan sea slugs display profound among-genera patterns in using seaweeds: virtually all ascoglossan slugs feed only on chlorophyte macrophytes, all shelled species feeding from the single genus *Caulerpa*, and all species occur on three or fewer host genera (Jensen 1997; Marin and Ros 2004). This extremely restricted diet is analogous to specialist lepidopterans feeding on leaves of tropical tree species (Novotny et al. 2002). In contrast with the slugs, gammaridean amphipods in the family Ampithoidae are not as restricted in their host use. A recent compilation of host records from 102 species of ampithoids (of 127 known species) finds amphipod species on 20 orders and all three divisions of macroalgae (Phaeophyta, Chlorophyta, and Rhodophyta) and from ten genera of seagrasses. Individual amphipod species are also found on a wide variety of hosts, with 30% of the species inhabiting hosts from two or more divisions of macroalgae. Thirty-six percent of the amphipod species were recorded from only one host genus; however, the majority of these species (83%) had data from only one study and thus the apparent specialization likely reflects the limited amount of information on those taxa rather than dietary specialization (Poore et al., unpublished data). Not surprisingly, a detailed analysis of the gut contents from dozens of herbivorous fishes (Randall 1967) revealed the broadest and most polyphagous diets, feeding on seaweeds from up to 25 distinct genera.

Species that include algae with lipophilic metabolites in their diets tended to have a broader algal range relative to herbivores that do not include chemically rich algae (Fig. 10.2; Poore et al., unpublished data). This was assessed by assigning genera as producing (or not producing) classes of lipophilic chemistry that are known to deter some marine herbivores (e.g., terpenes, acetogenins). The effect of including chemically rich macroalgae on the diet breadth was particularly dramatic for herbivorous fishes (from ~3 to 12 genera) and ampithoid amphipods (from ~1.5 to 5.5 genera), and largely absent for slugs. These patterns suggest that for ampithoid amphipods and fishes, one evolutionary advantage in evolving a resistance to chemically rich seaweeds may be that it increases the availability of appropriate seaweed hosts (i.e., enlarges the resource base). For ascoglossan slugs that generally utilize a far more restricted host range, it suggests that the slugs specialize at equal rates on chemically rich and chemically depauperate seaweeds, and suggests that their evolution may be driven by factors other than the presence of lipophilic chemistry.

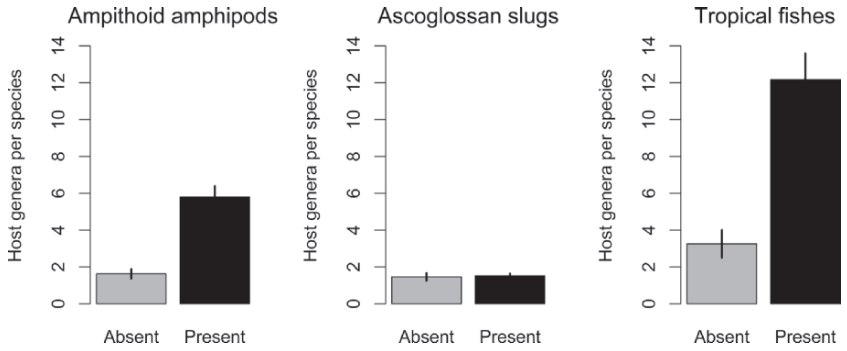

Fig. 10.2 The host ranges of species that utilize hosts that produce ("Present") or do not produce ("Absent") lipophilic chemistry (Poore et al., unpublished data). The more generalized amphipods and fishes have a broader host range when incorporating chemically rich seaweeds in their diet, while the host range of the more specialized slugs does not display any effect of secondary metabolites

Thus, there is an interesting contrast between terrestrial and marine systems in the relationship between specialization and detoxification of secondary metabolites. Among terrestrial insects and mammals, specialists seem to be associated with chemically "difficult" plants (Berenbaum et al. 1996), while in marine systems, generalists tend to consume chemically rich plants (e.g., amphipods and fishes).

These patterns should be reflected in the detoxification ability of specialist versus generalist herbivores of these groups. The detoxification mechanisms of generalist amphipods and fishes should require an "all-purpose", functionally versatile suite of enzymes, capable of degrading a broader range of algal allelochemicals. In contrast, the fish and amphipod specialists that tend to avoid chemically rich foods should have little to no ability to detoxify lipohilic allelochemicals. These predictions differ with sea slugs, who are specialists either on chemically rich or chemically depauperate seaweeds. Slugs specializing on chemically rich prey should have a highly efficient and specific suite of enzymes relative to the generalist fish and amphipods, and relative to the slugs that specialize on chemically depauperate seaweeds.

10.3.4 Are There Phylogenetic Constraints on Tolerance of Lipophilic Metabolites?

There is growing evidence that phylogenetic heritage explains a substantial amount of between-species variation in macrophyte use and tolerance for secondary metabolites. Further, it is possible that some portion of these phylogenetic constraints is mediated by the evolution of detoxification systems. For example, although ampithoid amphipods were generalized in host use, species in the genus *Peramphithoe* utilize a smaller number of host algal genera and orders than do species in *Ampithoe, Cymadusa*, or *Exampithoe* (Poore et al., unpublished data). An analysis of the compositions of these

host ranges indicates two broad patterns: *Peramphithoe* is the only genus that does not regularly utilize dictyotalean seaweeds (e.g., the genera *Padina* and *Dictyota*), while *Exampithoe* is unique because it does not use *Sargassum*. The notion that *Peramphithoe* has a more limited diet relative to other genera is consistent with preliminary observations (Conlan and Chess 1992; Poore and Steinberg 2001) that this genus is largely limited to algae from the Fucales and Laminariales. In areas where *Peramphithoe* species co-occur with dictyotalean seaweeds, their performance has been poor (e.g., *P. parmerong* on *Padina crassa* in Australian estuaries (Poore and Steinberg 2001)), and the amphipods are strongly deterred from feeding on dictyotalean lipophilic chemistry (Poore and Steinberg 1999). In contrast, there is profound variation among species of *Ampithoe* in utilizing dictyotalean species. *Ampithoe longimana* is regularly found on *Dictyota menstrualis* and *D. ciliolata* in North Carolina estuaries, and their lipophilic extracts do not strongly alter the performance of *A. longimana* (Sect. 10.4). The co-occurring *A. valida*, however, is never found on *Dictyota* and is strongly deterred by their lipophilic extracts (Duffy and Hay 1994; Cruz-Rivera and Hay 2003). Thus, one would predict that *Peramphithoe* would lack the ability to detoxify lipophilic compounds such as diterpenes.

10.3.5 Do Herbivores "Eavesdrop" on Their Macroalgal Hosts?

Jasmonates are plant-produced hormones that signal plant genes to, among other functions, synthesize chemical defenses in response to herbivore attack or microbial infection (Baldwin 1998; Stanjek et al. 1999). This group of hormones has similar effects in red and brown macroalgae, suggesting that these compounds may be used as ubiquitous signaling molecules in photosynthetic organisms. Insect consumers on plants have learned how to "eavesdrop" on these plant signals and activate the expression of their cytochrome P450 genes responsible for the detoxification of the plants' allelochemicals and, in doing so, increase their own chances of survival (Li et al. 2002). The ability of herbivores to cue on universal plant hormones, which signal the induction of several biosynthetically distinct plant secondary metabolites, and activate P450 counterdefense genes would be of great value to polyphagous species not only in the terrestrial environment. It is not inconceivable that generalist marine herbivores may have similar eavesdropping abilities, especially on seaweeds that induce lipophilic secondary metabolites (e.g., diterpene alcohols of *Dictyota menstrualis*: Cronin and Hay 1996).

10.4 Using Population-Level Variation in Herbivore Traits as an Analytical Tool

A powerful approach to elucidating the ecology and evolution of any phenotypic trait can come from an explicit manipulation of genetic differences in the trait among populations of a single species, a research focus of evolutionary ecologists

since and including the studies of Charles Darwin. Yet despite this analytical power, there are only a handful of marine ecologists who have utilized the power of within-species variation in understanding herbivore traits. To our knowledge, there are three marine examples of population-level differentiation in tolerance for secondary metabolites produced by algal prey. In each case, allopatric populations of herbivores are confronted with locally distinct algal communities, and display highest affinity for, or fitness on, local algal species.

The first two examples revolve around the evolutionary effects of harmful algal blooms (or HABs) on their consumers. The dinoflagellate *Alexandrium* spp. bloom in estuaries throughout the geographic range of the herbivorous copepod *Acartia hudsonica*, but the historical frequency and toxicity of these blooms is far greater in the north (i.e., Maine and Canadian estuaries) than in the south (i.e., New Jersey estuaries). When fed toxic *Alexandrium*, field-collected females from northern regions had higher ingestion and egg production rates than did females from southern populations, while no geographic differences were observed when control foods were offered (Colin and Dam 2002). These differences appear to be genetically mediated and can be generated after only three generations of exposure to toxic *Alexandrium* (Colin and Dam 2005). Such rapid evolution to a HAB by this herbivore indicates that evolutionary and ecological responses should be considered when modeling the dynamics of algal-herbivore interactions.

The toxic *Alexandrium* spp. also bloom frequently in the Bay of Fundy. Bricelj et al. (2005) found that the Bay of Fundy softshell clams (*Mya arenria*), when exposed to toxic algae, burrowed more effectively after 24 h and had greater survivorship after 16 days than did clams from the outer coast of Nova Scotia where *Alexandrium* rarely blooms. The reason was not because "tolerant" clams were better at detoxification: in fact, Bay of Fundy clams attained a mean toxicity 5-fold greater than did the "sensitive" clams. Rather, the difference in tolerance for the secondary metabolites (which, in this case, were the neurotoxins saxitoxin and tetradotoxin, which can ultimately cause paralytic shellfish poisoning in vertebrates) was mediated by the molecular evolution of the sodium channel that acts as the target of the secondary metabolite. In particular, a transgenic brain channel was used to confirm that a single substitution from glutamic acid to aspartic acid at position E945 yields a conformational change in the receptor site of the sodium channel, thus decreasing its binding affinity to the neurotoxin. This fantastic study suggests that the resistance mutation may represent "an important risk factor for human PSP resulting from consumption of this species."

In estuaries of the southeastern Atlantic coastline of North America, the amphipod *Ampithoe longimana* – a small herbivorous crustacean (adults < 1 cm length) – readily consumes tropical brown seaweeds in the genus *Dictyota*. However, the tropical *Dictyota* is unavailable to more northerly populations (i.e., amphipods from Virginia to Maine). In a series of laboratory-based experiments with transplanted populations, southern populations of *A. longimana* that are sympatric with *Dictyota* displayed stronger feeding preference for *Dictyota* than did populations that are north of *Dictyota*'s geographic endpoint (Sotka and Hay 2002; Sotka et al. 2003). Further, the offspring of southern mothers had higher relative fitness when

raised on *Dictyota* than did those of northern mothers. Bioassay-guided fractionation showed that southern populations were more tolerant of the diterpene alcohols produced by *Dictyota* than were northern populations. These latitudinal differences in feeding behavior and performance are genetically mediated, since the patterns were maintained even after 5+ generations were reared on a variety of green and red seaweeds (Sotka 2003). This is consistent with the notion that predators of amphipods strongly select for those with high preference for *Dictyota* among southern amphipod populations (Duffy and Hay 1994).

10.5 Conclusion

When confronted with the profound variation in tolerance for chemically rich seaweeds that has been documented among herbivores, a biologist may be tempted to throw up one's hands and "give up" on herbivores (a tendency that certainly has aided the overwhelming focus on algal defenses). Alternatively, one can view this variation as a challenge to evolutionary ecology, forcing us to focus on why this variation exists (ultimate mechanisms) and how this variation exists (proximate mechanisms). To meet the challenge, we suggest a new effort should be made to foster collaborations between physiologists, biochemists, and field ecologists. In particular, ecological questions that emerge from field observation and a careful reading of the literature should be tested using biochemical and genetic assessments of detoxification enzymes. In our view, the development of a framework for understanding the diversity of algal-herbivore interactions requires an understanding of both algal defenses and herbivore offenses.

We also suggest that marine ecologists pay close attention to the terrestrial herbivores. Arguably, our depth of knowledge on the biochemical and molecular basis of detoxification is equivalent to that of insect ecologists in the 1970s. Despite the fact that "seaweeds are not wet trees and marine herbivores are not soggy insects" (Hay and Steinberg 1992), parallels between the chemistry of marine and terrestrial organisms do exist and will undoubtedly help guide the study of biochemical resistance mechanisms.

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11
Secondary Metabolite Defenses Against Pathogens and Biofoulers

A.L. Lane and J. Kubanek(✉)

11.1 Introduction

Competition for space and resources is intense in benthic marine environments (McClintock and Baker 2001). In these habitats, macroalgae constitute a seemingly ideal substrate for growth of microorganisms and other epibionts, presenting these organisms with a living space rich in organic material. Some associations between macroalgae and microbes are mutualistic, benefiting both host and symbiont. For example, algal-associated bacteria may produce metabolites that protect hosts from biofouling (e.g., Boyd et al. 1999; Armstrong et al. 2001), and recent reports have indicated that epibiotic hydroids may enhance growth of the kelp *Macrocystis pyrifera* (Hepburn and Hurd 2005). Other algal associates are clearly detrimental to hosts, as evidenced by reports of algal disease and fouling-associated fitness costs (e.g., D'Antonio 1985; Correa 1997; Ruesink 1998). Red spot disease in the commercially valuable kelp *Laminaria japonica* (Sawabe et al. 1998) is caused by *Pseudoalteromonas bacteriolytica* bacteria; similarly, white rot disease in the kelp *Nereocystis luetkeana* is caused by an *Acinetobacter* sp. bacterium (Andrews 1977). Some bacteria act as secondary pathogens, accelerating disease progression following attack of a primary pathogen (Correa et al. 1994). Fungi can also act as seaweed pathogens, including *Lindra thallasiae*, an Ascomycete, which causes raisin disease in *Sargassum* spp. brown algae and *Thalassia* disease in seagrasses (Kohlmeyer 1971; Andrews 1976; Porter 1986). In addition to bacterial and fungal pathogens, some species of endophytic multicellular algae, cyanobacteria, and amebas have been implicated as causes of disease in macroalgae (Andrews 1977; Correa et al. 1993; Correa and Flores 1995).

In addition to pathogens, micro- and macrofoulers negatively impact a variety of macroalgal hosts. Biofouling by the diatom *Isthmia nervosa* is related to declines

A.L. Lane
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta,GA 30332-0400, USA

J. Kubanek
School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230, USA.
e-mail: julia.kubanek@biology.gatech.edu
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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in growth and reproduction of the red alga *Odonthalia floccose* (Ruesink 1998). Biofoulers may indirectly reduce algal fitness by increasing drag and susceptibility to tissue breakage in turbulent water and by increasing herbivore attraction (Dixon et al. 1981; D'Antonio 1985; Wahl and Hay 1995).

Although pathogens and biofoulers both negatively influence host fitness, there are fundamental differences between these two algal colonizers. Specifically, pathogens must exhibit virulence against hosts, while such pathogenesis is absent in biofouling. Chemical defenses against both biofoulers and pathogens are included in this chapter, because each involve relatively long-term intimate associations with host algae, both benefit from association with hosts, and both pose negative fitness effects on hosts.

Despite some reports of algal disease and the seemingly favorable conditions that hosts provide for pathogens and biofoulers, reports of widespread algal destruction remain surprisingly uncommon. This suggests that macroalgae have evolved mechanisms to resist deleterious microorganisms. One strategy is to disrupt colonization or growth of parasites with physical defenses, including production of a mucilaginous covering, outer cell layer shedding, and erosion of the distal ends of blades to remove parasites from macroalgal surfaces (Mann 1973; Filion-Myklebust and Norton 1981; Moss 1982; Nylund and Pavia 2005). Algae may also prevent colonization of their tissues through oxidative burst, in which algae respond to microbial challenge through the release of reactive oxygen species (see Chap. 12), or by other rapidly activated responses. Another resistance mechanism is the use of secondary metabolites as chemical defenses (e.g., Boyd et al. 1999; Wikstrom and Pavia 2004; Engel et al. 2006; Puglisi et al. 2006). As pathogens and foulers first select, settle, and attach to hosts, algae may prevent tissue damage by harboring secondary metabolites that circumvent this stage. Following parasite attachment, secondary metabolites may inhibit the growth, survival, virulence, or reproduction of these organisms.

Antimicrobial and antifouling chemical defenses have been reviewed previously for macroalgae, marine invertebrates, and other marine organisms (Engel et al. 2002; Steinberg and de Nys 2002; Paul and Puglisi 2004; Dobretsov et al. 2006; see also Sects. 2.5.6 and 3.4.1). Thus, we do not aim to cover the breadth of this area, but instead to explore a few recent studies illustrative of the strategies used by macroalgae to thwart parasites at each stage of the infection or biofouling process. We will focus particular attention on evidence for the role of algal-associated microbes in host chemical defense and on the specificity of antimicrobial secondary metabolites. These two themes are inherently intertwined, as broad-spectrum versus highly targeted antimicrobial defenses could differentially impact the diversity of microbial communities living with macroalgae and, in turn, influence chemical defense profiles of algal-associated microbes.

11.2 Defenses Against Settlement and Attachment

Algal chemical defenses that inhibit the settlement and attachment of pathogens or biofoulers represent the first line of defense against microbial challenge. Unlike compounds that function through growth inhibition or lethality, most settlement and

attachment defenses impact microbial behavior, and as a result, may put less selective pressure on microbes to develop resistance (Rasmussen and Givskov 2006).

11.2.1 Larval Attachment Defenses of Ulva reticulata

Biofoulers, including many species of bryozoans, crustaceans, tunicates, and polychaetes, are abundant off the coast of Hong Kong (Harder and Qian 2000). Larvae of these animals attach to abiotic or biotic substrates and remain throughout development. Not all potential hosts are equally affected by biofoulers, suggesting some algae are defended. For example, the green alga *Ulva reticulata* was observed to be unscathed by biofoulers, leading Harder and Qian (2000) to hypothesize that this alga is chemically defended, inhibiting attachment or metamorphosis of biofoulers. In a relatively simple laboratory-based assay, larvae of the polychaete *Hydroides elegans* or the bryozoan *Bugula neritina* were placed in a Petri dish containing seawater in which *U. reticulata* had been previously soaked. Both *H. elegans* and *B. neritina* larvae attached to the Petri dish substrate and metamorphosed at a significantly lower rate in seawater conditioned with *U. reticulata* than in control seawater (Harder and Qian 2000; Harder et al. 2004). Although precautions were taken to minimize damage in transferring *U. reticulata* from the field, the stress of collection could have resulted in the release of compounds that might not otherwise be present, potentially confounding results. Furthermore, the laboratory settlement assay (in still water) could not address whether inhibitory compounds(s) are effective in natural flow regimes, and may have exposed larvae to unnaturally high concentrations of *U. reticulata* exudate.

In an effort to reduce stress to *U. reticulata* during generation of antisettlement cues, exudate was collected in the field by enclosing *U. reticulata* blades in transparent plastic bags for 1 h. Laboratory assays were conducted with the conditioned water, and the results of this experiment further supported *U. reticulata* deterrence of larval attachment (Harder et al. 2004). Although this experiment provided additional evidence for waterborne algal compounds acting as settlement inhibitors, *U. reticulata* could still have been exposed to unnatural stress while enclosed in the plastic bag; utilizing a gas-permeable but water-impermeable bag to collect exudates may be more appropriate (e.g., Kubanek et al. 2002).

Since the antifouling compounds(s) of *U. reticulata* appeared to be effective within the water column surrounding the source plant, these compounds may inhibit larval settlement on nearby macroorganisms as well. If competitors are indeed protected by *U. reticulata* defenses, then natural selection could favor "cheaters," mutant conspecifics that benefit from a neighbor's defenses without paying the costs of producing the defense (Foster and Kokko 2006). At the same time, local species diversity could increase if undefended heterospecifics are protected by associating with a defended neighbor (e.g., Hay 1986).

Although *U. reticulata* lacks significant larval biofouling, it harbors a variety of epibiotic bacterial species. Dobretsov and Qian (2002) evaluated the antifouling effects of seven bacterial species cultured from *U. reticulata* surfaces. The extract

of the cell-free supernatant from one *Vibrio* sp. significantly inhibited settlement and metamorphosis of *Hydroides elegans*, but not biofilm-forming bacteria, indicating that settlement inhibitor(s) from *Vibrio* sp. target larval foulers, not other bacteria. However, antibacterial effects of *Vibrio* sp. extracts were addressed solely through disc-diffusion assays, which are poor mimics of natural conditions, as they cannot expose bacteria to natural concentrations of test compounds (Jenkins et al. 1998). Furthermore, bacteria grown in liquid culture may produce compounds different from those produced by epiphytic bacteria.

