29 Anthropogenic Introductions of Microalgae

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29.1 Potential Transport Vectors for Microalgae

The geographic range of microalgal species can expand depending on natural factors (climate change, catastrophic storm events, ocean currents, transport of spores via wind or bird feet) or human-mediated vectors. The present chapter focuses on anthropogenic transport by ships' ballast water, and translocation of aquaculture products such as shellfish. Other potential vectors such as fouling on ships' hulls, and transport via dredging or aquaculture equipment have not been adequately investigated to date with respect to microalgae. The potential impact from escape of aquaculture microalgal feedstocks also remains to be assessed. The global transport of microalgal species via ships' ballast water commenced in the 1870s (Carlton 1985). Annual transport on a worldwide basis is now at an estimated volume of 2–3 billion t, and has received considerable attention in the past two decades (reviewed by Hallegraeff 1998). Not surprisingly, the very first claim, made 100 years ago, of cargo vessel ballast water as a vector in the dispersal of non-indigenous marine organisms refers to a microalga (Ostenfeld 1908). To prove that a particular species of microalga has been introduced, however, is much more complex than, for example, for macroalgae (Wyatt and Carlton 2002). Because of the apparent continuity of the world's oceans, similar hydrological environments in different oceans tend to have morphologically similar phytoplankton assemblages ("latitudinal cosmopolitanism") and some scientists suggest that marine protists have had ample evolutionary time to reach and inhabit all suitable environments (Finlay 2002). While this may be true for oceanic diatoms and dinoflagellates, or for some widespread ecologically tolerant coastal diatoms, other species such as estuarine dinoflagellates can have fastidious nutritional requirements, e.g., with regard to humic substances from land runoff. For cyst-producing estuarine dinoflagellates that cannot cross oceanic boundaries by ocean currents, there is every reason to believe that transport in ballast water has played a role in the apparent global spreading of selected species. If the dogma of widespread cosmopolitanism of microalgae

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is rejected in favor of largely underestimated microalgal diversity (Sarno et al. 2005), human-mediated translocations are likely to have been seriously underestimated (McCarthy and Crowder 2000). Once successfully established in a new water body, especially when the introduced microalga produces sexual resting stages, it cannot be eradicated. Impacts on shellfish and finfish aquaculture operations may result in cases of toxin-producing microalgae, while broader environmental impacts from microalgal invasions causing altered food webs have not yet been assessed. In this context, we therefore treat the introduction of all non-indigenous microalgae, whether or not toxic, as potentially harmful to the environment.

29.2 Vector Surveys for Microalgae

The first direct examinations of ballast water at the end of a voyage were not made until the 1970s (Medcof 1975, cited in Hallegraeff 1998). Whereas initial ballast tank surveys mostly produced species lists from preserved samples (e.g., http://www.ku.lt/nemo/EuroAquaInvaders.html; including 250 diatoms, 126 dinoflagellates), later studies progressed to culturing and genetic analyses of ballast water algae. In extensive Australian ballast water surveys, 80 % of ships contained up to 30 culturable diatom species (including *Coscinodiscus, Odontella* as well as several potentially toxic *Pseudo-nitzschia* (Forbes and Hallegraeff 2001). Viable cultures of Paralytic Shellfish Poisoning producing dinoflagellates (*Alexandrium catenella, A. tamarense*) were produced from ballast water entering Australia from Japan and Korea (Hallegraeff and Bolch 1992; 5 % of ships), as well as from ships entering British ports (*Alexandrium minutum, A. catenella /tamarense*; Hamer et al. 2001; 17 % of samples). In one case, a single ballast tank was estimated to contain as many as 300 million viable *A. tamarense* cysts (Hallegraeff and Bolch 1992), amply demonstrating the significance of ballast water as a vector in increasing the geographic range of toxic species. Both commercial ships as well as recreational boats have been implicated in the dispersal of the brown tide *Aureococcus anophagefferens* into the North American Lakes (Doblin et al. 2004b). Viable transport of dinoflagellate cells and cysts of *Pfiesteria piscicida, P. shumwayae, Karenia brevis, K. mikimotoi, Alexandrium monilatum, A. tamarense* and *Prorocentrum minimum*, after passage though the digestive tract of shellfish, have also been demonstrated (Scarratt et al. 1993; Shumway et al. 2004). This latter vector can introduce unwanted harmful microalgae directly into sensitive aquaculture areas. The contribution of shellfish translocation to the spread of the dinoflagellate *Heterocapsa circularisquama* in western Japan has been discussed by Imada et al. (2001).