This discovery suggests that inhibitory compound(s) originally attributed to *U. reticulata* might instead be produced by the *Vibrio* sp. symbiont. Thus, Harder et al. (2004) applied bioassay-guided fractionation to isolate defensive compound(s) from (1) *U.-reticulata*-conditioned seawater and (2) *Vibrio* sp. culture. Following desalting of crude extracts, ultrafiltration resulted in concentration of active metabolites in the >100 kDa molecular weight fraction, suggesting a bioactive protein, polysaccharide, or glycoconjugate from both sources. In both, elimination of bioactivity by β-glucuronidase and α-amylase suggested that the bioactive components contained large polysaccharide units. However, the active fractions were differentially susceptible to proteolytic enzymes, suggesting that *U. reticulata* may be defended from biofoulers by multiple chemical defenses: glycoprotein(s) from *U. reticulata* and polysaccharide(s) or nonproteinaceous glycoconjugate(s) from *Vibrio* sp. Analogously, extracts from *Pseudoalteromonas* spp. bacteria associated with *U. lactuca* have been reported to inhibit a suite of common biofoulers, although the responsible compound(s) have not been characterized (Egan et al. 2001).

The macromolecular bioactive metabolites from *U.-reticulata*-conditioned seawater and from *Vibrio* sp. are unique among antifouling compounds characterized to date, since other reported larval-deterrent molecules from macroalgae have included phlorotannins and nonpolar terpene alcohols (Schmitt et al. 1995; Lau and Qian 1997; Brock et al. 2007). The apparent lack of proteinaceous antifouling metabolites in the literature may represent a bias in extraction methodology, since most extractions have used solvents that would have denatured or failed to extract high molecular weight, water-soluble proteins.

Through methodological advancements, chemically mediated relationships among bacterial symbionts, host macroalgae, and biofoulers may be more fully elucidated. Advanced methodology may be especially important in assessing the benefit of defensive-metabolite-producing symbionts to host organisms. One possible direction is the application of molecular biology methods to create mutant symbionts for which the genetic ability to produce bioactive secondary metabolites is knocked out. By comparing biofouling of algae harboring symbionts capable of producing defensive metabolites to mutants without this ability, the role of such microbial metabolites may be analyzed in a more ecological context than before. Furthermore, field-deployed mass spectrometry technology has improved greatly in recent years (Short et al. 2006), and such equipment may in the future be used to determine the natural concentrations and dynamics of some compounds released by marine algae and associated microbes.

11.2.2 Disruption of Microbial Communication Pathways: An Effective Inhibitor of Settlement and Attachment

The defense of the red alga *Delisea pulchra* against biofouling is exceptionally well-characterized, and has been previously discussed in a number of excellent reviews (e.g., Steinberg et al. 1997; Rice et al. 1999; Steinberg and de Nys 2002; Paul and Puglisi 2004; de Nys et al. 2006). Thus, only a brief overview and recent developments will be provided herein.

D. pulchra produces a variety of structurally related halogenated furanones (Fig. 11.1), which protect this alga from bacterial settlement and attachment (Kjelleberg et al. 1997; Maximilien et al. 1998). The structures of these furanones resemble acylated homoserine lactones (AHL) (de Nys et al. 1993), which have gained widespread attention as bacterial communication signals that regulate behavior, such as swarming, of many gram-negative bacteria and are important in bacteria-host interactions (Daniels et al. 2004). Manfield et al. (1999) demonstrated that algal furanones effectively inhibit bacterial swarming and subsequent attachment by acting as competitive inhibitors for LuxR, a major transcriptional activator for coordinated bacterial behavior that is activated by AHLs.

With this mechanistic understanding of the biological activity of *D. pulchra* furanones, it is possible to predict the bacterial taxa against which this alga is defended. Because gram-negative bacteria rely heavily upon the AHL signaling system (Konaklieva and Plotkin 2006), it is not surprising that *D. pulchra* exhibits antibiosis against a variety of these microorganisms (Maximilien et al. 1998). Further, furanones also target the autoinducer-2 (AI-2) signaling system, which is present in many genera of gram-positive and gram-negative marine bacteria

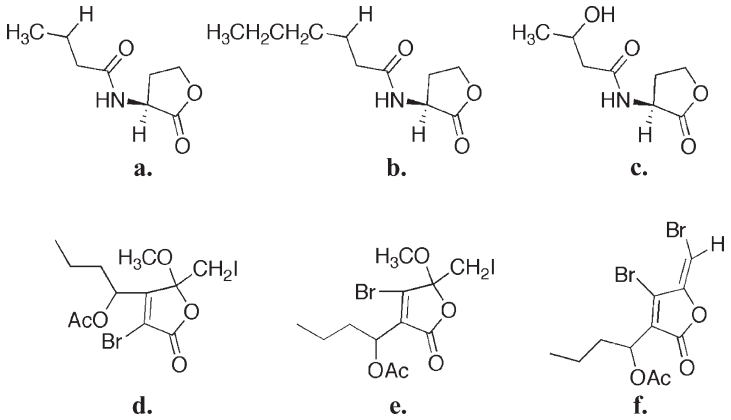

Fig. 11.1 Characteristic acyl homoserine lactones (AHLs) (**a–c**) are structurally similar to representative halogenated furanones (**d–f**) reported from *Delisea pulchra* (de Nys et al. 1993; Gould et al. 2006)

(Ren et al. 2001; McDougald et al. 2003). This discovery is surprising, given the lack of obvious structural similarity between AI-2 and furanones.

In addition to bacterial inhibition, furanones from *D. pulchra* directly inhibit settlement and attachment of some species of biofouling larvae and zoospores (de Nys et al. 1995; Dworjanyn et al. 2006), demonstrating the broad-spectrum antifouling activity of these molecules. However, the molecular mechanisms by which furanones deter larvae and zoospores are not known.

In contrast to the inhibitory activity of *D. pulchra* furanones against some zoospores, recent studies have indicated that AHLs act as chemoattractants to zoospores of the biofouling green algae *Ulva* spp., and are important in zoospore habitat selection in the laboratory (see Chap. 14; Joint et al. 2002; Tait et al. 2005). Analogously, bacteria associated with the red alga *Gracilaria chilensis* produce AHLs or AHL analogs, which induce spore liberation and facilitate recruitment of the algal epiphyte *Acrochaetium* sp. to *G. chilensis* hosts in the laboratory (Weinberger et al. 2007). Further investigation of the responses of biofouling algal species to AHLs and related molecules, especially in the field, will yield further insights into the ecological functions of these molecules.

11.3 Lethal and Growth-Inhibitory Antimicrobials

The chemical defenses of *U. reticulata* and *D. pulchra* discussed so far illustrate deterrence of potential pathogens and biofoulers during settlement and attachment. Yet, algal hosts may still successfully ward off these organisms even after colonization. Although surveys have suggested that antimicrobial and antifouling chemical defenses are widespread among macroalgae and their microbial symbionts (e.g., Boyd et al. 1999; Nylund et al. 2005; Engel et al. 2006; Puglisi et al. 2006), fewer studies have gone on to elucidate the chemical structures of bioactive metabolites (e.g., Jensen et al. 1998; Paul et al. 2006). In the two examples that follow, isolation of antimicrobial compounds was guided by laboratory assays using ecologically relevant microbes, leading to the characterization of structurally unique natural products.

11.3.1 Lobophorolide: A Potent Antifungal Chemical Defense

In a survey of antimicrobial chemical defenses from 55 species of Caribbean seaweeds, extracts from *Lobophora variegata*, a common brown alga, were found to be exceptionally potent in growth inhibition assays using *Lindra thallasiae*, a marine Ascomycete pathogenic to some algal species but not to *Lobophora* spp., and *Dendryphiella salina*, a saprophytic marine Deuteromycete (Kubanek et al. 2003). Bioassay-guided fractionation of whole tissue extracts of *L. variegata* resulted in the isolation of lobophorolide, a novel polycyclic macrolide of presumed polyketide origin (Fig. 11.2a). Furthermore, the molecule was present in macroalgal

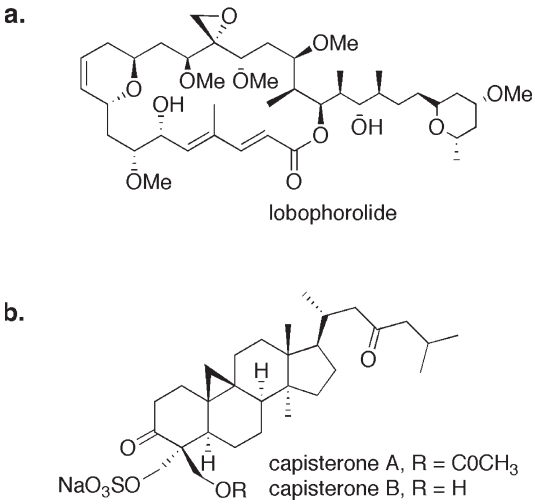

Fig. 11.2 Previously identified macroalgal secondary metabolites that inhibit growth of pathogenic and saprophytic fungi. (**a**) Lobophorolide was isolated from *Lobophora variegata*, but is likely of cyanobacterial origin (Kubanek et al. 2003). (**b**) Capisterones A and B were isolated from *Penicillus capitatus* and *P. pyrinformis* (Puglisi et al. 2004)

surface extracts at concentrations sufficient for fungal growth inhibition, supporting its role as a chemical defense.

Although lobophorolide bore a novel carbon skeleton, it is largely a structural hybrid of previously identified tolytoxins, scytophycins, and swinholides (Kitagawa et al. 1990; Tsukamoto et al. 1991; Todd et al. 1992; Andrianasolo et al. 2005). Tolytoxin and scytophycins were first isolated from cultures of free-living freshwater and marine cyanobacteria (Carmeli et al. 1990, 1993), and more recently tolytoxin-23-acetate was isolated from a cephalaspidean mollusk, although this compound is likely synthesized by cyanobacteria and concentrated via the food web (Nakao et al. 1998). Swinholides have been found in marine sponges of the genus *Theonella* and more recently in free-living *Symploca* and *Geitlerinema* cyanobacteria (Tsukamoto et al. 1991; Todd et al. 1992; Andrianasolo et al. 2005). The true producer of a variety of plant- and animal-associated polyketides has been debated heavily in the literature (Hildebrand et al. 2004a; Piel 2004). Although it is possible that previous researchers have overlooked these pathways in plants and animals, it is more likely that the actual producers of polyketides, such as lobophorolide, are microbes living in or on host tissues.

Direct evidence for the true producers of some polyketide metabolites has come from culture-independent methods based on the cloning of biosynthetic genes from symbiotic microbes. These efforts have been hindered by the complexity of locating genes of interest within the multitude of microbial genomes generally associated with marine macroorganisms. Despite these difficulties, polyketides of the pederin family, originally attributed to beetles and sponges, were recently determined to be produced by bacterial symbionts (Piel 2002; Piel et al. 2004). Piel (2002) identified the polyketide-producing beetle symbiont as a *Pseudomonas* sp. Using similar cloning techniques, the uncultured γ-proteobacterium "*Candidatus* Endobugula sertula" was identified as the producer of bryostatins, potent anticancer and antipredation polyketides isolated from bryozoans (Davidson et al. 2001; Hildebrand et al. 2004b; Sudek et al. 2007).

Lobophorolide represents another likely example of a chemical defense produced by a microbial symbiont; however, no direct evidence links this molecule to cyanobacteria associated with *L. variegata*. Kubanek et al. (2003) reported observation of a variety of bacteria, including cyanobacteria, on *L. variegata*. The variable but low concentration of lobophorolide in *L. variegata* ($1.2 \times 10^{-4} \pm 0.3 \times 10^{-4}$% of plant dry mass) also indirectly supported a microbial source, since plant secondary metabolites typically range in concentration from 0.1 to 10% of dry mass (Paul 1992). It should also be noted, however, that this low abundance could simply represent an optimized strategy to tune defense concentrations to microbe sensitivity, which ranged 1–2 orders of magnitude below natural concentration. Conclusive evidence of the actual producer of lobophorolide will ultimately come only through metagenomic analyses analogous to those applied by Piel (2002), or by future identification of this compound from microbes cultured from *L. variegata*.

Lobophorolide targets a variety of filamentous fungi, including not only ecologically relevant *Linda thalassiae* and *Dendryphiella salina*, but also the human pathogen *Candida albicans*. However, the ecological activity of lobophorolide appears limited to these higher fungi, as this compound did not inhibit growth of the thraustochytrid *Schizochitrium aggregatum* or the bacterium *Pseudoalteromonas bacteriolytica*, known to be pathogenic to selected macroalgae, nor did it deter feeding by herbivorous fishes (Kubanek et al. 2003). Hence, unlike furanones isolated from *D. pulchra*, which showed multifunctional biological activity against bacteria and biofoulers, lobophorolide may have evolved as a more targeted, specific defense, albeit one apparently functional against a variety of higher fungi.

Chemical defenses targeted against specific challengers are likely to have implications for algal-associated epibiont communities, that are quite different from those of defenses that effectively deter all parasites. As it is becoming increasingly clear that a number of antimicrobial natural products isolated from macroorganisms are actually microbial natural products, perhaps antagonistic interactions among microbes originally selected for the evolution of antimicrobial defenses that now protect hosts from microbial parasites. Illustrating the complexity of interaction between microbial competitors on algal surfaces, Franks et al. (2006) demonstrated that algal-associated *Pseudoalteromonas tunicata* produces secondary metabolites that inhibit fungal colonization, thereby giving bacteria a competitive advantage in colonizing algal surfaces. Recently, 16*S* ribosomal RNA sequencing has been applied to evaluate both cultured and uncultured bacterial diversity in nature (Webster et al. 2001; Hentschel et al. 2002), suggesting that this methodology may be invaluable in relating algal chemical defense profiles to the diversity of associated microbes.

11.3.2 Antifungal Chemical Defenses of Penicillus spp.

Recent investigations of antimicrobial chemical defenses in green algae of the genus *Penicillus* indicate that, similar to *L. variegata*, these abundant macroalgae also harbor potent defenses against fungal pathogens. From *P. capitatus*, Puglisi

et al. (2004) isolated two novel triterpene sulfate esters, capisterones A and B, with antifungal activity against *Lindra thalassiae* at natural whole-tissue concentrations (Fig. 11.2b). Like lobophorolide, the growth inhibitory activity of capisterones A and B appears limited to higher fungi. Although literature examples of cycloartane class triterpenoids such as capisterones A and B are rare, all marine examples of these molecules have come from algal species such as the red alga *Tricleocarpa fragilis* and the green alga *Tydemania expeditionis* (Govindan et al. 1994; Horgen et al. 2000). This fact, together with biosynthetic studies demonstrating the capacity of algae to produce a variety of isoprenoids (although not these specific triterpenes), suggests that capisterones A and B are produced by *P. capitatus* itself and not by a symbiotic microbe.

More recent investigations by Engel and Fenical (unpublished data) have revealed that capisterones are concentrated in the cap filaments of *Penicillus canitatus* and are not present at detectable levels in their heavily calcified stalks. By combining pulse amplitude modulated (PAM) fluorometry in the field with culture studies and chemical analyses in the laboratory, Engel and Fenical (unpublished data) have demonstrated that photosynthetically active cap filaments harbor low levels of culturable fungi and mean in situ capisterone concentrations of 10 μg/mL. These studies have shown a clear positive correlation between photosynthetic activity and in situ capisterone concentrations, as well as a strong negative correlation between fungal abundance on cap filaments and in situ capisterone concentrations. These results imply that healthy, photosynthetically active individuals maintain high capisterone concentrations and are thus more effective at controlling associated fungi. Although surface concentrations of capisterones were not evaluated, the low minimum inhibitory concentration (MIC; 0.1–0.7 μg/mL) of capisterones against cultured fungi suggests that natural concentrations are likely sufficient to inhibit fungal infection. Furthermore, capisterones are amphiphilic, possessing a lipophilic terpenoid core and a hydrophilic sulfate group. This may result in aggregation of capisterones at algal surfaces, since the lipophilic portion could interact with algal tissue while the hydrophilic moiety is strongly attracted to surrounding seawater.

Engel and Fenical (unpublished data) also evaluated antifungal chemical defenses of other *Penicillus* species from the tropical Atlantic. Similar to those of *P. capitatus*, cap filaments of *P. pyriformis* contained capisterones and closely related natural products at concentrations similar to those found in *P. capitatus*. In contrast, *P. dumetosus* contained only trace amounts of these triterpenoids. All *Penicillus* spp. examined contained indole-3-carboxaldehyde at whole tissue concentrations sufficient to inhibit the algal fungal pathogen *Lindra thalassiae*. Interestingly, this compound has thus far only been reported as a phytoalexin in terrestrial plants (Tan et al. 2004), produced in response to fungal infection. Further studies are needed to examine whether fungal infection also induces the production of this metabolite in *Penicillus* spp. Induction of macroalgal chemical defenses in response to mesograzers such as amphipods and snails has been reported (Cronin and Hay 1996; Pavia and Toth 2000; Toth and Pavia 2000); however, the induction of antimicrobial defenses has not yet been shown in macroalgae. In investigations of antimicrobial defense induction in terrestrial plants, wounding has been shown

to induce chemical defenses (e.g., Kristensen et al. 1999; Aneja and Gianfagna 2001; Rizhsky and Mittler 2001), probably because physical damage is a good proxy for the presence of pathogens. In contrast, Pavia and Toth (2000) demonstrated that physical damage alone was insufficient to induce antiherbivore defense production in algae. Hence, it will be interesting to determine whether wounding induces antimicrobial defenses in marine algae, and such studies are currently underway in our group.

One question that remains to be answered for lobophorolide, capisterones, and indole-3-carboxaldehyde is whether these antimicrobial molecules function by killing susceptible fungi or by slowing their growth and/or reproduction. The isolation of these antifungal natural products was guided using assays testing how fungi grow on agar media containing natural concentrations of macroalgal extracts. Reduced growth on treated agar relative to controls might indicate that a macroalgal chemical defense is lethal to fungi or that it slows fungal growth/reproduction, or a combination of both. Although perhaps not inherently important in assessing the ecological effects of antimicrobial defenses, determination of the mode of inhibition may have important implications for the coevolution of hosts and parasites. Compounds that inhibit microbial growth or reproduction could provide selective pressure for the evolution of resistance among microbes, since in cases where the defense is not lethal, small populations of pathogenic microbes might remain associated with algae for substantial periods of time, facilitating the emergence of a resistant phenotype. A competing hypothesis is that lethal compounds could strongly favor the evolution of resistance, since even one mutant resistant cell could rapidly dominate a macroalgal host. By developing ecological assays capable of distinguishing between lethal and growth/reproductive inhibitory effects, it may be possible to address such questions.

11.4 Future Perspective and Conclusions

The studies highlighted in this chapter provide a glimpse into the role of secondary metabolites in defending macroalgae against pathogens and biofoulers. These molecules operate at different stages of the infection or fouling process, and demonstrate that multiple strategies can be successful in controlling these organisms. As deterrents to settlement and attachment, molecules such as the polar, high molecular weight compounds from *U. reticulata* and an associated *Vibrio* sp. likely defend this alga from biofouling larvae. Furanones from *D. pulchra* disrupt bacterial settlement by inhibiting communication pathways necessary for bacterial quorum sensing and settlement. In contrast, chemical defenses, including lobophorolide and capisterones, defend macroalgae after settlement, by either killing or inhibiting the growth of fungal pathogens.

As illustrated by the cases of an antilarval defense in *U. reticulata* produced by a *Vibrio* sp. bacterium and by the likely cyanobacterial origin of lobophorolide,

microbial symbionts probably play an important role in synthesizing chemical defenses for host algal species. Harboring epiphytic or endophytic microbes that produce bioactive secondary metabolites may benefit hosts by eliminating metabolic costs for synthesizing and storing defensive compounds, and may reduce autotoxicity effects. These macroalgal-microbial associations may be mutualistic, although studies are needed to evaluate this hypothesis.

The specificity of most chemical defenses (e.g., Engel et al. 2006; Puglisi et al. 2006) is likely an important contributing factor to the structure of algal-associated microbial communities. Chemical defenses may affect marine communities by promoting some microbes on algal surfaces while deterring others, and by facilitating growth of macroalgae that would otherwise become overgrown by biofoulers. The likely microbial source of many defensive metabolites adds another interesting aspect in addressing the role of natural products in structuring communities. Although no studies to date have provided clear evidence for community consequences of antimicrobial defenses in macroalgae, the synergistic application of natural products chemistry, genetic engineering approaches, and field ecological experimentation may result in an advanced understanding of such relationships.

A better understanding of the dynamics of antimicrobial chemical defense production is also predicted for the future. In the more advanced field of marine plant-herbivore interactions, field experiments have demonstrated increased production of defensive metabolites in response to attack by specific herbivores (e.g., Cronin and Hay 1996; Toth and Pavia 2000). Given the likely costs of antimicrobial defenses, regulation of chemical defenses dependent upon risk of attack is expected to be similarly advantageous. It is possible that the most important chemically mediated battles are not between microbes and their hosts but instead among microbial species or populations co-occurring on or in a host. Evaluation of such hypotheses is inherently challenging and will necessitate significant methodological advancements and a better understanding of host-pathogen and host-biofouler interactions. Through the synergistic application of improved laboratory and field-based experiments, understanding of secondary metabolite defenses against pathogens and biofoulers will forge ahead.

Acknowledgments Research on marine chemical ecology by the Kubanek group has recently been supported by NSF grant OCE-0134843. We thank E. Prince and S. Engel for comments helpful in improving this manuscript and S. Engel and W. Fenical for sharing unpublished data.

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12
Oxidative Burst and Related Responses in Biotic Interactions of Algae

P. Potin

12.1 Introduction

Herbivores and pathogens can have strong effects on algal fitness, regulate population dynamics, and cause considerable damage in marine ecosystems. This was exemplified in kelp forests by dramatic changes associated with the reduction or extinction of local populations of some key predators controlling macroalgal grazers (Estes and Duggins 1995), or in coral reef ecosystems, where coralline lethal orange disease (CLOD), a disease affecting various coralline algae, has led to the destruction of thousands of kilometers of reefs (Littler and Littler 1995). Such diseases or predation may also be highly destructive in managed ecosystems, as reported for *Laminaria japonica* (Ishikawa and Saga 1989), *Porphyra yezoensis* (Fujita et al. 1972), and *Eucheuma/Kappaphycus* (Ask and Azanza 2002; Hurtado et al. 2006) aquaculture fields. In the context of global change, including human impacts and introduction of alien species, the frequency of pathogens and epidemics has increased in recent decades and sessile invertebrate and algal populations will have to adapt their defense strategies to cope with new challenges (Harvell et al. 1999, 2002; Mydlarz et al. 2006). Marine algae have evolved a variety of defensive mechanisms against grazers and pathogens (Pohnert 2004). They strongly depend on their chemical repertoire to influence interactions with other organisms and with the environment. A portion of these chemicals may provide constitutive barriers against grazers or parasites. Constitutive production of secondary metabolites provides antimicrobial compounds (see Chap. 11; de Nys and Steinberg 2002; Kubanek et al. 2003) and grazer deterrents (see Chaps. 2–6, 9; Paul and Puglisi 2004; Paul et al. 2006c). Considering the fundamental question of the investment of physiological resources in defense structures or metabolites (see Chap. 7; Amsler and Fairhead 2006; Ianora et al. 2006), however, it is obvious that marine algae have also developed activated and induced defense mechanisms. Nonetheless, in striking contrast with the knowledge

P. Potin
Station Biologique, Marine Plants and Biomolecules, Unité Mixte de Recherche 7139, Centre National de la Recherche Scientifique, Université Pierre et Marie Curie and LIA DIAMS, BP74, 29682 Roscoff, France. e-mail: potin@sb-roscoff.fr

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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on host-pest interactions in terrestrial crop or wild plants (e.g., Nürnberger et al. 2004), very little is known about signaling or defense induction and regulation in marine algae (Bouarab et al. 2001a; Potin et al. 2002).

In terrestrial plant-pathogen interactions, it is believed that most microorganisms fail to successfully colonize most plant species because plants possess an elaborate surveillance system that readily uncovers the presence of potentially pathogenic agents. Plant cells are able to sense this invasion, for example, by associated mechanical wounding or the detection of released plant wall fragments (John et al. 1997; De Lorenzo et al. 2001; Vorwerk et al. 2004) and conserved pathogen-derived molecules which are referred to as pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). A subset of the latter has been found to act as general elicitors of basal plant immune responses (Nürnberger et al. 2004; Nürnberger and Lipka 2005), part of which take place at the cell wall (Hauck et al. 2003; Schulze-Lefert 2004). Local generation of reactive oxygen species (ROS) at the cell periphery, for example, is a frequent plant response to attack by many microbial pathogens. ROS is a collective term that includes oxygen-derived small molecules, including oxygen radicals [superoxide (O_2•–), hydroxyl (•OH), peroxyl (RO_2•), and alkoxyl (RO•)], and certain nonradicals that are either oxidizing agents and/or are easily converted into radicals, such as hypohalous acid (HOX), ozone (O_3), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) (Halliwell and Gutteridge 1999). This transient and rapid production of large amounts of ROS associated with increased O_2 consumption is reminiscent of the oxidative burst, discovered several decades ago as a "respiratory burst" during phagocytosis in cells of the human immune system (Baldridge and Gerard 1933). The same phenomenon was described more recently in terrestrial plants (Doke 1983a, b), and it is recognized today as a ubiquitous characteristic of defense systems in these phyla (e.g., Baker and Orlandi 1995; Lamb and Dixon 1997; Wojtaszek 1997; Bolwell et al. 1998).

Although the role of ROS in mediating compatibility and/or resistance in plant-pathogen interactions is still controversial (for review, see Hückelhoven and Kogel 2003), these molecules are generally thought to function in oxidative cell wall cross-linking and plant defense signaling. The key role of reactive oxygen metabolism in the defensive responses of marine algae and invertebrates to environmental stresses and to infection by potential pathogens has been recently reviewed (Dring 2006; Lesser 2006). Thus, this review will concentrate on aspects that have received little attention in marine algae, such as methods to detect ROS in seaweeds, the oxidative-burst machinery, other sources of ROS, and also the ecological significance of the oxidative burst and related responses. Particular attention will be given to current views on algal defense elicitors and to recent developments in determining the identity of associated defenses, which are mounted concomitantly with the perception of such elicitors.

12.2 Reactive Oxygen Species and Detection Methods

ROS generation is generally a cascade of reactions that starts with the production of superoxide. Superoxide rapidly dismutates to hydrogen peroxide either spontaneously, particularly at low pH, or catalyzed by superoxide dismutase (SOD).

Other elements in the cascade of ROS generation include the reaction of superoxide with nitric oxide (NO) to form peroxynitrite (RNOO), peroxidase-catalyzed formation of hypochlorous acid from hydrogen peroxide, and the iron-catalyzed Fenton reaction leading to hydroxyl radical generation (Klebanoff 1980; Thannickal and Fanburg 2000). Nitrogen-containing oxidants, such as NO, are called reactive nitrogen species (RNS). ROS and RNS react with a large number of molecules, including other small inorganic molecules as well as proteins, lipids, carbohydrates, and nucleic acids. Through such interactions, ROS may irreversibly destroy or alter the function of the target molecule. Consequently, ROS are major contributors to cell damage in biological organisms but can be beneficial as a host defense. This latter point became particularly clear when the link was made between deficiency in ROS generation and reduced killing ability in leukocytes (Baehner and Nathan 1967) and more recently in plant cells with inactivated *rboh* genes (Torres and Dangl 2005). However, in the past decades ROS involvement has proven important in many reversible regulatory processes in virtually all cells and tissues (Thannickal and Fanburg 2000; for terrestrial plants, see Torres et al. 2006).

Facile detection of ROS and RNS in biologic systems is often problematic (for detailed methodological reviews, see Halliwell and Whiteman 2004; Tarpey et al. 2004). This is a result of numerous cellular mechanisms, both enzymatic and nonenzymatic, involved in their catabolism/decomposition, the complex and overlapping nature of their reactivities, as well as the often limited intracellular access of detector systems. In marine algae, the first monitoring of extracellular ROS emission upon mechanical damage was conducted using luminol-dependent chemiluminescence (Glazener et al. 1991) in the red alga *Eucheuma platycladum* (Collén et al. 1994). This technique is routinely used to monitor production in culture media of animal or plant cells and allows one to detect H_2O_2 and other forms of ROS such as O_2•– and •OH. Dring (2006) recently described reservations concerning the reliability of this method as an indicator of biological ROS. Luminol must first be oxidized in a one-electron step by H_2O_2 and a peroxidase or chemical catalyzer such as ferricyanide. Hence luminol may be an unreliable probe and should be used with appropriate controls to detect any contaminating oxidizing agent that could cause light emission. Nevertheless, this method was used in a number of studies depicting ROS emission in biotic interactions of marine algae (Bouarab et al. 1999, 2001b; Küpper et al. 2001, 2002, 2006; Weinberger et al. 2002, 2005a, b) and these results were validated using the following methods.

Since the pioneering work of Collén and Davison (1997) in *Fucus evanescens*, the use of fluorescent probes such as 2′,7′-dichlorohydrofluorescein diacetate (DCFDA) has also become popular to detect "cellular peroxides" in marine algae (Vardi et al. 1999; Weinberger et al. 1999; Collén and Davison 1999a–c; Coelho et al. 2002; Ross et al. 2005b). However, it is unlikely to be effective because it reacts slowly with H_2O_2 or lipid peroxides and because DCFDA can detect cellular peroxides only if they are decomposed to radicals. It appears that DCF fluorescence is an assay of global oxidative stress rather than of any particular ROS (Halliwell and Whiteman 2004). During the past decade, it was used in many studies of oxidative stress in marine algae (reviewed by Dring 2006) and is particularly suitable for cell imaging of an oxidative burst by using fluorescence and confocal microscopy (Vardi

et al. 1999; Küpper et al. 2001; Coelho et al. 2002; Ross et al. 2005b). Recently, diaminofluorescein diacetate (DAF2-DA), a specific reporting probe of NO production, was successfully used to detect NO in a wounded siphonous green alga (Ross et al. 2006) and in diatoms (Vardi et al. 2006). However, this probe did not detect RNS in lipopolysaccharide (LPS)-treated *Laminaria digitata* (Küpper et al. 2006).

Other indirect methods have also been used to detect or localize ROS production in marine algae. Küpper et al. (2001) used nitroblue tetrazolium salts to demonstrate O_2•– production in *L. digitata* challenged with alginate oligosaccharides. More recently, a method utilizing precipitation of cerium salts to detect subcellular sites of H_2O_2 production was successfully used in marine red algae (Weinberger et al. 2005a).

12.3 Inducers and Sources of ROS Emission in Biotic Interactions of Marine Algae

An increasing number of observations indicate that emission of ROS following inducer recognition mediates host-pathogen interactions in algae (Table 12.1). The physiological generation of ROS can occur as a by-product of other cellular reactions in mitochondria, chloroplasts and peroxisomes, cytochrome P450, and other cellular elements (Dring 2006). However, in biotic interactions, emission of ROS or RNS is most often triggered by cell-cell recognition, involving perception at the cell membrane of a signal from an invading organism or liberated from the host surfaces (referred to as elicitors in terrestrial plant pathology) (Fig. 12.1).

12.3.1 Elicitors and Sources of ROS in Terrestrial Plant-Pathogen Interactions

PAMPs include cell wall components of microorganisms such as the LPS of Gram-negative bacteria and peptidoglycans or lipoteichoic acid (LTA) of Gram-positive bacteria. In terrestrial plants they also include bacterial proteins or peptide motifs such as flagellin and elongation factor Tu (EF-Tu) (reviewed by Nürnberger and Lipka 2005; Abramovitch et al. 2006). LPS are now recognized as elicitors of innate immunity in terrestrial plants (Silipo et al. 2005). Challenges with various sources of LPS trigger the induction of an oxidative burst (Meyer et al. 2001; Gerber et al. 2004), activation of phosphorylation cascades (Gerber and Dubery 2004), as well as the production of NO (Zeidler et al. 2004). The relevance of elicitors such as oligosaccharins as in vivo participants in defense systems is supported by their possible natural occurrence during plant-microbe interactions (Fritig et al. 1998). Xyloglucan (Fry et al. 1993) and pectin (e.g., Boudart et al. 1998) elicitors are of endogenous origin (i.e., from the plant), whereas glucans (Klarzynski et al. 2000) and chitins are exogenous (released from the pathogen).

In most terrestrial plant-pathogen interactions a diphenylene-iodonium (DPI)-sensitive (O'Donnell et al. 1993), membrane-located, and receptor-activated NADPH oxidase generates superoxide radicals (Levine et al. 1994; Doke and Miura 1995; Lamb and Dixon 1997; Bolwell et al. 1998), which eventually dismutate into H_2O_2 and O_2 (Sutherland 1991). Apoplastic peroxidases (Bolwell et al. 1998; Martinez et al. 1998), as well as various oxidases such as oxalate oxidase (Zhang et al. 1995; Thordal-Christensen et al. 1997) or amine oxidase (Laurenzi et al. 2001; Rea et al. 2002), have also been identified as sources of ROS in higher plants.

12.3.2 A Growing Repertoire of ROS Inducers

Until recently, a few inducers of ROS emission were recognized as true elicitors of defense responses in marine plant-microbe interactions, and these have already been discussed in previous reviews (Potin et al. 1999, 2002; Pohnert 2004; Dring 2006). This list has slightly increased recently (Table 12.1).

An oxidative burst has been reported both after the perception of algal cell-wall-derived elicitors (Bouarab et al. 1999; Weinberger et al. 2001, 2005a; Küpper et al. 2001, 2002) and in response to PAMPs and MAMPs from various mammalian and marine pathogenic bacteria (Küpper et al. 2006). The most efficient elicitor of an oxidative burst in *L. digitata* is the LPS from *Salmonella arbotus equi,* which induced severe inflammatory responses, including oxidative burst in mammalian hosts. Although the oxidative burst induced by this LPS occurs considerably later, it has a range similar to that observed after elicitation by oligoguluronates in *L. digitata* and is also sensitive to DPI (Küpper et al. 2006). This study was the first report on an oxidative burst response to LPS perception in any algal group, although it is likely that recognition of LPS and other MAMPs will prove to be ubiquitous in several groups of algae. Precise structure-function studies will reveal the minimum structure required for perception, and it will be useful to isolate and test the LPS from algal pathogenic bacteria such as *Pseudoalteromonas bacteriolytica*, the causative agent of red spot disease in maricultured *L. japonica* (Sawabe et al. 1998).

12.3.3 New Insights into ROS Sources in Algae

In contrast with the ubiquitous response of higher plant cells to either oligogalacturonans or oligoglucans, the capacity to recognize a specific oligosaccharide structure seems to be confined to particular algal taxa. In a survey of 45 species belonging to 11 orders, the capacity to recognize alginate oligosaccharides as defense signals is limited to a few members of brown algae belonging to the orders Laminariales, Desmaretiales, Ectocarpales, and Fucales (Küpper et al. 2002). In red

Table 12.1 Host-released elicitors, pathogen-associated molecular patterns, and substrates of ROS-generating enzymes involved in algal defenses

Molecule	Source	Minimal structural motif required for defence activation	Biological response	Reference
Agar	Cell walls of agarophyte red algae	Agar hexasaccharide: neogarohexaose	Oxidative burst, and all associated responses in *G. conferta* in response to bacterial infection	Weinberger et al. 1999, 2001, 2005a
	Gracilaria conferta	Optimum degree of polymerization > 10	Tip bleaching in *G. gracilis*	Schroeder et al. 2003
Carrageenans	Cell walls of carrageophyte red algae	Carrageenan oligosaccharides	Modulation of the virulence of the endophytic green alga *Acrochaete operculata*. Oxidative burst + carrageenolytic enzymes for lambda + secretion of L-Asn for kappa	Bouarab et al. 1999, 2001
	Chondrus crispus	Lambda (3 sulfate per disaccharide repeating units) and kappa (1S) families		Weinberger et al. 2002, 2005b
Alginate	Cell walls of brown algae	Homo-oligomeric fragments of poly-α-1,4-L-guluronic acid (degree of polymerization > 15)	Oxidative burst, and all associated responses in *Laminaria digitata* and *L. japonica* + VHOCs	Küpper et al. 2001, 2002
			Induced resistance against bacteria and endophytes in kelps	Palmer et al. 2005; Teissier et al., unpublished results
			Gene-regulated responses	Cosse et al., unpublished results
Lipopolysaccharides	Human pathogenic and marine bacteria	Lipid A + various attached oligosaccharide decorations	Oxidative burst, and oxylipin production in *L. digitata*	Küpper et al. 2006

Unknown	Unidentified epiphytic bacterium of *G. conferta*	Small peptide (700–1,500 Da)?	ROS emission in *G. conferta*	Weinberger and Friedlander 2000
Unknown	Marine fungus *Lindra thalassiae*	Nonelucidated	ROS emission in the green alga *Dictyosphaeria cavernosa*	Ross 2005; www.sms.si.edu/2005final_screen.pdf
Unknown	Cell-free extracts of endophytic green alga *A. operculata*	Nonelucidated	Oxidative burst, and all associated responses in *Chondrus crispus*, including oxylipin pathway and VHOCs	Bouarab et al. 1999, 2004 Bouarab et al., unpublished results
Amino acid	Secreted by endophytic green alga *A. operculata* challenged with kappa-carrageenan oligosaccharides	L-Asparagine	ROS production by an extracellular amino acid oxidase	Weinberger et al. 2002, 2005b
Agar	Cell walls of agarophyte red algae *Gracilaria chilensis*	Agarose decasaccharide	ROS production by an extracellular agar oligosaccharide oxidase	Weinberger et al. 2005a
Agar	Cell walls of agarophyte red algae	Sulfated agars and porphyran-agaroid from *Porphyra* spp.	Appressoria formation in the specific pathogenic oomycete *Pythium porpyrae*	Uppalapaati and Fujita 2000

ROS reactive oxygen species, *VHOCs* volatile halogenated organic compounds

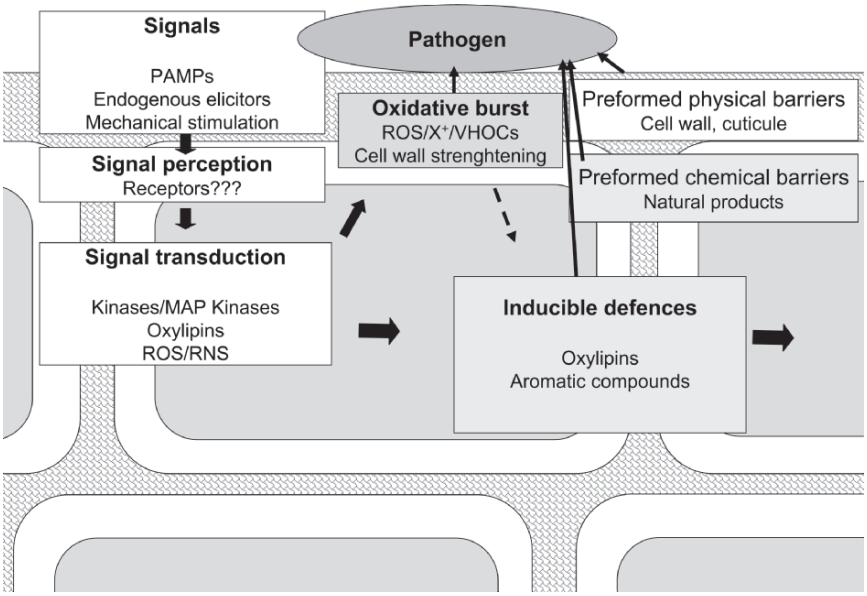

Fig. 12.1 Hypothetical scheme of the complex sensory, signaling and executive mechanisms following the oxidative burst induced by recognition of exogenous pathogen-associated molecular patterns (PAMPs) or endogenous elicitors (Table 12.1) based on the current knowledge about marine algae. The occurrence of receptors for PAMPs or oligosaccharides is not yet proven in algae. Pharmacological evidence and some direct measurements of ion fluxes indicate that initiation of signaling cascades controls ROS emission and activated responses and the subsequent gene-regulated inducible defenses. (Redrawn and adapted from a drawing of analogous mechanisms that constitute the basis of nonhost resistance in terrestrial plants (Nürnberger and Lipka 2005))

algae, respiratory bursts in response to agar oligosaccharides were observed in six species of the family Gracilariaceae but were not observed when other agarophyte or carrageenophyte red algae were tested (Weinberger et al. 1999). Sensitivity to agar oligosaccharides therefore seems to be a ubiquitous, but specific feature in Gracilariaceae. However, the respective sources of ROS production were not clearly determined in these previous reports. Recently, the two agar-producing red algae *Gracilaria chilensis* and *G. conferta* were compared for their respective capacity to generate H_2O_2 in the presence of agar oligosaccharides in their culture medium (Weinberger et al. 2005a). In *G. conferta*, a transient release of H_2O_2 was observed, followed by a refractory state of 6 h. This response was sensitive to DPI and other chemical inhibitors of NADPH oxidase, protein kinases, protein phosphatases, and calcium translocation in the cell, while it was insensitive to inhibitors of metalloenzymes. Transmission electron microscopic observation of the H_2O_2-dependent formation of ceriumperoxide from cerium chloride indicated that large amounts of oxygen were activated at the plasma membrane of *G. conferta* within few minutes after challenge with agar oligosaccharides. These results indicate that

a putative system consisting of a receptor specific to agar oligosaccharides and a plasma-membrane-located NADPH oxidase is responsible for the release of H_2O_2 in *G. conferta*. In contrast, subcellular examination of *G. chilensis* showed that the H_2O_2 release after exposure to agar oligosaccharides was located in the cell wall. It was sensitive to inhibitors of metalloenzymes and flavoenzymes, but not to DPI, and no refractory state was observed, indicating that the activation of an oxidase is not linked to a receptor. The release of H_2O_2 was correlated with accumulation of an aldehyde in the algal medium, suggesting that an oxidase is present in the apoplast of *G. chilensis*. The enzyme acted exclusively on agar oligosaccharides larger than disaccharides and was inhibited by reduced agar oligosaccharides. As revealed by native polyacrylamide gel electrophoresis, the expression of several isoforms of agar oligosaccharide oxidase was constitutive in *G. chilensis*. In contrast, elicitation of *G. conferta* with agar oligosaccharides resulted within 24 h in a strong expression of two isoforms of an agar oligosaccharide oxidizing enzyme, which were absent in nonelicited plants. Both species thus appear to be responding to agar oligosaccharides by releasing H_2O_2 but using different biochemical mechanisms. However, both responses display a defensive value. *G. chilensis* plants with a high potential for oxidation of agar oligosaccharides proved to be less susceptible to settlement by the epiphytic red alga *Acrochaetium* sp. than did plants with a low potential, and a single addition of agar oligosaccharides to the medium of *G. chilensis* also resulted in reduced settlement of *Acrochaetium* spores (Weinberger et al. 2005a).