29.3 Evidence for Successful Establishment of Non-Indigenous Microalgae

29.3.1 Absence in Historic Samples

The diatom *Biddulphia* (now *Odontella*) *sinensis*, well known from the tropical and subtropical coasts of the Indo-Pacific, was not reported in European waters until 1903, when it produced dense plankton blooms in the North Sea. Since it was unlikely that this large diatom could have been overlooked previously, and impossible that it could have been carried by currents from distant oceans, Ostenfeld (1908) suggested that this species was introduced by ship. This diatom species was subsequently confirmed by Gollash et al. (2000) from ballast water in a vessel traveling from Singapore to Germany. Whereas the apparent introduction of the diatom *O. sinensis* was without harmful effects, the arrival of the diatom *Coscinodiscus wailesii* (either via ballast or with Japanese oysters) in 1977 in the North Sea for a brief period caused problems to fisheries by clogging fishing nets with extensive mucus (Boalch and Harbour 1977). A range of other microalgae which only recently have bloomed in well-studied Northern European waters (e.g., *Karenia mikimotoi* =*Gyrodinium aureolum* since 1966; *Fibrocapsa japonica* since 1992) do not preserve satisfactorily and their historic absence therefore cannot be confidently verified in this way.

29.3.2 Sediment Cyst Cores

Analyses of dinoflagellate cysts (*Gymnodinium catenatum*) in radionuclide-dated sediment cores in Tasmania, Australia, demonstrated its appearance around 1973 (first plankton blooms in 1980) coinciding with the start-up of a woodchip mill, which initiated the introduction of international ballast water (McMinn et al. 1999). In contrast, sediment cores from the Atlantic coast of Portugal (first PSP outbreak in Spain in 1976) indicated the presence of *G. catenatum* cysts since the beginning of the 20th century (Amorim et al. 2004) and cores from New Zealand (first PSP outbreak in 2000) demonstrated its presence since at least the 1930s (Irwin et al. 2003). Unfortunately, the mucoid cysts of *Alexandrium* dinoflagellates do not routinely preserve in deep sediment cores, with the exception of the study of Mudie et al. (2002) who succeeded in tracking historical *Alexandrium* cyst peaks in eastern Canada back to 13000 B.P.

29.3.3 Increasing Molecular Evidence

Molecular tools increasingly offer the potential to detect non-indigenous microalgal strains and in some cases even track donor source populations (Scholin et al. 1995). A prime example is the detection in the Mediterranean ports of Sete and Barcelona of *Alexandrium catenella* with a temperate Asian ribotype not found anywhere else in Europe (Lilly et al. 2002; Vila et al. 2001; Fig. 29.1). Evidence of introduced ribotypes hiding among indigenous strains is also emerging for Australian *Alexandrium* (De Salas et al. 2001) and North Sea *Fibrocapsa* raphidophytes (Kooistra et al. 2001). The potentially ichthyotoxic dinoflagellate *Pfiesteria piscicida* has been confirmed by molecular probes in ballast water entering US and Australian ports (Doblin et al. 2004a; Parke and Hallegraeff unpubl.), and the brown tide *Aureococcus anophagefferens* was detected in the bilge water of small recreational boats (Doblin et al. 2004b). Genome exchange between resident and invader strains has the potential to generate genetic diversity and possibly even hybrid vigour (Fig. 29.2).