The interaction between the red alga *Chondrus crispus* and its green algal endophyte *Acrochaete operculata* provides one of the most studied host-pathogen interaction in marine algae. *C. crispus* cell wall matrix polysaccharides include either κ- or λ-type carrageenans, which are found in gametophytes and tetrasporophytes respectively. These were shown to control endophyte penetration, with λ-carrageenans increasing and κ-carrageenans reducing the endophyte virulence (Bouarab et al. 1999). Contact of *A. operculata* with κ-carrageenans enhances secretion of the nitrogen storage compound L-asparagine (L-Asn), which in turn induces a release of H_2O_2 by *C. crispus* (Weinberger et al. 2002). This is an interesting example in chemical ecology where the induced signal of an attacker serves directly as a substrate for the production of a chemical defense metabolite. Only the presence of an amino acid oxidase that can produce elevated levels of H_2O_2 is required to control the early stages of the infection by *A. operculata*. The H_2O_2 concentrations generated by *C. crispus* in the presence of physiologically relevant amounts of L-Asn were shown to be sufficient to significantly prevent settlement of *A. operculata* zoospores (Weinberger et al. 2005b). However, this is not the only way in which C. *crispus* uses ROS in the control of *A. operculata*.

Gametophytes of *C. crispus*, when challenged with cell-free extracts of *A. operculata*, generate an oxidative burst. This is inhibited by DPI, an inhibitor of flavoenzymes such as NADPH oxidases. Moreover, when incubated with DPI the gametophytes lost their resistance to *A. operculata* infection (Bouarab et al. 1999). These results suggest the involvement of a NADPH oxidase homologue in the generation of the oxidative burst and demonstrate that the oxidative burst is an essential element in *Chondrus* immunity. The identity of this oxidase was recently elucidated.

A single copy gene encoding a homologue of respiratory burst oxidase gp91^{phox}, named *Ccrboh* has been identified from the red alga *C. crispus* (Hervé et al. 2006). A search performed using the *Ccrboh* gene in available algal genome and expressed sequence tag (EST) databases identified sequences showing common features of NADPH oxidases in other algae, such as the two diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseunonana*, the red, unicellular *Cyanidioschyzon merolae*, and macroalga *Porphyra yezoensis*, whereas no sequence showing similarity to *rboh* genes has yet been found in the brown alga *L. digitata* (Hervé et al. 2006). Indeed, phylogenetic analysis revealed that homologues of NADPH oxidases in red algae and diatoms constitute an independent cluster, which emerged early in evolution from a common ancestor of the ferric reductase and NADPH oxidases (Hervé et al. 2006). These results invalidate the hypothesis of Lalucque and Silar (2003), which postulated that NADPH oxidase constitutes the redox signaling system that contributed to the emergence of multicellular organisms in the course of evolution.

Elicitor- or stress-activated, DPI-sensitive oxidases, presumably membrane-bound NADPH oxidases, have been reported in *C. crispus* (Bouarab et al. 1999) and other red (Weinberger et al. 1999), as well as in brown (Küpper et al. 2001; Coelho et al. 2002) and green siphonous algae (Ross et al. 2005b, 2006). The reactions catalyzed by these enzymes were shown to control infections by pathogens, including bacteria (Weinberger and Friedlander 2000; Küpper et al. 2001, 2002) and endophytic filamentous algae (Bouarab et al. 1999; Küpper et al. 2002).

12.4 Oxidative-Burst-Associated Responses

12.4.1 Emission of Volatile Halogenated Organic Compounds

A rapid response, likely to constitute a chemical defense against foulers, pathogens, and herbivores in marine algae, is the emission of volatile halogenated organic compounds (VHOCs). Reminiscent of the oxidative burst of mammalian phagocytes, rapid and intense production of ROS following pathogen recognition in marine algae is concomitant with production of hypohalous acids, which can halogenate various organic substrates (Weinberger et al. 1999). In mammals, neutrophil activation results in the production of cytotoxic, bactericidal compounds that contribute to protection from invading organisms such as bacteria and fungi (Hampton et al. 1998). The combination of H_2O_2 production by an NADPH oxidase and the release of myeloperoxidase by the activated neutrophils results in the production of hypohalous acids, including hypobromous acid and hypochlorous acid (Gaut et al. 2001). In marine algae, an increased production of iodinated, brominated, or chlorinated organic compounds is associated with oxidative stress induced by excess light (Mtolera et al. 1996), ultra-violet (UV) exposure (Laturnus et al. 2004), or temperature changes (Abrahamsson et al. 2003). An increased production of

VHOCs was also observed in response to grazing pressure in *Ascophyllum nodosum* beds (Nightingale et al. 1995). Their biogenesis involves vanadium haloperoxidases (vHPO), which catalyse the oxidation of halides (X^-) and generate X^+ to yield hypohalous acid (XIO) and other forms of oxidized halides (X_2, X_3^-). Marine organisms and especially seaweeds have been known for a long time to concentrate halides from their environment (for a recent review, see Leblanc et al. 2006).

In the brown alga *L. digitata*, the oligoguluronate-induced oxidative burst is followed by a rapid increase in the emission of iodine-containing halocarbons and molecular iodine (I_2) (Palmer et al. 2005). Similarly, agar oligosaccharides caused H_2O_2 release, which resulted in an immediate increase in the brominating activity within the red alga *G. conferta* (Weinberger et al. 1999). This led to the bleaching of thallus tips described earlier (Friedlander and Gunkel 1992) and was similar to the symptoms of "ice-ice" white powdery disease in *Eucheuma* and *Kappaphycus* species (Lavilla-Pitogo 1992; Largo et al. 1995). Recent investigations in red algae have also shown that elicitation of an oxidative burst by agar oligosaccharides in *G. conferta* (Weinberger et al., unpublished results) and by pathogen extracts in *C. crispus* (Bouarab et al., unpublished results; Bouarab 2000) triggers an upregulation of VHOC production. Interestingly, when interactions involved molecules that provide substrates for agar oxidases in *G. chilensis* (Weinberger et al. 2005a) or amino acid oxidases in *C. crispus* (Weinberger et al. 2002, 2005b), production of VHOCs remained unchanged between control and challenged thalli of these species (Bouarab et al.; Weinberger et al., unpublished results). This suggests that membrane-associated ROS production and signaling events are required to provide additional substrates for vanadium haloperoxidases.

More recently, a novel type of halogenating enzyme, named hydroperoxide halolyase, which generates halogenated aldehydes, has been described in the marine diatom *Stephanopyxis turris* (Wichard and Pohnert 2006). In other microalgae, halogenation of organic compounds was shown to mainly involve methyl halide transferases (Moore et al. 1996; Manley 2002), and no vHPO has yet been identified on genomic data obtained from diatoms (Scala et al. 2002; Armbrust et al. 2004). Clearly, these emissions are not directly associated with an oxidative burst.

12.4.2 Lipid Peroxidation and Generation of Oxylipins

Lipids can be oxidized by some ROS such as hydroxyl (•OH), but not by H_2O_2, NO, or O_2•– (Halliwell and Gutteridge 1999). However, both enzymatic and nonenzymatic cellular mechanisms can oxidize fatty acids. Challenging *L. digitata* sporophytes with lipopolysaccharides from various sources resulted in a rapid release of free fatty acids (FFAs) with a concomitant accumulation of oxidized derivatives of linolenic (C18:2) and eicosapentaenoic acid (C20:5) (Küpper et al. 2006). Other strong inducers of the oxidative burst, such as oligoguluronates, in *Laminaria* could induce neither the release of FFAs nor the oxylipin production. These results

suggest that different signaling pathways are implicated in the control of oxylipin production depending on the nature of ROS inducers (Küpper et al. 2006). In *C. crispus* gametophytes, the oxidative burst induced by cell-free extracts of *A. operculata* cultures is followed by activation of lipases, which liberate free fatty acids to fuel lipoxygenase pathways (Bouarab et al. 2004). Fatty acid hydroperoxides and hydroxides are produced, which were identified by a combination of liquid chromatography and mass spectrometry (LC-MS) in the first study which identified a function for oxylipin in mediating algal innate immunity mechanisms (Bouarab et al. 2004). Two lipoxygenase isoforms, which were specific for the metabolism of linoleic acid, were upregulated following the oxidative burst in *C. crispus*, and lipoxygenase inhibitors abolished the natural resistance of *C. crispus* gametophytes, showing that induced resistance, which is dependent on the oxidative burst, involved downstream activation of the oxylipin pathways (Bouarab et al. 2004). Although the wound response of *G. chilensis* was not shown to be linked to an oxidative burst (see Sect. 12.4.3), it involved release of free fatty acids as well as the hydroxylated eicosanoids, 8*R*-hydroxy eicosatetraenoic acid (8-HETE) and 7*S*,8*R*-dihydroxy eicosatetraenoic acid (7,8-di-HETE). While the release of free arachidonic acid and subsequent formation of 8-HETE is likely controlled by phospholipase A, 7,8-di-HETE production is independent of this lipase. This dihydroxylated fatty acid might be directly released from galactolipids that contained 7-HETE or 7,8-di-HETE (Lion et al. 2006). Further investigations of oxidative-burst-related FFA release and oxylipin production in algae are required to fully understand the involvement of these pathways in the regulation of defense responses either as signaling or as antimicrobial compounds.

12.4.3 Phenolics, Cell-Wall Cross-Linking, and Responses to Wounding

Reminiscent of the deposition of phenolics in higher plants in response to pathogen attack, *L. digitata* plantlets inoculated with the epi-endophytic pathogen *Laminariocolax tomentosoides* show a strong, localized accumulation of UV-blue autofluorescent compounds in the cortical cells surrounding the penetrating spore germ tubes. In contrast, oligoguluronate-treated plantlets resistant to endophyte penetration exhibit a less intense, but ubiquitous, cortical accumulation, suggesting that synthesis of aromatic secondary metabolites such as phlorotannins is induced following elicitation of an oxidative burst in *L. digitata* (Küpper et al. 2002). Remarkably, *C. crispus* gametophytes synthesize UV-absorbing compounds around the sites of *A. operculata* zoospore penetration, whereas this response is absent in the sensitive tetrasporophyte generation (Bouarab et al. 2004 and unpublished data). Although the exact structure of these UV-absorbing compounds remains to be identified in *C. crispus*, analysis by high-pressure liquid chromatography (HPLC) confirms that they are aromatic in nature, suggesting the involvement of phenylpropanoid

metabolism. Indeed, *A. operculata* extracts and several oxylipins activate two key enzymes in the induction of this pathway, shikimate dehydrogenase and phenylalanine ammonia lyase (Bouarab et al. 2004 and unpublished results). A pharmacological approach using two inhibitors of these pathways, glyphosate and L-α-aminooxy-β-phenylpropionic acid (AOPP), consistently inhibited activation of these enzymes, prevented the accumulation of UV-fluorescent compounds, and abolished the resistance of *C. crispus* gametophytes to *A. operculata* (Bouarab et al., unpublished data). In the filamentous brown alga *Pylaiella littoralis,* cells infected by the biotrophic parasite *Chytridium polysiphoniae* accumulate fluorescent compounds, indicating that an increase in nonphotochemical quenching is concomitant with the onset of active defense mechanisms in the infected cells (Gachon et al. 2006).

Vreeland and Laetsch (1990) and Vreeland et al. (1998) proposed a brown algal cell wall synthesis model in which oxidative cross-linking of extracellular alginate and phlorotannin polymers leads to adhesion, cell wall strengthening, or both. Assuming this model is correct, targeted cross-linking of cell wall material by haloperoxidase catalysis requires H_2O_2, which as described earlier, is abundantly produced during oxidative bursts. In vitro cross-linked polyphenols have been obtained using purified vHPO in the presence of H_2O_2, bromide, or iodide (Berglin et al. 2004; Bitton et al. 2006), leading to cell wall strengthening. Although it is known that oxidative burst responses in terrestrial plants provide mechanical protection and activate the metabolism against pathogens (Brisson et al. 1994) or herbivores (Orozco-Cardenas and Ryan 1999; Orozco-Cardenas et al. 2001), there are no published reports of an oxidative burst in response to direct herbivore attacks in algae.

ROS production in the red alga *E. platycladum* (Collén et al. 1994) and in the green alga *D. vermicularis* (Ross et al. 2005a) has been monitored following mechanical injuries. The latter study emphasized the pharmacological inhibition of a DPI-sensitive, putative NADPH oxidase that contributes to ROS accumulation. *D. vermicularis* is a coenocytic alga that upon injury is able to rapidly form wound plugs to prevent cytoplasmic loss (Ross et al. 2005b). DPI inhibition of ROS production suggests that the second phase of wound repair is based on activation of a putative NADPH oxidase enzyme, 35 min after injury, leading to micromolar-level production of H_2O_2. This latent oxidative burst is proposed to be involved, through catalysis by peroxidases, in oxidative cross-linking of coumarins during wound plug hardening and browning (Ross et al. 2005a). More recently, *D. vermicularis* have been shown to also produce RNS, including NO, and pharmacological approaches have suggested that the signaling pathways leading to the production of ROS and RNS share similarities with defense and wounding signal transduction in higher plant systems (Ross et al. 2006). Although other mechanisms of wound-activated responses such as transformation of halimedatetraacetate or esterase-mediated transformation of caulerpenynine exist (reviewed in Pohnert 2004), it is possible that a wound-induced oxidative burst may represent a common theme in the defense strategies of siphonous green and other marine algae.

12.4.4 Gene-Regulated Responses

The capability to monitor gene-regulated responses is essential to identify the various steps that lead from the perception of elicitors to their activation of the oxidative burst machinery for defense induction. Powerful genomic approaches have now become available for the main algal models mentioned in this chapter and have allowed investigators to mine putative defense genes.

Exploitation of libraries of Expressed Sequence Tags (EST) to compare transcripts in macroscopic sporophytes of *L. digitat*a with those in microscopic gametophytes (Crépineau et al. 2000) revealed that the sporophyte library contained higher levels of a vHPO transcript when compared with those of the gametophyte library (Crépineau et al. 2000). Using these ESTs and complementary proteomic approaches for cDNA cloning, several genes of vanadium-dependent bromoperoxidase (vBPO) and iodoperoxidase (vIPO) have been characterized, representing multigenic families in *L. digitata* (Colin et al. 2003). One constitutive vIPO (Colin et al. 2003) is likely responsible for iodide oxidation and transport in *L. digitata* sporophytes (Küpper et al. 1998; Leblanc et al. 2006). A cDNA library, utilized for transcript analysis of *L. digitata* protoplasts that were exposed to severe oxidative stress, revealed numerous copies of a vHPO that is markedly different from the constitutive isoform (Roeder et al. 2005). These may have special defense-related functions such as the halogenation of organic compounds. Surprisingly, ESTs coding for other key ROS-scavenging enzymes such as catalase, superoxide dismutase, gluthation peroxidase, or ascorbate peroxidase were lacking from the libraries (Roeder et al. 2005) even though these enzymes are expressed under oxidative stress conditions in terrestrial plants (Mittler 2002).

As with *L. digitata*, EST analysis of protoplasts from *C. crispus* gametophytes yielded a high proportion of detoxification and heat-shock proteins (Collén et al. 2006a), validating this approach for discovery of stress-related proteins in algae. A total of 10,154 ESTs are currently available from the leafy gametophyte and 10,265 for the filamentous sporophyte of the red alga *Porphyra yezoensis* (Asamizu et al. 2003). As mentioned earlier, a *rboh* gene was identified in the gametophyte library and was used for cloning the *Ccrboh* homolog in *C. crispus* (Hervé et al. 2006). Interestingly, in comparison with that in control thalli, expression of *Ccrboh* is induced and maintained at higher levels for at least 24 h following the inoculation of *C. crispus* gametophytes with zoospores of *A. operculata*. Induction of *Ccrboh* mRNA accumulation occurred when germinating zoospores attempt to penetrate through the host cell wall, confirming the involvement of a DPI-sensitive NADPH oxidase in the defensive oxidative burst response (Bouarab et al. 1999).

The many genes generated by EST libraries from *C. crispus* have been used to construct the first cDNA microarray for a macroalga and it was first used for expression profiling of *C. crispus* after exposure to the plant stress hormone methyl jasmonate (MeJA) (Collén et al. 2006b). The study showed that 6% of the genes responded to the addition of MeJA and the most dynamic response was seen after 6 h. A comparison between different functional groups showed an upregulation of stress-related genes and a downregulation of genes involved in energy conversion

and general metabolism. Interestingly, the DHAP synthase gene, which is the first enzyme in shikimate pathway, is upregulated by MeJA. This confirms the regulation of secondary metabolite synthesis by the oxylipin pathway identified using biochemical approaches (Bouarab et al. 2004).

A DNA filter array has been constructed in order to provide the first characterization of the transcriptional reorganization occurring after oligoguluronate perception in *L. digitata* sporophytes. The array contains about 100 genes related to stress and defense as deduced in EST libraries from either the *L. digitata* thallus (Crépineau et al. 2000) or the protoplasts (Roeder et al. 2005) or obtained in a subtractive library from plants challenged with or without oligoguluronates (Cosse et al., unpublished results). This filter has been used to study the kinetics of gene expression in response to elicitors of the oxidative burst response, and several genes under regulation were validated by real-time PCR experiments. This includes several transcripts involved in the pentose phosphate pathway and coding for vHPO, indicating that they are clearly important for establishing defense responses (Cosse et al., unpublished results). This first DNA filter array is currently being used to develop strategies to monitor defense-gene-regulated responses in field studies.

12.5 Functions of the Oxidative Burst in an Ecological Context, the Hallmark of Parasite or Disease Resistance

12.5.1 Ecological Functions of the Oxidative Burst

From the previously discussed studies, the oxidative burst response appears to integrate and/or orchestrate several defense responses. These provide an adaptive mechanism to counteract attacks on or injury to algal cells and thalli. As shown in Fig. 12.2, the occurrence of an oxidative burst associated with the perception of cell-free extracts of *A. operculata* was shown to play a crucial role in the natural resistance of *C. crispus* gametophytes (Bouarab et al. 1999).

In the kelp *L. digitata* (Fig. 12.3), the integration of diverse downstream responses to the oxidative burst was shown to control the growth of epiphytic, potentially pathogenic bacteria (Küpper et al. 2001, 2002). Elicitation with oligoguluronates also increased resistance of the alga to infection by its brown algal endophyte *Laminariocolax tomentosoides* (Küpper et al. 2002). Interestingly, programmed cell death (PCD), which is associated with hypersensitive response (HR) in higher plants, is not observed in response to a challenge of *L. digitata* with oligoguluronates, whereas it was described in *L. japonica* infected with alginic decomposing bacteria (Wang et al. 2004). This suggests that PCD in inoculated *L. japonica* may be induced by bacterial effectors or upon recognition of PAMPs (Abramovitch et al. 2006).

In the red alga *G. conferta*, agar oligosaccharide-induced oxidative bursts also result in the elimination of the bacterial epiflora (Weinberger and Friedlander 2000). Since a positive correlation was observed between the presence of agarolytic

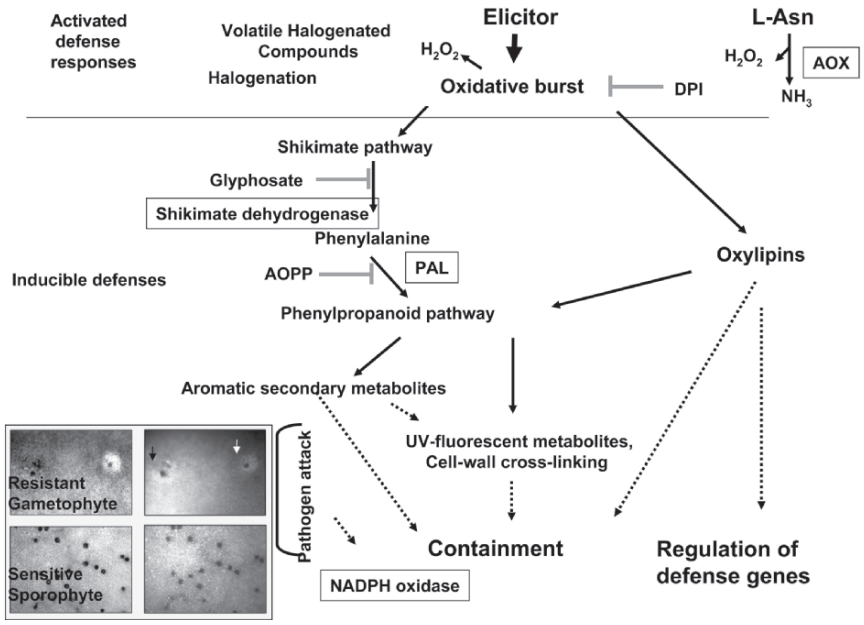

Fig. 12.2 Simplified scheme of defense reactions deciphered from studies of the interaction between the red alga *Chondrus crispus* and the green algal pathogenic endophyte *Acrochaete operculata*. Recognition of pathogen-derived elicitors triggers a diphenylene-iodonium (DPI)-sensitive oxidative burst and the production of signaling molecules, which either induce defense-specific biochemical pathways or activate halogenation reactions which directly affect pathogenic cells. In contrast, L-Asn provides a substrate for the AOX enzyme to yield H_2O_2 and NH_3 as by-products of amino acid oxidation. As illustrated in the *inset*, UV-fluorescent compounds accumulate in *C. crispus* gametophytes at the sites of attempted penetration by *A. operculata* zoospores, whereas sensitive sporophytes do not display any fluorescence. *PAL* phenylalanineammonia lyase, *AOPP* L-α-aminooxy-β-phenylpropionic acid, *AOX* L-amino acid oxidase

epiphytes and bacterial pathogenicity against *Gracilaria* spp. (Schroeder et al. 2003), it is clear that this mechanism provides a way to prevent the effect of virulence factors, such as bacterial exoenzyme agarase in these algae. Red algal halogenated furanones were recently reported to inhibit expression of exoenzyme virulence factors both in the phytopathogenic bacterium *Erwinia carotovora* (Manefield et al. 2001) and in the human pathogen *Pseudomonas aeruginosa* (Hentzer et al. 2003). Similar mechanisms are likely to occur with algal bacterial pathogens. In mammals, neutrophil activation results in the production of cytotoxic, bactericidal compounds that contribute to protection from invading foreign organisms such as bacteria and fungi (Hampton et al. 1998). The combination of H_2O_2 production by an NADPH oxidase and the release of myeloperoxidase by activated neutrophils results in production of hypohalous acids, including hypobromous acid and hypochlorous acid (Gaut et al. 2001).

Similarly, DPI-sensitive activation of H_2O_2 in marine algae and the concomitant formation of volatile and nonvolatile halogenated compounds are likely to play a

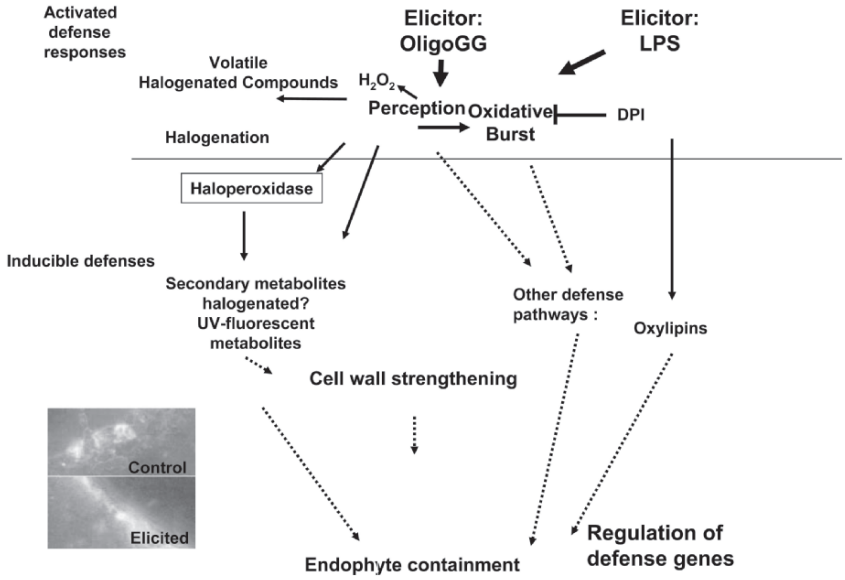

Fig. 12.3 Simplified scheme of defense reactions deciphered from studies of the oligoguluronate-induced oxidative burst in the brown alga *Laminaria digitata* in its association with the brown algal pathogenic epi-endophyte *Laminariocolax tomentosoides*. Recognition of cell-wall released oligoguluronate triggers a diphenylene-iodonium (DPI)-sensitive oxidative burst and production of signaling molecules, which either induce defense-specific biochemical pathways or activate halogenation reactions which directly affect pathogenic cells. In addition, lipopolysaccharide (LPS) triggers a DPI-sensitive oxidative burst and the production of oxylipins. In the *inset*, *L. digitata* plantlets inoculated with the epi-endophytic pathogen *L. tomentosoides* show a strong, localized accumulation of UV-blue autofluorescent compounds in cortical cells surrounding the penetrating spore germ tubes, whereas the oligoguluronate-treated plantlets resistant to endophyte penetration exhibit a less intense, but ubiquitous cortical accumulation

critical role in the defense systems of marine organisms. The correlation between stress-activated oxidative metabolism and increases in VHOC emission has led some authors to propose that halogenated compounds are only secondary waste products of ROS detoxification processes (Pedersén et al. 1996). Their specific selection as defensive compounds in algae has been controversial for a long time even though a number of these compounds are known to have potent antibiotic activities (Wever et al. 1991; Manley 2002). Algal halogenated compounds were shown to interfere with bacterial signaling systems such as the quorum sensing (QS) of Gram-negative bacteria and to induce the dispersal of bacterial biofilms (see Chap. 11). A natural haloperoxidase system from *L. digitata*, producing hypohalous acid (XOH) and X_2 plus X_3^-, was shown to inactivate acylated homoserine lactones (AHLs), which mediate QS communication between Gram-negative bacteria (Borchardt et al. 2001). Recent investigations of iodovolatilization, (i.e., molecular iodine emission and VHOCs emission) have shown that it follows oxidative stresses (Palmer et al. 2005). It is tempting to speculate that in iodine-concentrating brown

algae such as *L. digitata*, the oxidative bursts and concomitant iodine efflux in the presence of extracellular vHPOs are part of a very efficient early defense response that eliminates or controls microbes using molecular iodine. I_2 is formed chemically by oxidation of I^- in the presence of H_2O_2, but its rapid formation can be catalyzed by vHPOs. Strong antiseptic activities of iodine species have been known for a long time, and aqueous or alcoholic povidone-iodine solutions are commercially available as disinfectants. As I_2 is the most reactive chemical form of iodine, it provides the highest biocide potential (Gottardi 1999). Ohsawa et al. (2001) demonstrated that bromoform ($CHBr_3$) produced by the red calcareous algae *Corallina pilulifera* and *Lithophyllum yessoense* could eliminate epiphytic organisms, especially diatoms, from the macroalgal surface and this antiproliferating effect was dependent on vBPO enzyme activity. In the red alga *Asparagopsis armata*, $CHBr_3$ and dibromoacetic acid are the dominant brominated compounds among an impressive number of metabolites (Kladi et al. 2004) stored in specialized gland cells (Paul et al. 2006a). When released at the surface of the thallus, they displayed antibiotic activity against epiphytic bacteria (Paul et al. 2006b). They also function as mesograzer feeding deterrents as inferred from the increased consumption of bromide-starved algal cultures (Paul et al. 2006c). In *C. crispus* gametophytes, three VHOCs were upregulated after defense elicitation and were toxic to spores and germlings of *A. operculata* (Bouarab et al., unpublished results). Indeed, these compounds fulfil all criteria necessary to assign a natural antifouling role: they provide resistance to epi-/endophytism, they are produced on the surface, and they affect spore settlement at the thallus surface. In red algae, VHOCs seem to play an important physiological role in activated defense responses, acting as biocidal or repelling substances against microorganisms and herbivores, and also in controlling the development of epi-/endophytic, parasitic, or pathogenic algae.

Thus, together these studies suggest that the oxidative burst machinery has evolved before the crown diversification of eukaryotes (Baldauf 2003) to provide marine algal lineages with natural and induced innate immunity mechanisms. These play a role similar to the HR in terrestrial plants infected by incompatible pathogens and they share important common traits with the innate immunity response of mammalian phagocytes.

12.5.2 Toward New Approaches to Test the Ecological Relevance of Oxidative-Burst-Associated Responses

Monitoring the occurrence and dynamics of an oxidative burst in natural populations and testing its functions in field experiments is not a simple task. Some indirect evidence of the occurrence of oxidative bursts may be inferred from chlorophyll fluorescence parameters (Collén and Davison 1999a, 1999c; Gachon et al. 2006) and/or lipoperoxide accumulation, but it will be difficult to distinguish these responses from other environmental stresses. Indirect approaches need to be developed to investigate the impact of an oxidative burst on the fitness of algal species.

An epidemiological survey of complementary symptoms, reminiscent of the HR of terrestrial resistant plants, such as tip bleaching or accumulation of fluorescent aromatic compounds around the site of penetration of parasite propagules (Figs. 12.2 and 12.3), may facilitate the evaluation of the impact of host-microbe interactions in natural populations (Correa and Sanchez 1996; Ellertsdottir and Peters 1997; Bouarab et al. 2001a). This could allow one to test the importance of oxidative-burst-associated defense reactions in various biotic interactions.

A sophisticated, but likely more precise approach would be to develop comparative monitoring of defense-responsive gene expression patterns in controlled mesocosm experiments or in natural populations experiencing varying grazing or fouling intensities or infectious diseases. Using quantitative screening methods for the induction of transcripts identified as being upregulated, stable, or downregulated, one might identify gene networks and the interplay of defense pathways in response to specific pests. As mentioned earlier, development and optimization of screening tools, such as dedicated DNA chips or filter arrays, has been conducted in *C. crispus* and *L. digitata* (Collén et al. 2006b). These new molecular tools are available to start assessing patterns of differential gene expression and to identify major defense-responsive genes whose expressions vary within a population or are dependent on environment cues. These new approaches will also benefit the development of quantitative high- or medium-throughput methods for metabolite profiling of several individuals or within a population (La Barre et al. 2004). Knowledge of the complete genome of the brown alga *Ectocarpus siliculosus* by the end of 2007 (Peters et al. 2004) should also be of enormous help in identifying new candidate defense genes and in identifying gene function in mutants impaired in the resistance against pathogens or grazers. Furthermore, the availability of pangenomic DNA microarrays will be an important tool for investigating the signaling function and pathways of defense regulators, including waterborne signals (Toth and Pavia 2000). Although this favors *Ectocarpus* as a model alga for research in defence signaling, there is no doubt that some of the available molecular and genetic tools, such as invalidation of master or specific genes in other ecologically relevant species, will allow an integration of molecular ecology with more classical approaches in marine chemical ecology.

12.6 Conclusions

This chapter was an attempt to summarize how much important work has been done in the context of the oxidative-burst-associated responses in algal cells. The past decade has witnessed a substantial progress in our understanding of ROS metabolism (Dring 2006), the generation of halogen oxidants (Leblanc et al. 2006), and the oxidative metabolism of fatty acids in algae (Bouarab et al. 2004; Küpper et al. 2006). These active defense pathways against pathogens and possibly foulers and herbivores are likely to be important in terms of control of ecological interactions and in contributing a major role in the structure and function of ecosystems

based on macroalgal communities. Until recently, a common view of algal defense mechanisms was based on a lack of cell-based immunity mechanisms in algae. As it is true that algae, like terrestrial plants, do not display acquired immunity specialized cells, they have evolved several innate immunity traits that are conserved with other eukaryotes and that provide them with an efficient system to cope with pathogen aggression and to fight diseases. The dynamics of these activated and induced defense responses, most often orchestrated by an oxidative burst and/or by RNS production, is a key element to take into account in the study of the ecological roles of defense strategies based on the production of secondary metabolites (Chap. 11).

Acknowledgments Research on biotic interactions by the Potin group has recently been supported by Institut Français de la Biodiversité (Program biodiversity and global change), Britanny Region (PRIR 560408; A3CBL9), and CNRS. I warmly thank my former collaborators Drs. K. Bouarab, F. Küpper, and F. Weinberger who provided, and continue to bring, so much to this new field of research and also my current research group for continuing this effort. Dr. B. Kloareg's constant support is gratefully acknowledged. I also warmly thank Profs. J. Guern, A. Pugin, and J. Correa, Dr. J.-P. Salaün, and Profs. G. Pohnert and P. Leonardi for helpful discussions, advice, and support, which facilitated the development of assay methods.

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13
Defense Strategies of Algae and Cyanobacteria Against Solar Ultraviolet Radiation

U. Karsten

13.1 Introduction

Ultraviolet radiation (UVR) is a natural fraction of the solar radiation, and therefore has always influenced life in aquatic ecosystems. The development of oxygenic photosynthesis 2.5–2.7 billion years ago (Holland 1984) led to drastic chemical changes in the Earth's oceans and atmosphere. The gradual increase in photosynthetically produced oxygen over millions of years was accompanied by a strong enrichment of it in the atmosphere, which ultimately acted as precursor for the ozone (O_3) layer in the stratosphere.

The stratosphere as part of the atmosphere is located 10–50 km above the earth's surface, and represents a layer characterised by intense interactions among radiative, dynamic and chemical processes, in which horizontal mixing of numerous gaseous components such as ozone proceeds much more rapidly than vertical mixing. The stratospheric accumulation of ozone is primarily responsible for absorbing parts of the solar UVR before it can reach the biosphere, and its formation is a delicate and complex matter. A finely balanced equilibrium between ozone producing and degrading processes in the stratosphere maintains the efficient solar radiation-filter function of this trace gas. Anthropogenic influences through the emission of man-made halogenated volatile substances (e.g. CFCs, chlorofluorocarbons) over the last eight decades have resulted in a stratospheric enrichment of these compounds, which may persist for decades. Because of the high chemical reactivity of halogens they efficiently destroy ozone in the protective layer. This is particularly well reflected in the strong ozone decline over Antarctica each spring, which can account for more than 75% depletion, a phenomenon known to the public as 'ozone hole' (Wessel et al. 1998; for details see Whitehead et al. 2000).

U. Karsten
Institute of Biological Sciences, University of Rostock, Albert-Einstein-Str. 3, 18059 Rostock, Germany. e-mail: ulf.karsten@uni-rostock.de

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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13.1.1 Solar Spectrum and UVR

The emission characteristics and distance of the sun to the earth determine the intensity and spectral distribution of the radiation reaching our planet. Solar radiation entering the earth's atmosphere exhibits a typical spectrum characterised by UVR (190–400 nm), photosynthetically active radiation (PAR: 400–700 nm) and infrared radiation (IR: >700) (Fig. 13.1). UVR is differentiated according to the CIE definition (Commission Internationale de l'Eclairage 1935) into three wavebands – UVC: 190–280 nm, UVB: 280–315 nm and UVA: 315–400 nm. However, UVC does not reach the earth's surface, as it is completely absorbed by oxygen on its way through the atmosphere. Because of the absorption features of ozone, it is the UVB range that is likely to increase as a consequence of stratospheric ozone destruction. Calculations indicate that a 10% decline in column ozone would result in an ~5% increase of surface irradiance at 320 nm while the same decline would be accompanied by a 100% increase at 300 nm (Frederick et al. 1989).

In contrast to UVC and UVB, UVA more easily penetrates the atmosphere (Fig. 13.1). Typical UVA and UVB values for temperate regions such as Hamburg, Germany (53°N), at noon on a sunny day in early summer can amount to about 45 and 2.2 W m^{-2}, respectively. In tropical regions UVA can increase to >60 W m^{-2} and UVB to about 4 W m^{-2}. However, there are many factors that determine the UVR at the earth's surface. Besides clouds and atmospheric particles, changes in day length, season, latitude and altitude are particularly responsible for a high variability in the radiation conditions of terrestrial ecosystems, many of which are inhabited by aeroterrestrial algae, cyanobacteria and algal or cyanobacterial lichens. The picture is even more complicated for aquatic ecosystems (Whitehead et al. 2000).

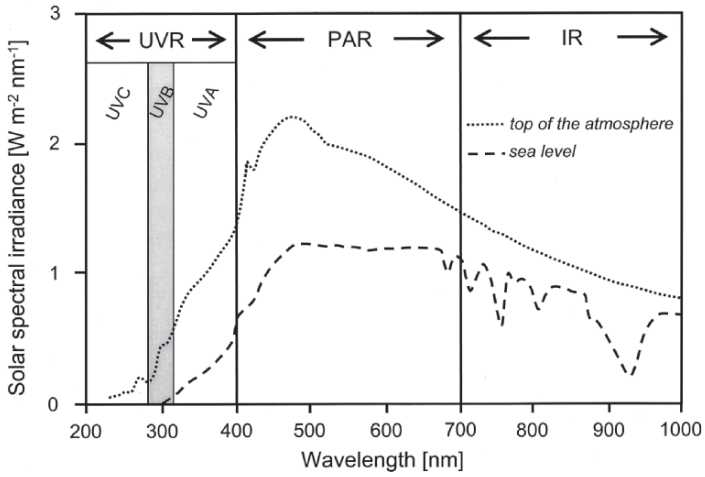

Fig. 13.1 Spectral characteristics of the solar radiation at the top of the atmosphere and after passing through the atmosphere to sea level

13.1.2 UVR in Aquatic Ecosystems

In addition to the factors leading to high UVR variability in terrestrial ecosystems, the optical properties of the water column, as well as waves and currents, have to be considered in aquatic ecosystems. While clear water, which can be found in the oceans only off-shore such as in the Sargasso Sea (Jerlov 1976), as well as in high-altitude lakes (Sommaruga and Psenner 1997), generally exhibits a very high transparency for UVA and UVB, any dissolved substances such as the riverine 'Gelbstoffe' and suspended particles strongly increase absorption and scattering of these wavelengths. Therefore, coastal waters are often optically opaque. Consequently, the attenuation of UVA and UVB in aquatic ecosystems is highly variable, and UVB penetration expressed as 1% of surface conditions can range from only few decimetres in eutrophic and turbid coastal waters (Piazena and Häder 1994) to 60–70 m in clear Antarctic waters (Smith et al. 1992). Depending on their vertical distribution, algae and cyanobacteria living in aquatic ecosystems can be regularly exposed to high UVA and UVB or never ever experience these short wavebands throughout their life (Fig. 13.2).

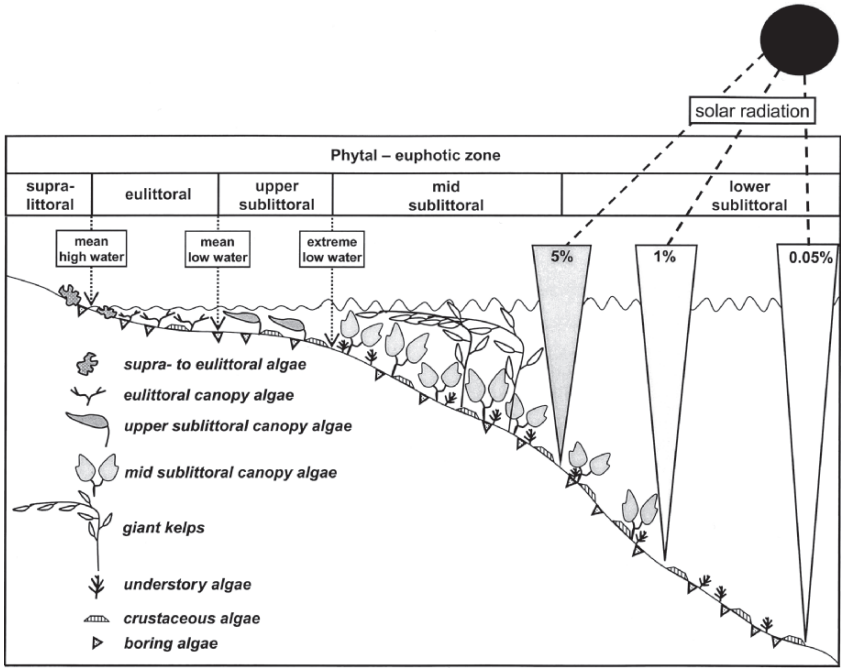

Fig. 13.2 Typical vertical zonation pattern of macroalgae in the littoral zones of rocky shores, and the penetration depths of solar radiation expressed as percentage of the incident surface radiation (adapted from Lüning 1985)

13.2 Effects of UVR on Algae

Solar radiation is essential for life on earth. In the process of photosynthesis, algae, cyanobacteria, and higher plants absorb light energy with their photosynthetic antennae and use it to chemically fix inorganic carbon into energy-rich organic compounds. However, an increase in UV-radiation can inhibit many biological processes. The major cellular targets of UVB are different biomolecules, which directly absorb this radiation, or which are indirectly affected by various UV-induced photochemical reactions. The biological and, ultimately, ecological consequences are numerous.