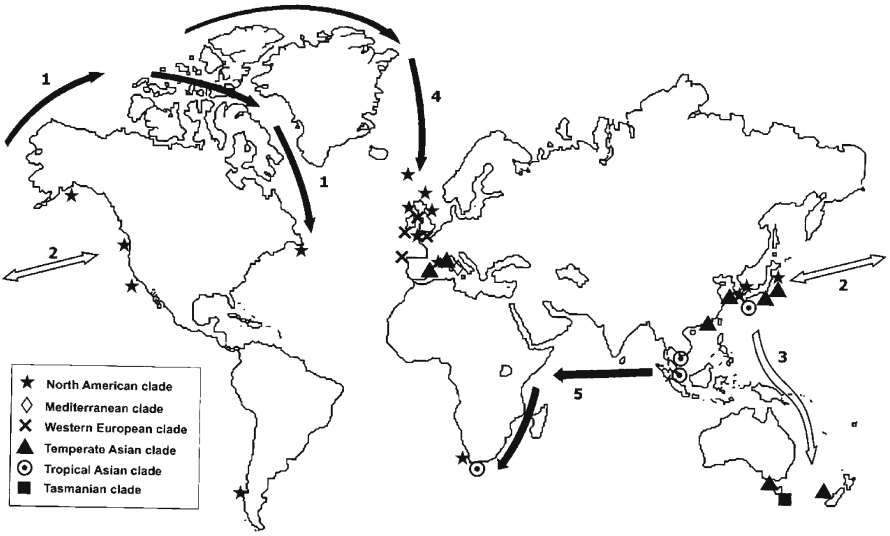

Fig. 29.1. Molecular biogeography of LSU ribotypes in the *Alexandrium tamarense/catenella* dinoflagellate species complex. *Black arrows* indicate natural dispersal, while *clear arrows* suggest human-assisted dispersal. The appearance of temperate Asian ribotype in 1983 in the Mediterranean can only be explained by human-assisted introduction. (After Scholin et al. 1995; Sebastian et al. 2005)

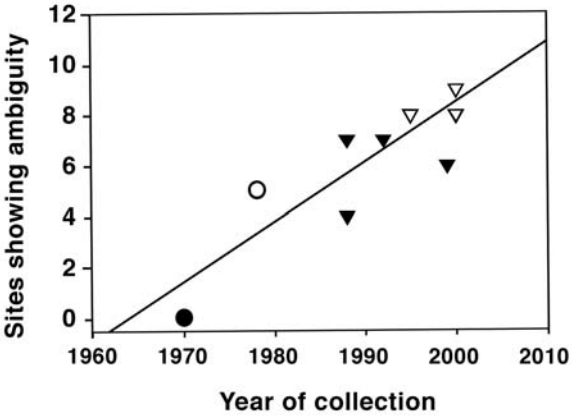

Fig. 29.2. Evidence for a temporal increase of genetic diversity (ambiguities at ten positions in the ITS region) of global cultures of the raphidophyte *Fibrocapsa japonica* (from Kooistra et al. 2001)

29.4 Management Options to Reduce Risk of Introductions

With the growing awareness of the problem of introduced marine pests, in the past two decades a number of national and international regulations have been developed to reduce the risk of transfer of non-indigenous organisms. These include an ICES Code of Practice on the Introductions and Transfer of Marine Organisms (latest version 2003; see www.ices.dk). As an example of an application, during the 2000 *Gymnodinium catenatum* bloom in northern New Zealand, a ban was instigated on the transfer of contaminated shellfish spat (L. MacKenzie, pers. comm.). On a national front, both Canada and Australia introduced mandatory ballast water guidelines in 2001, and the US Coast Guard implemented mandatory ballast water management in 2004. This culminated in the adoption in 2004 of the IMO International Convention for the Control and Management of Ships' Ballast Water and Sediments, which prescribes strict performance standards for ballast water exchange (>95 % volumetric exchange) and treatment processes (discharge shall not exceed ten viable organisms per m^3 >50 µm minimum dimension; 10 viable organisms per mL <50 µm and >10 µm minimum dimension; and a microbial standard). The far-reaching implications of these IMO regulations (see www.imo.org) for global shipping will be explored below.

29.4.1 Warning System for HABs in Ballast-Water-Uptake Zones

Prevention is better than to cure. The most-effective measure to prevent ship uptake of harmful algal blooms would be to avoid taking on ballast water during known harmful bloom events in the world's ports (IMO resolution A. 868(20)). This has been applied e.g., during the 1988 *Chrysochromulina*

polylepis bloom in Norwegian coastal waters (K. Tangen, pers. comm.), and the 1993 *Karenia mikimotoi* blooms in New Zealand. Precautionary procedures need also be developed when taking on ballast water in shallow areas with known sediment cyst beds of harmful species.

29.4.2 Ballast Water Exchange Studies on Phytoplankton

The IMO Convention prescribes exchange of ballast water in open oceans more than 200 (or if not possible 50) nautical miles from the shore. Mid-ocean exchange of ballast water is believed to be currently the most reliable method in order to minimize the risk of transfer of unwanted organisms on existing vessels. Compared with coastal waters, deep-ocean waters generally contain fewer organisms, and species occurring in open ocean waters are generally not able to survive in coastal zones and vice versa. Analyses of differences in colored dissolved organic matter (CDOM) between coastal and open-ocean waters are being explored as a method of verification of mid-ocean ballast water exchange (Murphy et al. 2004). The efficiency of removal of organisms as distinct from the original ballast water is a complex issue, which will be affected by the nature and behavior of organisms in the tanks, the design of tanks, mixing within the tanks and the types and behavior of sediments. Another problem is the possible retention of dinoflagellate cysts in ballast-tank sediments. In a study of 32 vessels, which claimed to have exchanged ballast water in mid-ocean, 14 were still found to contain significant amounts of sediment, including dinoflagellate cysts (Hallegraeff and Bolch 1992). There are currently three methods by which a mid-ocean exchange of ballast water may be achieved:

Empty/Refill (Reballasting)

This is usually only possible for smaller ships such as woodchip vessels <40,000 t dead weight, which should be able to achieve a nearly 100 % water exchange. In examining the effectiveness of open ocean reballasting in reducing the number of diatoms and dinoflagellates in ballast water from container ships traveling from California to Hong Kong, Zhang and Dickman (1999) reported that the Master estimated that 95 to 99 % of the original water was removed, and their analyses showed that 87 % of the diatoms and harmful dinoflagellates had been removed. In a similar study (Dickman and Zhang 1999) for container ships traveling from Manzanillo, mid-ocean reballasting resulted in 48 % removal of diatoms and dinoflagellates (again with the Master estimating 95–99 % water replacement). The differences between the two studies were attributed to the different ship ages and varying efficiency of ballast water exchange systems.

Continuous Flow-through of Ballast Water (Ballast Exchange)

A continuous flow-through system allows continuous sea-to-sea circulation of ballast water while the ballast tanks remain filled, i.e., seawater is pumped into the ballast tanks while the tank is simultaneously overflowed from the top. This system does not impose excessive bending moments or shearing forces and minimizes stability problems. Trials have shown that three-times volumetric exchange of ballast water results in approximately 95 % removal of viable algal cells (Rigby and Hallegraeff 1994).

Dilution Method

The dilution method is a variation of the continuous flow-through technique. Continuous ballasting may be carried out from the top of the tanks via one pipe system and at the same time continuous deballasting occurs by a second pipe system at the bottom of the tank. In a trial on an oil carrier a water exchange efficiency of 90 % was achieved with a phytoplankton exchange of 96 %, while chlorophyll *a* exchange was estimated as 86 % (Villac et al. 2001).

Location of Ballast Water Exchange

The precise location of ballast water exchange needs to be carefully chosen. MacDonald and Davidson (1998) reported that during ballast water exchange in European seas the diversity of diatoms and dinoflagellates increased in 69 and 85 % of cases, and abundance increased in 31 and 85 % of cases, respectively. Forbes and Hallegraeff (2001) reported that 80 % of woodchip ships operating between Japan and Tasmania reballast in coastal waters off the Philippines and Papua New Guinea and bring into Tasmania a new viable tropical/cosmopolitan inoculum, mixed with remnants of old Japanese plankton. Monitoring of woodchip vessels, which claimed to have exchanged 100 %, indicated that 80 % of ships still contained up to 30 culturable diatom species (including potentially harmful *Pseudo-nitzschia*).

Is 95 % ballast water exchange of HAB species sufficient?

Even if it is assumed that the efficiency of removal of organisms in ocean exchange is the same as the water replacement efficiency, it is important to realize that large numbers of harmful organisms may still be present in the water discharged into the receiving port. This is especially true when ballasting occurs during an algal bloom. Some *Alexandrium* toxic plankton blooms reach cell densities of 10^5 cells/L, of which approximately 40 % can successfully produce cysts (i.e., resulting in 40,000 cysts/L). Assuming a cargo vessel taking on 60,000 t of ballast water in such bloom conditions, a single ship could theoretically carry up to $2–4x10^{12}$ cysts. This compares with an actual estimate of $3x10^6$ *Alexandrium* cysts contained in a 25,000 t woodchip carrier entering the Australian port of Eden after ballasting during a confirmed *Alexandrium* dinofla-

gellate bloom in the Japanese port of Muroran (Hallegraeff and Bolch 1992). In a strict sense a *single* viable dinoflagellate cyst would constitute a viable inoculum, but taking into account limited losses from cysts germinating under unfavorable water conditions, 100 to 1,000 cysts would pose an inoculum capable of attempting to colonize their new environment for many years. To prevent such a threshold of dinoflagellate cyst introduction would require a ballast water treatment efficacy better than 99.9 %. This exercise raises serious doubts about the value of ballast water exchange for this particular target species while favoring technologies, which can achieve 100 % efficacy.