13.2.1 Molecular Targets

Nucleic acids such as DNA represent one of the most UV-sensitive biomolecules, and UV-induced damage occurs directly by the absorption of UVB quanta by the aromatic residues. The absorbed energy can be dissipated by different mechanisms involving single bases (e.g. single-strand breaks) or interactions between adjacent bases (e.g. dimerization) and between non-adjacent bases (i.e. inter- or intrastrand crosslinks) (Karentz et al. 1991). The structural consequences are conformational alterations such as the often observed formation of cyclobutane dimers and pyrimidine (6–4)–pyrimidone (6–4)–photoproducts (Lois and Buchanan 1994). Such UV-induced DNA damage can significantly compromise the accuracy of nucleic acid transcription and replication, causing misreading or erroneous replication, leading to an increasing number of mutations. A higher mutation rate can result in reduced gene expression and, hence, debilitation or even increased mortality of algal cells.

Proteins are built up by 20 different amino acids, of which tyrosine, phenylalanine, tryptophan and histidine contain aromatic residues capable of absorbing UVB. Besides UV-induced DNA photoproducts, damage to protein molecules represents the second major effect of UVB in algae. Typical target proteins are those associated with the plasmalemma or involved in photosynthesis such as the D1 protein of photosystem II and the enzyme Rubisco in the Calvin cycle (Campbell et al. 1998; Bischof et al. 2000). Furthermore, disulphide bonds between cysteine residues in proteins can be easily photo-oxidatively cleaved by absorbed UVB (Vass 1997). These bonds are responsible for protein folding, and thus, for the native three dimensional structure, which is a prerequisite for any specific function of the protein. Any structural damage to proteins can be reflected in loss of cell vitality because these molecules have multiple roles as enzymes, hormones and structural components. However, since proteins typically occur in numerous copies inside the cell, any UV-induced damage is less severe compared with DNA-damage (Harm 1980). On the other hand, degradation and replacement of damaged protein molecules require energy, which might otherwise support more essential processes such as DNA repair.

Pigments of the photosynthetic apparatus can also be destroyed after UV-exposure, with the phycobilins (main pigments of red algae and cyanobacteria) being the most sensitive, and carotenoids generally being less affected than chlorophylls (Teramura 1983).

13.2.2 Induction of Reactive Oxygen Species

In aquatic ecosystems, UV radiation may induce the formation of reactive oxygen species (ROS) by photoactivation of dissolved organic mater, photochemical degradation, and liberation of excited electrons that initiate the reduction of molecular oxygen resulting in superoxide anion radicals (Cooper and Zika 1983). A second reduction step of the superoxide radical followed by protonation yields hydrogen peroxide, which is a powerful oxidant because of its relatively long half-life that allows long distance diffusion (Asada 1994). Stratospheric ozone depletion and the concomitant UVB enhancement could have biologically significant effects on the input of hydrogen peroxide to shallow or stratified surface waters such as tidal flats, which are typically rich in benthic diatoms. While normal hydrogen peroxide concentrations in the marine water column range between 20 and 300 nM, lakes can exhibit up to 900 nM (Price et al. 1992). However, during summer, in tidal pools in Northern Europe and Antarctica hydrogen peroxide concentrations between 2,000 and 5,000 nM can be measured (Abele-Oeschger et al. 1997; Abele et al. 1999).

Besides these external processes, formation of ROS may also take place intracellularly. Photooxidative stress, including UVB, stimulates various cellular processes leading to the production of superoxide radicals and hydrogen peroxide, as well as singlet-oxygen and hydroxyl radicals. The sources and production sites of ROS are mainly related to photosynthetic activities such as the pseudocyclic photophosphorylation and the Mehler reaction, which stimulate the accumulation of hydrogen peroxide (Asada 1994; Elstner 1990).

UV-induced ROS are extremely toxic to cells by causing oxidative damage to all biomolecules (Sies 1991). For instance, lipids, which are major compounds of all biological membranes, may be destroyed by ROS. After a first initiation reaction an unsaturated fatty acid is converted to a peroxyl radical, which in turn attacks another unsaturated fatty acid finally leading to free radical cascades. This photochemical peroxidation of unsaturated fatty acids may be particularly damaging for membrane structure and function (Bischof et al 2006a).

13.2.3 Ultrastructure of Cells

Many UVB-induced physiological effects such as declining photosynthetic rates can be related not only to damaged biomolecules, but also to ultrastructural changes in organelles or membranes (Holzinger and Lütz 2006). Typical alterations include

swollen mitochondrial cristae, disrupted thylakoids or detached phycobilisomes in chloroplasts, bent-shaped dictyosomes, and damaged plasmalemma. An intact ultrastructure of the algal cell is a prerequisite for optimum functioning of all physiological processes.

13.2.4 Physiological Processes

As a consequence of the UV-induced damage of biomolecules or ultrastructure, all physiological processes are potentially impaired. Photosynthesis is probably the most intensively studied process in plant biology. Due to its biochemical complexity, numerous sites can be affected by UVB. These include, for example, inhibition of energy transfer within the PS II reaction centre, the water splitting complex, or the light-harvesting complex. In addition, key enzymes such as Rubisco and ATPase are typical targets. The common consequences of UVB on photosynthetic function are decreased or even fully inhibited CO_2-fixation, and hence a decline in primary and biomass production (Franklin and Forster 1997; Bischof et al. 2006a).

In addition, other physiological processes such as nutrient uptake, motility, reproduction and growth can be strongly affected by UVB. Growth studies reveal that cellular processes other than photosynthesis are impaired (Michler et al. 2002). Hence, growth represents an important indicator to assess UV stress particularly in long-term experiments due to the integration of all positive and negative influences.

13.2.5 Ecological Consequences

Although the ecological consequences of enhanced UVB exposure to algal species are still largely unexplored, some data exist and some assumptions can be made. Based on the differential adaptation and acclimation capabilities in different algal species, UVB may, even under non-depleted ozone conditions, substantially affect the structure of communities, as well as modulate productivity, reproduction, vertical distribution, biodiversity and succession, competition, and alga–herbivore interactions (Bischof et al. 2006a).

The information available on UV-susceptibility of different developmental stages in kelps indicate the unicellular zoospores as being most sensitive (Wiencke et al. 2000, 2004). However, zoospore UV-sensitivity varies species-specifically as a function of depth distribution of the sporophyte, i.e. kelps from shallow water are more tolerant than plants from deeper vertical positions. Consequently, any increase in UVB-induced spore mortality will result in impaired reproductive success and finally reduce fitness of the population. In addition, elevated UVB will penetrate deeper into the water column, which may result in a shift of the upper distribution limit of seaweed communities to deeper waters (Wiencke et al. 2006).

13.3 Protective Mechanisms to Counteract Harmful UV Effects

If cyanobacteria and algae are regularly confronted with UV-radiation in their natural habitats they rely on a number of different strategies to mitigate or even avoid biologically harmful UV-effects to guarantee long-term survival (Fig. 13.3). These include avoidance, numerous protective mechanisms and repair of essential biomolecules. Some recent reviews well cover also older references about UV-effects on and UV-adaptive mechanisms in algae and cyanobacteria (Bandaranayake 1998; Bischof et al. 2006a; Cockell and Knowland 1999; Dunlap and Shick 1998; Franklin and Forster 1997; Shick and Dunlap 2002).

13.3.1 Avoidance

Many benthic microalgae (e.g. diatoms) and cyanobacteria are closely associated with the substrate and produce mat like structures, which can range from a thickness of few micrometers to decimeters. Mat systems are considered to be joint ventures of different taxa in which the inhabiting microorganisms benefit each other by the release of protective substances. Two distinct cyanobacterial layers occur in a mat associated with a subtropical mangrove system growing fully exposed to solar radiation (Karsten et al. 1998a). While the 0.5-mm thick top layer was dominated by *Lyngbya aestuarii*, which is characterised by having a sheath with

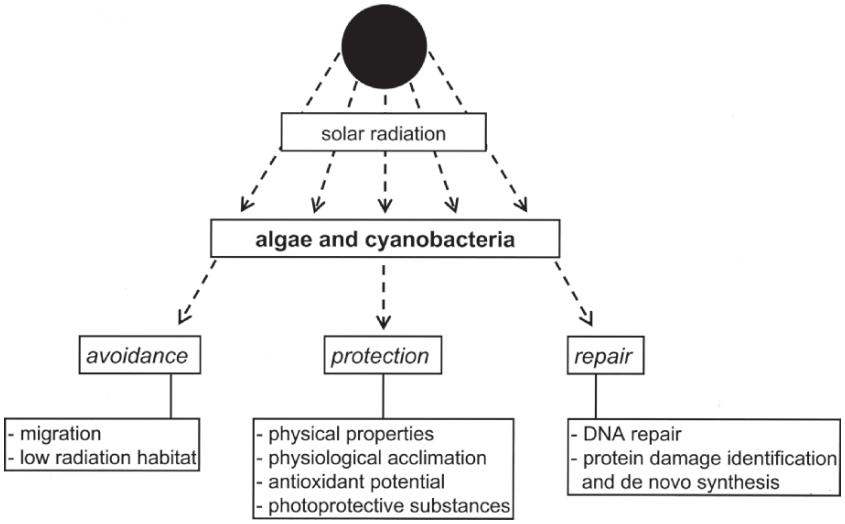

Fig. 13.3 Strategies of algae and cyanobacteria to counteract biologically harmful UV radiation

the UV-absorbing yellow–brown pigment scytonemin, the lower layer was mainly formed by the bundle forming *Microcoleus chthonoplastes*, a species unable to produce this particular UV-sunscreen. Scytonemin produced by *Lyngbya* served as a passive photoprotective mechanism ('umbrella') for *M. chthonoplastes* too (Karsten et al. 1998a).

Migration and vertical movement in the mats or sediments definitely appears to be an efficient strategy for motile cyanobacteria and microalgae to avoid long-term exposure to high UV radiation and hence to save energy for photoprotective acclimation. *Microcoleus chthonoplastes* from a microbial mat isolated in Solar Lake, Egypt, showed highest incidence of migration in response to UVB compared with UVA and PAR, indicating that this cyanobacterium was able to sense UVB directly, most probably due to the presence of a specific photoreceptor (Bebout and Garcia-Pichel 1995). Physically moving away from harmful UVB is one of the most effective avoidance strategies. However, it always requires an ability to detect this waveband. Furthermore, all algae and cyanobacteria have a dilemma with regard to solar radiation. While sufficient PAR is an essential prerequisite for photosynthesis, too much UV radiation can be harmful.

Many aeroterrestrial cyanobacteria are known to live endolithically inside mineral crusts or rocks, which requires low radiation adaptation but also guarantees protection against UVB (Büdel 1999). Other cyanobacteria and various unicellular green algae together with fungi form composite, symbiotic organisms known as lichens. Since many lichens synthesise unique UV-absorbing compounds and since the phototrophs are located inside the thallus, these microorganisms avoid elevated radiation (Büdel et al. 1997).

Phytoplankton species are often motile, or are able to sink and float, thereby minimizing the risk of any photodamage (Villafane et al. 2003).

In contrast to motile benthic microalgae and phytoplankton, most macroalgae are sessile and hence have to cope with the prevailing radiation conditions. If these plants grow deep in the water column they are never exposed to UV (Fig. 13.2) (Karsten et al. 2001). In contrast, in the intertidal zone (eulittoral) or in sheltered coastal lagoons, macroalgae have to often cope with high radiation. Here, green algae of the annual genera *Ulva* and *Chaetomorpha* often form mat-like communities, which exhibit steep gradients of UVB, but also of physiological responses. While top layers are exposed to high surface irradiance and hence often bleach, bottom layers permanently experience very low radiation conditions or even remain in darkness (Bischof et al. 2002, 2003, 2006b). Consequently, photosynthetic activity is inhibited in the top layers, performs at maximum rates in intermediate thallus layers and, due to light limitation, is again strongly reduced in bottom layers (Bischof et al. 2002). Another strategy for small macroalgae to avoid high radiation in more shallow water is to grow under the protecting canopy of larger macroalgal taxa such as kelps. Indeed, recruitment of juvenile kelps under the canopy of adult plants is considered to be an adaptive behaviour, which effectively protects these early life stages from stress, therefore minimizing ecological cost for defense and hence enabling allocation of more photosynthate for growth (Herms and Mattson 1992).

13.3.2 Physiological Acclimation

The response of any alga towards UVB exposure is determined by the interplay of genetically fixed adaptation and physiological acclimation (Bischof et al 2006a).

Many micro- and macroalgae, particularly from the supralittoral, eulittoral and upper sublittoral (Fig. 13.2), can cope with high radiation conditions in summer because of their ability for dynamic photoinhibition, a photoprotective mechanism by which excessive energy absorbed is rendered harmless by thermal dissipation (Krause and Weis 1991). The appropriate species exhibit a more or less pronounced decrease in photosynthetic activity during high radiation stress especially at noon, followed by a fast and full recovery during subsequent exposure to low radiation conditions, for example, at late afternoon (Hanelt et al. 1997). In contrast, all deep water and understory algae from the mid to low sublittoral recover only partly and slowly, indicating chronic photoinhibition. While dynamic photoinhibition is a reversible physiological mechanism, chronic photoinhibition is reflected by photodamage of components of the photosynthetic apparatus such as the D1 protein (Bischof et al. 2006a) and requires de novo synthesis of proteins.

13.3.3 Physical Properties

The periphery and extracellular structures of algae and cyanobacteria can provide substantial protection against UV exposure. In particular, cell walls and membranes might represent an effective optical barrier attenuating incident UV radiation before reaching intracellular organelles and biomolecules (Holzinger and Lütz 2006 and references therein). This effect can be amplified by calcification, cell wall incrustations, extracellularly deposited mucoid substances, and the presence of any epibiota. In various kelp species, individuals with thicker thalli generally exhibited a higher UV-tolerance than those with thin thalli (Dring et al. 1996). Furthermore, the outer silicate wall of diatoms can absorb up to 30% of incident UVB, which represents a significant primary UV defense (Davidson et al. 1994).

13.3.4 DNA Repair

As outlined earlier, a particularly harmful effect of UVB exposure is DNA damage. Consequently algae and cyanobacteria have developed strategies to prevent damage or to efficiently repair existing damage. Repair of UV-induced DNA photoproducts can take place by various mechanisms that include photoreactivation (light-mediated repair) or nucleotide and base excision repair (Britt 1996). The light-induced DNA repair is based on the activity of the key enzyme photolyase, which binds to complementary DNA strands and efficiently breaks pyrimidine dimers. However, this enzyme only functions as a DNA repair mechanism when visible

light is available for activation (preferentially in the violet to blue range of the spectrum). In contrast, the nucleotide and base excision repair includes numerous molecular steps such as damage recognition by specific proteins, assembly of a DNA repair complex, incision of the DNA backbone on either side of the damage, removal of the damaged strand, and filling of the remaining gap by DNA polymerase followed by attachment of the replacement DNA to the rest of the strand with a DNA ligase (Britt 1996).

Susceptibility of DNA to UV-damage is highly dependant on the developmental stage of the alga. In kelps, haploid zoospores are more sensitive to UV-induced DNA damage compared with the young diploid sporophytes (Wiencke et al. 2000). In addition, the UV-sensitivity of the zoospores can be related to the depth distribution of the adult sporophytes, i.e. more resistant taxa preferentially inhabit shallow waters. Less genetic damage was incurred in diploid red algal carpospores compared with haploid brown algal zoospores (Roleda et al. 2004). Zoospores were, however, more efficient in DNA damage repair. In a sexual organism, the advantage of both ploidy states can be combined by spending much of the life cycle in the haploid state, then fusing to become diploid. During the diploid state DNA damage can be repaired, since there are two copies of this biomolecule in each cell and one copy can be presumed to be undamaged. The ecological impact of UVB-induced DNA damage on the early life stages of macroalgae is important in shaping up community structure and zonation pattern (Bischof et al. 2006a; Wiencke et al. 2006).

13.3.5 De Novo Protein Biosynthesis

The replacement of any UV-damaged protein by de novo biosynthesis contributes to counteract UV damage. The molecular mechanisms are well studied in the cyanobacteria *Synechocystis* sp. and *Synechococcus* sp., and might be similar in micro- and macroalgae. In both organisms, exposure to moderate doses of UVB resulted in an increased turnover rate of the D_1 and D_2 reaction centre subunits of photosystem II, thus rapidly replacing damaged proteins by newly synthesised polypeptides (Campbell et al. 1998; Máté et al. 1998). A key step in this repair process is the UVB induced differential transcription of reaction centre protein encoding genes.

13.3.6 Antioxidative Potential

Cyanobacteria and algae have evolved a complex defense system against ROS, including non-enzymatic antioxidants like carotenoids, tocopherols (vitamin E), ascorbic acid (vitamin C) and reduced glutathione (Asada 1994).

Carotenoids exhibit multiple physiological roles, i.e. as light-harvesting pigments in photosynthesis, as quenching solutes for the triplet state of chlorophyll *a* to

dissipate excess photochemical energy, and as a general radical trapping antioxidant to prevent lipid peroxidation (Burton and Ingold, 1984; Edge et al., 1997). Long-term exposure of cyanobacteria and algae to high radiation typically leads to increasing carotenoid to chlorophyll *a* ratios, i.e. protective pigments are accumulated (Lakatos et al. 2001).

Tocopherols are the second major class of lipid-soluble antioxidants in photosynthetic membranes. Their primary function is to protect cells from lipid peroxidation. The water-soluble ascorbic acid also significantly acts against lipid peroxidation and DNA damage. However, these low-molecular weight antioxidants are chemically consumed and hence not considered the most efficient detoxifying agents.

In contrast, antioxidant enzymes can efficiently counteract all UV-induced ROS (Aguilera et al. 2002). These enzymes are represented by superoxide dismutase (SOD), catalase and glutathione peroxidase as well as those involved in the ascorbate–glutathione cycle, such as ascorbate peroxidase, mono-dehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase. One of the most important classes of antioxidant enzymes is the SOD family, which eliminate noxious superoxide radical anions. Different metalloforms of SOD exist (Fe, Mn, CuZn and Ni), which due to their intracellular localisation protect different cellular proteins (Lesser and Stochaj 1990).

13.3.7 Photoprotective Substances

For all phototrophic organisms exposed to UV radiation for substantial parts of their life cycles, strategies that passively screen UV radiation will contribute to preventing UV-induced direct and indirect damage to essential biomolecules. In addition, UV-screening may also save metabolic energy by reducing the need for constantly active avoidance and repair processes.

Mycosporine-Like Amino Acids

The most common photoprotective sunscreens in many, but not all algal taxa and cyanobacteria are the mycosporine-like amino acids (MAAs), a suite of chemically closely related, colourless, water-soluble, polar and at cellular pH uncharged or zwitterionic amino acid derivatives (Fig. 13.4).

MAAs are related to fungal mycosporines, which were first isolated from sporulating mycelia (Leach 1965; Favre-Bonvin et al. 1976), and consist of aminocyclohexenone or aminocyclohexenimine rings. The various MAA structures result from N-substitutions of different amino acid moieties to the cyclohexenone or cyclohexenimine chromophore. At present, there are only few known aminocyclohexenone-derived MAAs such as mycosporine-glycine, which typically exhibit their absorption maximum in the UVB range. All the other ~20 described MAAs are derivatives of the aminocyclohexenimine structure, which absorb maximally at

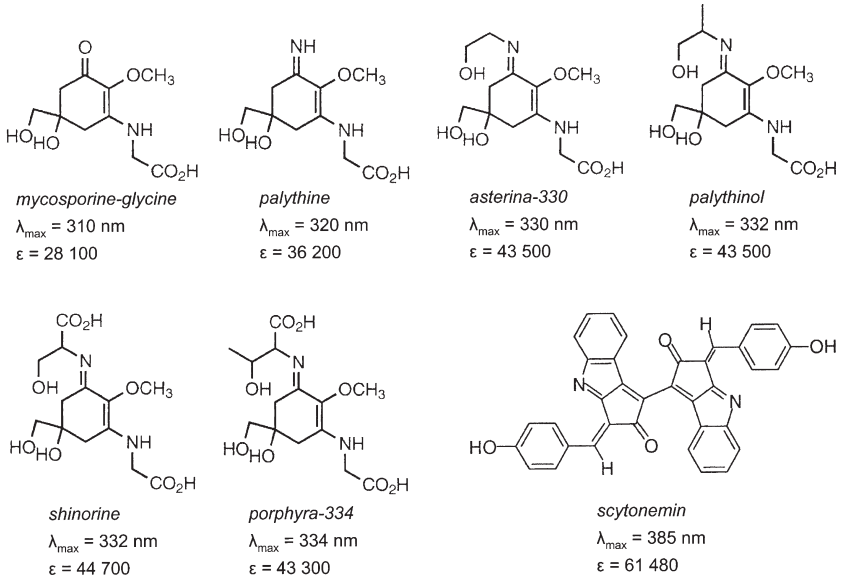

Fig. 13.4 Chemical structure, absorption maximum and molar extinction coefficient of typical algal mycosporine-like amino acids (MAAs) and cyanobacterial scytonemin

UVA wavelengths (Cockell and Knowland 1999; Karentz 2001; Shick and Dunlap 2002). Many cyanobacteria contain multiple MAAs, of which some have been identified. However, most chemical structures have still to be elucidated (Castenholz and Garcia-Pichel 2000). MAAs are proposed to function as passive shielding solutes by dissipating the absorbed short wavelength radiation energy in the harmless form of heat without generating photochemical reactions (Bandaranayake 1998). These biomolecules exhibit extremely high molar absorptivity for UVA and UVB (molar extinction coefficients between 28.000 and 50.000), and have been reported to be photochemically stable structures, both of which are prerequisites for their sunscreen function (Conde et al. 2000).

The anabolic and catabolic pathways for MAA biosynthesis and degradation have not yet been experimentally elucidated. However, various studies indicate that MAAs are most likely synthesised through the shikimate pathway, products of which are involved in the formation of aromatic amino acids (Shick 2004). The shikimate pathway is restricted to algae (and higher plants), cyanobacteria, bacteria and fungi, and hence all animals have an obligate dietary requirement for ingestion of some aromatic amino acids. In addition, since many aquatic animals contain MAAs, but lack the biosynthetic capability to produce these compounds, a dietary origin from grazing on algae and cyanobacteria is the only plausible explanation. Indeed, algal diets can regulate MAA concentration and composition in aquatic invertebrates and fish (Karentz 2001; Shick and Dunlap 2002). The ingested MAAs can be specifically bioaccumulated in the most UV-susceptible tissues or reproductive

structures (e.g. eggs) (Adams and Shick 1996). In addition, algal-derived MAAs can be interconverted to animal-specific MAAs in the digestive track by animal enzymes or by endosymbiotic bacteria. Finally, these compounds may serve as precursors for the degradative conversion to gadusol and 4-deoxygadusol, which are considered as substrates for MAA biosynthesis (Dunlap and Shick 1998). Since MAAs are not uniformly distributed between different tissues of invertebrates and fish, a selective partitioning of these compounds into specific cells must take place. However, the underlying mechanisms such as postulated active transport systems have not been studied (Karentz 2001).

Among phototrophic organisms, MAAs occur in most microalgal groups and cyanobacteria, although not in all taxa (Jeffrey et al. 1999; Karentz 2001). Dinoflagellates and diatoms tend to have the greatest and fastest capacity to synthesise and accumulate MAAs within hours after exposure to enhanced radiation conditions (PAR and UV) (Caretto et al. 1990). Among the macroalgae, MAAs are mainly found in red algae. Brown algae and most green algae typically lack these compounds, except the green macroalga *Prasiola crispa* ssp. *antarctica* and some closely related taxa, which contain high concentrations of a unique MAA with an absorption maximum at 324 nm, the so-called 324-nm MAA (Hoyer et al. 2001; Karsten et al. 2005).

From an ecological standpoint, the ephemeral, tufty *Prasiola* is intriguing due to its capability to grow aeroterrestrially on bark, soil and rock, as well as in the supralittoral zone of marine rocky shores and on anthropogenic surfaces such as harbour piers. In polar regions, species of this genus prefer habitats rich in nitrogen-containing faeces of birds such as underneath or near seagull or penguin colonies (Holzinger et al. 2006). Considering a relation between the MAA concentration and nitrogen availability in different species of the red alga *Porphyra* (Korbee et al. 2005), as well as a nitrogen-dependency of photoacclimation in *Ulva rotundata* (Henley et al. 1991), it becomes obvious that this nutrient might be a critical factor for the physiological performance of *Prasiola* under aeroterrestrial conditions. As a consequence of living under subaerial conditions this alga experiences strong amplitudes of the prevailling environmental parameters. Seasonal studies on *Prasiola* indicate some variation in MAA concentrations, but always with high minimum steady-state amounts (Jackson and Seppelt 1997; Karsten et al. 2005). In this respect *Prasiola* species resemble the so-called *MAA-type III* consisting of red algal taxa that contain high contents of MAAs under all environmental conditions (Hoyer et al. 2001; see later). Typical members of this group include the supralittoral to higher eulittoral genera *Bangia* and *Porphyra*, which seem to accumulate MAAs not only in the field after exposure to high solar radiation, but also under long-term low-light laboratory conditions, indicating genetic rather than environmental control of the concentration of UV-absorbing compounds (Karsten and West 2000; Hoyer et al. 2001, 2002). Consequently, it appears that the 324-nm MAA in *Prasiola* species is expressed constitutively.

The function of MAAs as intracellular screening agents has been inferred in numerous red algae from a decrease in concentration with increasing depth (Hoyer et al. 2001, 2003). Supra- and eulittoral red algal species typically experience

the strongest insolation, and consequently synthesise and accumulate high MAA contents, which generally are positively correlated with the natural UV doses (Karsten et al. 1998b; Huovinen et al. 2004). This is also well reflected when red algae from different biogeographic regions are compared, i.e. tropical species contain much higher MAA amounts than similar taxa from temperate to polar waters (Karsten et al. 1998b, 2000). In contrast, many red algal taxa growing in the deep sublittoral are biochemically not capable of producing MAAs (Hoyer et al. 2001, 2002; Karsten et al. 2001), which explains their strong sensitivity to high ambient solar radiation. These species do not experience UV exposure in nature, and, hence, there is no physiological need to synthesise and accumulate metabolically expensive nitrogen-containing MAAs. This in turn saves energy to better support other essential pathways such as the biosynthesis of light-harvesting phycobilisomes to guarantee sufficient PAR absorption under the prevailling low-light conditions.

The screening function of MAAs was evaluated for various cyanobacteria (Garcia-Pichel and Castenholz 1993), and it could be shown that supplemental UV radiation led to a strong induction of these compounds. Up to 26% of incident UV radiation of 320 nm can be attenuated due to the accumulation of MAAs. The additional presence of the UV-absorbing scytonemin, a compound specific to cyanobacteria (see later), may increase the total attentuation at 320 nm up to 60% (Garcia-Pichel et al. 1992). While MAAs are thought to be intracellularly localised, most probably homogeneously distributed in the cytoplasm, the cyanobacterium *Nostoc commune*, which inhabits extreme arid and polar habitats exhibits unique MAAs that are extracellularly deposited and covalently linked to oligosaccharides of the glycan coat (Ehling-Schulz et al. 1997). Since these specific MAAs are accumulated in very high concentrations of up to 10% of the species dry weight, they attenuate about 70% of the incident UV radiation, and hence provide very efficient photoprotection, including substantial protection against UVB-induced photobleaching of chlorophyll *a* (Ehling-Schulz et al. 1997). In addition, various physiological and cell biological experiments indicated that the presence of MAAs provided a significant benefit to the cells such as higher growth rates under UV exposure (Garcia-Pichel et al. 1992).

Field experiments in the Arctic with the red alga *Devaleraea ramentacea* showed a continuous decrease in photosynthetic performance under UV treatment with increasing collecting depths between 1 and 5 m. The total MAA concentration was also correlated with the original sampling depth, i.e. shallow water isolates contained much higher amounts than algae from deeper waters, also indicating a strong correlation between the MAA contents and the degree of sensitivity of photosynthetic activity in *D. ramentacea* (Karsten et al. 1999). In the Antarctic red alga *Palmaria decipiens*, juveniles collected in the upper to mid sublittoral during winter contained low MAA concentrations while mature plants collected in late spring and summer exhibited significantly higher values, indicating strong seasonal effects, which are related to the changing daylengths and radiation conditions (Post and Larkum 1993).

Based on the MAA concentrations and the induction patterns after exposure to different radiation conditions red algae can be physiologically classified in three categories (Hoyer et al. 2001): *Type I* – no MAAs at all; *Type II* – MAAs inducible

in variable concentrations and *Type III* – permanently high MAA values. While *Type I* represents deep-water red algae of the lower sublittoral, *Type II* and *III* species grow from the supra- and eulittoral to the upper and mid sublittoral zone. Experiments under defined radiation conditions proved that the induction, biosynthesis and accumulation of MAAs is a very flexible and species-specific process. While some taxa synthesise MAAs particularly under UVB, others prefer UVA or higher PAR only (Hoyer et al. 2003). Although experimental evidence for a particular trigger mechanism is still missing, it is reasonable to assume that a signal transduction pathway must be involved in the overall process leading to high MAA concentrations. Based on the different types of MAA induction patterns in red algae the presence of various photoreceptors, most probably between the blue light and UVB range, must be considered (Kräbs et al. 2002).

The entire algal thallus does not respond uniformly to ambient solar conditions. Young apical or marginal zones, i.e. growing cells, synthesise and preferentially accumulate MAAs leading to cross sectional and longitudinal concentration gradients (Hoyer et al. 2001). Populations of *Devaleraea ramentacea* collected from very shallow water typically exhibit green apices and red basal parts. The more exposed green tips contain 5-fold higher MAA contents than the red bases (Karsten et al. 1999). Older tissue regions usually exhibit thicker cell walls and a leathery texture, and are therefore optically opaque. The higher MAA concentrations in the most exposed thallus regions are essential to guarantee protection of the more delicate meristematic cells.

Besides the stimulating effect of increasing solar radiation on the biosynthesis and accumulation of MAAs in many algae and cyanobacteria, other environmental factors may also influence them. Nutrient availability (e.g. ammonia) in particular has positive effects on MAA concentrations (Korbee et al. 2005).

As well as functioning as natural UV-protective compounds, some MAAs such as mycosporine-glycine also have moderate antioxidant activity (Dunlap and Yamamoto 1995). In addition, the presumed biochemical precursor of MAAs, 4-deoxygadusol exhibits strong antioxidant activity (Dunlap et al. 1998). The photo-physicochemical properties of MAAs guarantee both a high UV-protective effectiveness in combination with antioxidant capabilities.

Other physiological functions of MAAs in phototrophic organisms such as organic osmolytes have been suggested, because very high concentrations can be found in cyanobacteria living in hypersaline environments (Oren 1997). However, salt shock experiments with the marine cyanobacterium *Microcoleus chthonoplastes* did not indicate any major involvement of MAAs in the process of osmotic acclimation (Karsten 2002), and hence their proposed function as osmolytes has to be questioned.

Carotenoids

As mentioned in Sect. 13.3.6, carotenoids serve mainly as accessory pigments in the light harvesting complexes of the photosynthetic apparatus, and additionally as cellular protection against ROS. Whether carotenoids also act as passive UV-sunscreens is

still somewhat controversial (Cockell and Knowland 1999). However, some cyanobacteria such as *Nostoc commune* increase their outer-membrane carotenoids echinenone and myxoxanthophyll by about 50% within few hours after exposure to UVB radiation (Ehling-Schulz et al. 1997). In addition, by genetic manipulation the endogenous carotenoid content can easily be modified in the cyanobacterium *Synechococcus* sp. leading to either increased amounts or a shifted composition towards the formation of zeaxanthin (Götz et al. 1999). Because of the increased overall concentrations of carotenoids or the preferential accumulation of zeaxanthin, photosynthesis of the transformants exhibited a much higher tolerance against UVB radiation compared with the wild type.

Scytonemin

Scytonemin has been reported from all cyanobacterial groups which have taxa that posses extracellular sheaths and that occur in high radiation habitats (Garcia-Pichel and Castenholz 1991), as well as in cyanobacterial lichens (Büdel et al. 1997).

Unlike the colorless MAAs, scytonemin has a yellowish-brown color, and exhibits an in vivo absorption maximum in the UVA range at 370 nm, although shorter and longer wavelengths are also well filtered. This pigment becomes red in the reduced state and green after oxidation. Scytonemin is an extracellular, lipid soluble dimeric molecule with a molecular weight of 544 Da and a structure based on indolic and phenolic subunits (Fig. 13.4). It is formed by condensation of tryptophan and phenyl-propanoid derived subunits (Proteau et al. 1993). The underlying biochemical pathways are still unknown.

The biosynthesis of scytonemin is strongly induced by UVA and PAR, and most cyanobacteria have a minimum photon fluence rate at which scytonemin begins to be synthesised. Because of its high extinction coefficient it may prevent 90% of incident UVA entering the cell, thus, efficiently functioning as a UVA sunscreen (Garcia-Pichel et al. 1992; Brenowitz and Castenholz 1997). The biosynthesis of scytonemin is followed by deposition in the extracellular sheath where this compound remains chemically unchanged under natural conditions. Photodegradation does not occur, which is evident from its persistence of up to decades in terrestrial cyanobacterial crusts or dried mats (Brenowitz and Castenholz 1997). Scytonemin effectively reduces UVA-induced inhibition of growth and photosynthesis as well as the photobleaching of chlorophyll *a* in *Chlorogloepsis* sp. (Garcia-Pichel et al. 1992). In strains of *Nostoc commune* that lack MAAs, but contain scytonemin, UVB treatment completely destroyed chlorophyll *a*, indicating that scytonemin does not act as UVB-sunscreen (Ehling-Schulz et al. 1997).

For cells that occur under layers or inside colonies of scytonemin-producing taxa, the attentuation of UVA may be even more effective. The ‘umbrella’-function of scytonemin produced by surface layers of *Lyngbya aestuarii* in microbial mats for other cyanobacteria living underneath has already been described (Sect. 13.3.1) (Karsten et al. 1998a).

Phenolic Compounds

A special class of polyphenolic compounds are phlorotannins, which represent secondary metabolites that are exclusively found in brown macroalgae (Ragan and Glombitza 1986). Several functions are commonly accepted, including a role in deterring herbivores and microbes and in adhesion and strengthening cell walls (Schoenwaelder 2002a; Amsler and Fairhead 2006). In addition, phlorotannins absorb UV radiation, mainly UVC and partly UVB, with maxima at 195 and 265 nm (Ragan & Glombitza 1986, Pavia et al. 1997), and posses a high antioxidant activity. Consequently, phlorotannins are considered as efficient UV-sunscreen compounds, which is supported by various studies.

The presence of high concentrations of phlorotannins in the outer cell layers of the intertidal *Hormosira banksii* protect against strong photodamage during the Australian summer (Schoenwaelder 2002b). Although the outer cell layers are damaged and disrupted by high natural solar radiation, phlorotannins are released from physodes (phlorotannin containing vesicles) into the cytoplasma where they easily oxidise. As a consequence, the oxidised phlorotannins become brownish and form a photoprotective cellular layer for the underlying photosynthetic tissue. In addition, strong differences in UV-tolerance between intertidal *Fucus spiralis* and intertidal to upper sublittoral *Fucus serratus* can be related to the number of physodes in egg and zygote cells (Schoenwaelder et al. 2003), i.e. a lower number led to higher sensitivity.

An induction of phlorotannins after a 2-week exposure to artificial UVB radiation was first described in *Ascophyllum nodosum* (Pavia et al. 1997). Similarly, an increase in the size of physodes was observed in various Laminariales from Spitsbergen after UVB treatment, indicating an induction of phlorotannin biosynthesis (Wiencke et al. 2004). This has recently been verified in zoospore suspensions of the UV tolerant brown algae *Alaria esculenta* and *Saccorhiza dermatodea*, which exhibited strong increases in absorbance after UVB exposure, whereas the absorbance of spore suspensions of the UVB sensitive species *Laminaria digitata* remained unchanged (Roleda et al. 2006).

An exudation of phlorotannins in response to artificial UVB radiation was observed in *Macrocyctis integrifolia*, which led to a strong reduction in UVB transmission through the water column, thereby protecting germinating zoospores of *Laminaria groenlandica* against UV radiation (Swanson and Druehl 2002). Therefore, high phlorotannin content and high exudation rates may reflect an adaptation of brown algae against UV radiation stress.

Other UV-Absorbing Compounds

Several other UV-absorbing compounds have been described as potential photoprotectants in cyanobacteria and algae, but these substances are not as common as MAAs or scytonemin.

A possible role of pterins as UV-protecting compounds has been suggested for the marine cyanobacterium *Oscillatoria* sp. since UVA radiation effectively induced

biosynthesis of a biopterin glucoside compound (Matsunaga et al. 1993). The pteridine-related biopterin glucoside exhibits strong absorbance in the UVA-range, and its presence led to an enhanced UVA tolerance in *Oscillatoria* sp. In contrast, a seasonal study of the variations in amounts of scytonemin, MAAs and pterins in a cyanobacterial mat growing on an intertidal mangrove sediment clearly documented that in contrast to increasing scytonemin levels, during summer the concentrations of pterins consistently remained very low (Karsten et al. 1998a). Since the average content of pterins accumulated by cyanobacteria do not attain concentrations high enough to provide a significant optical effect, a photoprotective role as sunscreen can be discounted, at least for this particular microbial mat.

Dasycladus vermicularis is a siphonous green macroalga inhabiting littoral zones in the temperate Atlantic and some regions of the Mediterranean. This species has been reported to excrete UV-absorbing coumarins into the surrounding medium when the alga is manipulated (Pérez-Rodríguez et al. 1998). Maximal concentrations of the dominant 3,6,7-trihydroxycoumarin were found in the apical part of the thallus, which is the most sun-exposed part of the plant. At a cellular level, this compound is mainly localised in the internal part of the cell wall and around the vacuolar membrane. The coumarin layer close to the cell wall of *Dasycladus vermicularis* absorbed 88% of the incident UVA radiation at 346 nm, thus well supporting its function as a natural sunscreen. Besides the sunscreen function, 3,6,7-trihydroxycoumarin exhibits a strong antioxidative potential similar to that of vitamin C. In addition, the release of this substance into the medium under different stress conditions provided an efficient UV filter for other co-occurring macroalgae (Pérez-Rodríguez et al. 2003). This seems to be of ecological relevance particularly when *Dasycladus vermicularis* grows in shallow water enclosed habitats such as rock pools.

Flavanoids and isoflavanoids represent typical UV-absorbing derivatives of the phenylpropanoid pathway in higher plants. Their function as UV-sunscreens have been experimentally documented by the prevention of UV-induced DNA damage (Kootstra 1994). Most interesting, there are indications that isoflavanoids might also occur in cyanobacteria and green microalgae (Prof. Klejdus, University of Brno, Czech Republic, personal communication), but further studies are required to address this open question.

Sporopollenin is a complex polymer made up of a diversity of aromatic and aliphatic residues, which occurs in many algal cell walls where it non-specifically attenuates UV radiation (Xiong et al. 1997). It may therefore act as a constitutive UV sunscreen compound.

Other chemically non-characterised UV-absorbing compounds have been reported in the water column near phytoplankton blooms such as the so-called P380, which exhibits a broad absorbance between 300 and 470 nm with a maximum at about 380 nm (Llewellyn and Mantoura 1997).

More recent studies have documented the occurrence of a specific UVB-absorbing compound with an absorption maximum at 294 nm in the green macroalga *Ulva pertusa* (Han and Han 2005). UVB-radiation strongly induced this compound and its accumulation resulted in a higher photosynthetic performance under UV exposure,

as well as to a higher antioxidative capacity. However, the chemical structure still has to be elucidated.

13.4 Conclusions

Algae and cyanobacteria inhabit almost all photic habitats on Earth, and many of them are strongly influenced by high insolation, particularly biologically harmful UVB radiation. However, in these phototrophic organisms various photoprotective strategies have evolved to tolerate UV exposure. Consequently, algae and cyanobacteria represent excellent model systems to study and understand the underlying mechanisms. With new developments in genomics, proteomics, metabolimics and analytical chemistry new types of UV-sunscreen compounds will continue to be discovered and their biosynthetic and regulatory mechanisms elucidated.

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14
Algal Sensory Chemical Ecology

C.D. Amsler

14.1 Introduction to Sensory Chemical Ecology

Sensory chemical ecology is the branch of chemical ecology that focuses on chemical communications between organisms and chemical sensing of the environment by organisms. Algae are well known to have numerous physiological responses to variations in their chemical environment, particularly with respect to nutrients (Lobban and Harrison 1994). However, with respect to environmental sensing it is typical for "chemical ecology" to be restricted to behavioral responses and I have followed that restricted definition here. Sensory chemical ecology occupies only a single, short chapter in this book on algal chemical ecology. This is an illustration of one of if not the major way in which the current field of algal chemical ecology differs from chemical ecological studies of other organisms. If a current, comprehensive book on terrestrial chemical ecology was available, it could easily be dominated by chapters on sensory chemical ecology (cf. more focused works by Roitberg and Isman 1992; Eisner and Meinwald 1995; Cardé and Millar 2004; Dicke and Takken 2006). Even in nonalgal marine chemical ecology, sensory ecology is relatively well studied, particularly with respect to prey detection via odor plumes and to chemical cues for larval settlement (e.g., reviews by Pawlik 1992; Atema 1995; Hadfield and Paul 2001; Trapido-Rosenthal 2001; Steinberg et al. 2002). The sensory chemical ecology of algae is best known with respect to chemical communication either in gamete attraction involving pheromones or in chemical induction of gametogenesis or gamete release. Most such reports have examined freshwater green algae and marine brown algae. More limited studies have involved chemoattractive behaviors of macroalgal spores or microalgal vegetative cells in a wider variety of taxa or involved chemical cues for green or brown macroalgal spore settlement. However, perhaps the most exciting areas of recent progress have been with respect to settlement cues in macroalgal spores.

C.D. Amsler
Department of Biology, University of Alabama at Birmingham, Birmingham, AL 25294-1170, USA. e-mail: amsler@uab.edu

C.D. Amsler (ed.), *Algal Chemical Ecology*.

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Known chemoattractive behaviors in vegetative cells, gametes, and spores usually involve swimming. Among nonflagellated algae, only the desmid *Closterium* and the diatom *Amphora*, both of which utilize gliding motility, are known to have chemoattractive responses (Cooksey and Cooksey 1988; Sekimoto 2005). Chemoattraction via amoeboid movements is well known in other organisms (e.g., Manahan et al. 2004; Manes et al. 2005; Bagorda et al. 2006). Thus, there is no reason to expect that other macroalgal cells that move via gliding motility or amoeboid movements, including single celled red algae, red algal spores, and other desmids and diatoms, many of which are known to exhibit phototactic behaviors (Nultsch and Hader 1979, 1988; Pickett-Heaps et al. 2001), could not also exhibit chemoattractive behaviors. The lack of other reports on algae using mechanisms other than flagella for motility seems likely to be the result of a lack of other efforts to look.

Chemoattractive behaviors in swimming algae can be either chemotactic or chemokinetic (Amsler and Iken 2001). In true chemotaxis, cells orient their swimming directly towards or away from a chemical source, while in chemokinesis, net movement towards or away from a source is accomplished via modulation of swimming speed or the rate at which swimming direction is changed (Fraenkel and Gunn 1961; Dusenbery 1992). However, in some organisms chemokinetic behaviors have historically been referred to as "chemotaxis" in the literature (cf. Amsler and Iken 2001).

14.2 Sexual Communication

14.2.1 Gamete Attraction

Mechanisms such as chemical attraction, which improve the chances that compatible gametes of a species will encounter one another, should obviously be selectively advantageous. Chemoattraction of male gametes by female gametes in the model green algal genus *Chlamydomonas* has been reviewed by Maier (1993) and Govorunova and Sineshchekov (2005). Although gamete attraction has been reported in several *Chlamydomonas* spp. (Maier 1993), it is best documented in the heterogamous species *C. allensworthii* (Govorunova and Sineshchekov 2005). Female gametes of *C. allensworthii* secrete plastoquinone-related pheromones called lurlenes that are bioactive at picomolar concentrations (Jaenicke and Marner 1995; Starr et al. 1995; Jaenicke and Starr 1996). Different strains of *C. allensworthii* produce and respond to lurlenes with slightly different chemical structures (Jaenicke and Starr 1996).

Sexual reproduction in another group of green algae, the desmids, involves a number of different pheromones at various stages (Sekimoto 2005), including for attraction of gametes via gliding motility. In *Closterium ehrenbergii* and *Cosmarium botrytis*, mating type plus (mt^+) gametes are attracted to mating type minus (mt^-)

regardless of the strain combination but mt^- cells are only attracted to mt^+ in particular combinations (Brandham 1967; Coesel and de Jong 1986). In *C. ehrenbergii*, the pheromone that attracts mt^+ gametes to mt^- has been identified as a 20-kDa protein (Fukumoto et al. 1998).

Chemoattraction of spermatozoids and androspores in the filamentous green alga *Oedogonium* spp. has been reviewed by Maier (1993) and Sekimoto (2005). In macrandrous species, spermatozoids released by male filaments fertilize oogonia on female filaments, while in nannandrous species, male filaments produce androspores, which attach next to developing oogonia on female filaments and germinate into dwarf male filaments that later release spermatozoids (Graham and Wilcox 2000). In the macrandrous species *O. cardiacum*, spermatozoids are attracted to the oogonia by a pheromone (Hoffman 1960), which has been partially characterized as a water-soluble, nonpolypeptide pigment, with a molecular weight between 500 and 1,500 Da (Machlis et al. 1974). In nannandrous species, both androspores and spermatozoids are attracted by pheromones secreted by oogonia (Rawitscher-Kunkel and Machlis 1962). In *O. donnellii* the androspore attractant has been determined to be a nonpolar compound soluble in dichloromethane (Hill et al. 1989).

Chemoattraction of male gametes to female gametes is widespread in the brown algae and very well characterized in numerous species. There are several recent reviews of these behaviors and the pheromones responsible (Amsler and Iken 2001; Pohnert and Boland 2002; Amsler and Fairhead 2006) and the review of Maier (1995) is particularly comprehensive. Readers are referred to these reviews for in-depth detail. Briefly, there are 12 specific volatile, C_8 or C_{11} hydrocarbon pheromones produced by over 60 brown algal species. The best characterized responses are a complex chemokinetic behavior displayed by gametes of *Ectocarpus siliculosus* in response to the pheromones ectocarpene and pre-ectocarpene (Müller 1978; Maier and Calenberg 1994; Pohnert and Boland 2002) and a true chemotactic response in spermatozoids of *Laminaria digitata* in response to the pheromone lamoxirine (Maier 1982; Maier and Müller 1990).

14.2.2 Inducers of Gamete Production or Release

In addition to gamete attraction, pheromones can also have critical roles in stimulating the production or release of gametes in some green and brown algae (reviewed by Maier 1993; Sekimoto 2005). The most in depth studies have involved species of the colonial green alga, *Volvox*, in which the process has been examined at cellular, molecular, and biochemical levels (Sekimoto 2005). A high frequency mutation initiates sexual maturation of some male colonies, which release glycoprotein pheromones that induce sexual maturation of other colonies into both males and females (Sekimoto 2005). In desmids, at least three different small protein or glycoprotein pheromones are involved in the induction and completion of sexual differentiation in *Closterium* spp. in addition to the chemotactic pheromone described

above (also reviewed by Sekimoto 2005). In three of the more morphologically complex orders of brown algae (Laminariales, Desmarestiales, and Sporochnales), the same pheromones responsible for chemoattraction of male gametes also triggers their release from antheridia (reviewed by Maier 1993, 1995).

14.3 Chemoattraction to Nutrients

Although something of an oversimplification, one can say that the two main things that algae need to grow and reproduce are light and nutrients. It has long been known that many microalgae as well as the spores and gametes of macroalgae are phototactic (Nultsch and Hader 1979, 1988; Amsler et al. 1992) and although there have been fewer studies of chemoattraction to nutrients, these same types of cells can behaviorally respond to nutrients and thereby potentially optimize their nutrient microenvironment, at least under some physical flow regimes. In fact, in the algal species best studied mechanistically for both phototaxis and chemotaxis, *Chlamydomonas reinhardtii*, there is evidence for integration of the signaling pathways of both processes soon after the initial signal recognition events (Govorunova and Sineshchekov 2003). Chemotaxis in vegetative cells of *Chlamydomonas* spp. has been known since the work of Hagen-Seyfferth (1959) and has recently been reviewed by Govorunova and Sineshchekov (2005). *Chlamydomonas* spp. exhibit positive chemotaxis to ammonium and are attracted or repelled by organic nitrogen sources such as amino acids and the culture media components tryptone and peptone (Govorunova and Sineshchekov 2005). Chemoattraction to ammonium is influenced by circadian rhythms and lost during gametogenesis (Sjoblad and Frederikse 1981; Ermilova et al. 2003). Other unicellular green algae, *Chlorococcum minutum*, *Dunaliella tertiolecta*, and *D. salina*, are also chemotactic to several amino acids (Ermilova and Gromov 1988; Amsler and Iken 2001). *C. reinhardtii* vegetative cells can be grown heterotrophically and are attracted to several sugars (Ermilova et al. 1993; Govorunova and Sineshchekov 2005). They also accumulate around sources of organic acids although this appears to be a chemokinetic response via decreased swimming speed (Hirschberg and Rodgers 1978). The colonial green alga *Astrephomene gubernaculifera* is attracted by acetate, on which it is able to grow heterotrophically (Hoops et al. 2002).

Amsler and Iken (2001) reviewed chemoattraction to nutrients in several groups of marine microalgae, including attraction of cryptomonads and dinoflagellates to inorganic and organic sources of nitrogen, attraction of a raphidophyte to phosphate, and attraction of diatoms to sugars that can support heterotrophic growth. There are several newer reports of interest dealing with chemoattraction in dinoflagellates. Cancellieri et al. (2001) reported that zoospores of the potentially ichthyotoxic dinoflagellates *Pfiesteria piscicida* and *P. shumwayae* can be attracted by sterile-filtered mucus and excreta from several species of fish that are potential prey for the dinoflagellates. The attraction was strongest in zoospores of the most actively toxic dinoflagellate strains, absent in nontoxic strains, and intermediate in strains

with reduced levels of toxicity caused by an extended absence of fish cues. In a mathematical model of the accumulation of another harmful algal bloom forming dinoflagellate, *Karenia brevis*, Janowitz and Kamykowski (2006) reported that chemotaxis to nutrients was very important in allowing frontal zone accumulations of cells during the simulations. Onshore of accumulations, chemotaxis to surface nutrients (flowing out from coastal bays) along with phototaxis significantly aided upward migration while offshore of the front, chemotaxis to upwelled nutrients from the bottom aided downward migration. Although chemotaxis to nutrients has usually been assumed to be of importance only on small spatial scales such as within benthic biofilms (e.g., Amsler et al. 1992; Amsler and Iken 2001), this model indicates that chemotaxis could also play a role in much larger scale patterns of algal distribution, even in the open ocean.

Although perhaps not an attraction to nutrients in a strict sense, symbiotic dinoflagellates (zooxanthellae) have been known for some time to be attracted to host invertebrates (which ultimately provide them with nutrients), presumably via chemical cues from the hosts (Kinzie 1974; Fitt 1984). These cues may include ammonia and nitrate released from the host (Fitt 1984). Recently Pasternak et al. (2004) demonstrated chemoattraction by *Symbiodinium* sp. to cell-free homogenates of juvenile soft coral polyps, which did not previously have symbiotic zooxanthellae, but not to adult polyps, which did already have symbiotic algae. The attraction was subsequently shown to be a true chemotactic response with an additional chemokinetic effect of the algae swimming slower in the presence of host chemical cues (Pasternak et al. 2006).

In macroalgae, spores of the kelps *Macrocystis pyrifera*, *Pterygophora californica*, and *Laminaria japonica* are attracted to or repelled from a number of macro- and micronutrients (Amsler and Neushul 1989; Fukuhara et al. 2002) as has been reviewed in some detail by Amsler and Iken (2001) and Amsler and Fairhead (2006). In general, spores are attracted to nutrients but, as is best documented with *M. pyrifera*, they are repelled from higher concentrations of some nutrients at concentrations that inhibit growth or gametogenesis of the gametophytic filaments that develop from spores after germination even though lower, stimulatory concentrations of these nutrients attract the spores. In contrast, spores of the small, filamentous brown alga *Ectocarpus siliculosus* display no chemotactic or chemokinetic responses to nutrient mixtures (Amsler et al. 1999).

14.4 Sensory Ecology of *Ulva* Spores

Spores of green algae in the genus *Ulva* have been very important models for studies of macroalgal spore biology and biofouling, particularly species with tubular thalli that until recently were separated into the genus *Enteromorpha*. The spores and gametes have long been known to be phototactic (e.g., Christie and Evans 1962; Woodhead and Moss 1975) and to respond to microtopographical features of surfaces during settlement (e.g., Christie 1973; Callow et al. 2002; Carman et al. 2006).

They are also known to respond to chemical and physicochemical cues. *Ulva compressa* spores are stimulated to settle on surfaces coated with several fatty acids, in particular myristic acid (Callow and Callow 1998). This is almost certainly because of chemoattraction of the spores by the fatty acids (Callow and Callow 1998) and is probably a chemotactic response (M. Callow, personal communication).

With respect to physicochemical cues, *Ulva* spores display marked settlement responses to variations in surface wettability (hydrophobicity). Recent studies in this area have utilized self-assembled monolayers (SAMs), which allow investigators to construct chemically defined surfaces varying only in the specific functional groups that are exposed at the surfaces, and have demonstrated that *Ulva linza* spores settle preferentially on more hydrophobic surfaces (Callow et al. 2000; Finlay et al. 2002; Ista et al. 2004). However, this is not simply a response to wettability alone since the response differed between surfaces of the same numerical wettability value, which were constructed with different functional groups (Ista et al. 2004). When presented with alternating sectors of hydrophobic and hydrophilic surfaces, swimming spores were observed in greater densities over the hydrophobic areas, suggesting that the motile spores are able to sense the physicochemical nature of the underlying surface (Callow et al. 2000). Gregarious settlement (preferential settlement of spores next to already settled spores) was also increased on hydrophobic surfaces (Callow et al. 2000) although if diffusible chemical cues from settled spores promotes gregarious settlement as has been postulated by Callow et al. (1997), this might not be a direct effect of the surface chemistry. Surprisingly, even though gregarious settlement increases resistance of spores to dislodgement, overall *U. linza* spores adhere more strongly to SAMs of decreasing hydrophobicity (Finlay et al. 2002). Although settling preferentially on surfaces that provide the weakest attachment appears quite maladaptive, in nature an algal spore would virtually always be settling on a biofilm-coated surface (Callow and Callow 2006) rather than a clean surface such as those presented by SAMs. Presumably, in the microenvironments within a biofilm, selective settlement on relatively hydrophobic surfaces is adaptive, but this remains to be demonstrated experimentally.

Not only do macroalgae normally settle within biofilms, but it has been known for over 20 years that the biofilm bacteria can either stimulate or inhibit the settlement of *Ulva* spp. spores (e.g., Thomas and Allsopp 1983; Hölmstrom et al. 1996; Patel et al. 2003). In one of the most exciting recent advances in algal chemical ecology, Joint et al. (2002) demonstrated that *Ulva* sp. spores are stimulated to settle by diffusible cell-to-cell signaling molecules used by biofilm bacteria for quorum sensing. Such signal molecules are utilized by bacteria to coordinate gene expression in a cell density dependent manner (Chhabra et al. 2005). *Ulva* sp. spores sense *N*-acylhomoserine lactone (AHL) produced by the bacterium *Vibrio anguillarum* and settle at higher rates in its presence (Joint et al. 2002). The effect was also seen in *Escherichia coli* strains producing recombinant AHL but not in mutant *V. anguillarum* or *E. coli* strains which do not produce AHL. Tait et al. (2005) confirmed these observations and demonstrated that the settlement stimulation was also reduced in wild type *V. anguillarum* transformed to produce a lactonase enzyme, which is known to degrade AHLs in vivo. These authors also demonstrated that the

increased settlement within the AHL-producing biofilms is not random but rather that the *Ulva* spores settle preferentially directly on top of the bacteria producing AHL. This was not observed in biofilms of non-AHL-producing mutant bacteria and did not appear to be the result of the surface microtopography resulting from the settled bacteria (Tait et al. 2005). Modified AHLs with a variety of side chain lengths and substitutions were effective in stimulating spore settlement so long as the side chain was more than four carbons in length (Joint et al. 2002; Tait et al. 2005).

Wheeler et al. (2006) examined the behavior of *Ulva* spores in gradients of AHLs and found that the spores accumulate around an AHL source using a unique chemokinetic response. Spores that are in close contact to a surface markedly decrease their swimming speeds the closer they get to an AHL source, thereby accumulating in the vicinity of the source. This effect was not seen in spores swimming somewhat above and out of contact with a surface, demonstrating a thigmotactic component to the behavior. As the authors point out, this is likely important since slowing swimming when not in contact with the bottom could increase the chances that a spore would be resuspended out of the benthic boundary layer and, therefore, loses its chance to settle. The response when in contact with surfaces is not simply a slowing because of the settlement process. It was observed across the range of individual swimming speeds and in spores clearly not in the early phases of settlement. In addition, the chemokinetic effects of AHLs did not interfere with spore phototaxis. Existence of a "true" chemotactic response (i.e., directed swimming oriented to the AHL source) as a component of the observed response was examined and shown to be lacking (Wheeler et al. 2006).

The ecological relevance of *Ulva* spp. spore attraction to AHLs in particular or to biofilms formed by particular bacterial species in general remains unknown. It has been known for many years that normal morphogenesis in species of *Ulva* and some other similar green algae is dependent upon factors derived from bacteria (e.g., Fries 1975; Provasoli and Pinter 1980) and Matsuo et al. (2005) have recently identified a specific bacterial metabolite responsible. Consequently, an obvious possibility is that the spores are attracted to bacteria that produce substances required for normal morphogenesis. However, Marshall et al. (2006) tested this hypothesis by isolating 20 different bacterial strains from three species of *Ulva* and found that although some strains promoted normal morphogenesis and/or faster growth of germlings and some strains stimulated spore settlement, there was no correlation between the two effects. Understanding the ecological relevance of bacterially-stimulated algal spore behaviors remains an important goal for future research.

14.5 Chemical and Physicochemical Modulation of Spore Settlement in Brown Algae

Spores of the kelps *Macrocystis pyrifera* and *Pterygophora californica* are stimulated to settle more rapidly by the presence of nutrient mixtures but the behavior is only observed five or more hours after they are released (Amsler and Neushul 1990).

No such effect was observed in spores of the filamentous brown alga *Ectocarpus siliculosus* but the assays could only be done with newly released spores so it is possible that this behavior could develop in spores that have been swimming for longer periods (Amsler et al. 1999). In *M. pyrifera*, individual nutrients that were previously observed to chemotactically attract spores (Amsler and Neushul 1989) also stimulated settlement but there were several exceptions (Amsler and Neushul 1990). Phosphate, which did not elicit chemotaxis stimulated settlement while cobalt, which was an effective chemoattractant, did not effect settlement. Along with the fact that chemoattraction by nutrients is observable in younger spores (Amsler and Neushul 1989), this indicates that chemoattraction and chemical stimulation of settlement are mechanistically distinct behaviors. Nevertheless, both behaviors probably increase the likelihood that a spore will settle in a nutrient microenvironment suitable for growth and development of the gametophyte stage that the spores germinate into. They also differ in that chemotaxis could be either positive or negative while only settlement stimulation was observed. Both ammonium and iron are stimulatory for gametophytic growth or reproduction at low concentrations but inhibitory at higher concentrations and both of these are chemoattractants at low concentrations and chemorepellants at high concentrations (Amsler and Neushul 1989). But in settlement experiments, both ammonium and iron stimulated settlement at low and high concentrations (Amsler and Neushul 1990). It is possible that this difference is because spores in a chemotaxis assay are presented with a gradient of the chemical while in the settlement experiments, the compounds were either present at a uniform concentration or absent.

Even though growth inhibitory concentrations of nutrients did not decrease kelp spore settlement (Amsler and Neushul 1990), body wall extracts from sympatric echinoderms that decrease germination could decrease spore settlement in the filamentous brown alga *Hincksia irregularis* although in many cases settlement rates were significantly higher than controls, particularly at low concentrations of the extracts (Greer et al. 2003). Germination inhibitory compounds can be detected at even lower concentrations utilizing computer-assisted analysis of spore swimming because they cause spores to swim slower and more erratically (Iken et al. 2001, 2003) and this method can be used to track compounds responsible through bioassay-guided isolation procedures (Greer et al. 2006). In settlement bioassays there were marked differences between the effects of the echinoderm extracts related to the physicochemical nature of the settlement surface (hydrophobic, negatively charged, or positively charged because of differences in exposed functional groups). This could have been caused by physicochemical interactions between the compounds and the surfaces, functional interactions between separate effects of the compounds and surfaces on the spores, or a combination of both (Greer et al. 2006). Both *H. irregularis* and *Ectocarpus siliculosus* respond to physicochemical cues and settle at significantly higher rates on hydrophobic surfaces compared with positively or negatively charged hydrophilic surfaces (Amsler et al. 1999; Greer and Amsler 2002, 2004; Greer et al. 2003, 2006). Light, and particularly the presence of light–dark boundaries, also effect spore settlement in *H. irregularis* and the physicochemical nature of the surface (hydrophobic vs. negatively charged hydrophilic) influences the settlement patterns observed in response to those

boundaries (Greer and Amsler 2002). This indicates that the spores are integrating their behavioral responses and have the capacity for relatively complex responses to environmental variables. Furthermore, the settlement responses to combined light and physicochemical environments varies between different clones of *H. irregularis* with minor differences between clones from the same population in North Carolina and with marked differences between those and a clone from a geographically distant population in the northern Gulf of Mexico (Greer and Amsler 2004). Recent genetic analysis of the clones has indicated that all have identical nucleotide sequences for the nuclear-encoded ITS region but that the Gulf of Mexico clone has a significantly divergent sequence for the chloroplast-encoded rbcL gene compared with those from North Carolina (M.O. Amsler, unpublished). Regardless of the taxonomic implications of those data, as with the responses of *Ulva* spores described in the preceding section, the ecological relevance of these complex behavioral responses in brown algal spores is an important topic for future investigations.

Acknowledgements I am grateful to M. Callow, M. Amsler, P. Bucolo, and two anonymous reviewers for constructive comments on earlier versions of the manuscript, to M. Callow for coordinating the anonymous review of this chapter, and to M. Amsler for brown algal genetic analyses. Manuscript preparation and the genetic analyses were supported in part by NSF award OPP-0442769.

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