29.4.3 Treatment Options

Filtration. Filtration of ballast water is one of the most environmentally sound, albeit expensive, methods available (Table 29.1). Recent investigations with screen sizes of 50 and 100 μm have achieved microalgal removal efficiencies of 90 % at a nominal flow rate of 340 m^3/h (Cangelosi 2002; Parsons and Harkins 2002). It is noted that the flow rates used in these pilot treatments are low compared to the flow requirements on board ship and the efficacy achieved is still lower than that possible with 95 % ballast water exchange. The effectiveness of filter systems can be increased by the use of an additional technique such as heat, biocides or UV as secondary treatment. Cyclonic sep-

Table 29.1. Indicative comparative ballast water treatment costs. Adopted from Taylor et al. (2002)

Treatment option	Costs per m^3 (US$)
Exchange of ocean water (no additional equipment). Costs are reduced by 50 % (empty/refill option) if gravity ballasting can be accomplished	0.01–0.02
Exchange of ocean water including capital costs associated with additional equipment (for safe or effective operation)	up to 0.18
Heating/flushing process	0.03
Using recycled process water	0.04
Heating/flushing process using Hi Tech system involving recirculation, higher temperatures and additional heat exchange equipment	0.05
Hydrocyclones	0.06–0.26
Continuous backflushing filtration (with high capital cost component)	0.07–0.19
UV irradiation	0.10–0.39
Chemical treatment (based on operating cost alone)	0.14–24.10
UV combined with hydrocyclone	0.16–0.58
UV combined with filtration	0.17–0.51
Land based treatment	0.20–8.31
Dedicated treatment ship	0.33
Use of fresh water	0.50–0.72

aration technologies have proved ineffective for removing planktonic organisms from ballast water because most plankton are negatively buoyant with densities too close to water for effective separation.

Heat Treatment. Temperatures of 35–38 °C for a period of 4–5 h effectively killed vegetative cells of most harmful microalgae. With many organisms, the temperature required will generally be lower for longer periods of heating. For example, 30–90 s exposure to temperatures above 40 °C were effective in killing cysts of the dinoflagellates *Gymnodinium catenatum* and *Alexandrium tamarense*, whereas temperatures as low as 35 to 38 °C were sufficient after 4 h of heating. These laboratory findings were confirmed in full-scale shipboard trials, where the ship's pipework was modified to enable waste heat from the main engine-cooling circuit to heat the water in one of the ballast tanks by flushing with the heated water which reached 37–38 °C (Rigby et al. 2004). Heating of ballast water also has the added advantage that organisms contained in sediments would be subjected to these temperatures, but this technology may not be appropriate for short (domestic) voyages or where heat losses to the ocean are high (for example where sea temperatures are low). A successful application of heat treatment was also undertaken to "clean" oyster spat from contaminating dinoflagellate cysts during the 2000 New Zealand *Gymnodinium catenatum* bloom.

Ultraviolet Irradiation. UV irradiation is commonly used for sterilizing large amounts of potable water or wastewater, and for water purification in aquaculture and fisheries. Experiments with phytoplankton showed that a short exposure at high irradiance was found to be more effective than long exposure at low irradiance. Hallegraeff et al. (1997) and Montani et al. (1995) cited in Hallegraeff 1998 demonstrated that germination of cysts of *Alexandrium*, *Gymnodinium*, *Protoperidinium*, *Scrippsiella* and *Gyrodinium* occurred after exposure to UV radiation, most likely because many cyst walls are impermeable to UV. Other problems with UV treatment include the possibility that some smaller organisms could pass the UV unit without any treatment in the shadow of larger organisms or suspended solids, the reduced penetration of UV irradiation in turbid ballast waters, and the recovery of the phytoplankton following exposure to UV irradiation.

Chemical and Biocidal Inactivation of Ballast Water. A large number of chemical disinfectants are commercially available that have been used successfully for many years in land-based potable and wastewater treatment applications. Biocides suggested for use with ballast water include hydrogen peroxide, chlorination (chlorine dioxide, hypochlorite), ozonation, oxygen deprivation using reducing agents (e.g., sulphur dioxide, sodium sulphite), coagulants, antifouling paints as ballast tank coatings, organic biocides (formaldehyde, glutaraldehyde, peracetic acid, vitamin K) and others. Hydrogen peroxide

(100–2,500 ppm) offers considerable potential as an environmentally friendly biocide even for toxic dinoflagellate cysts (Ichikawa et al. 1993; Hallegraeff et al. 1997, cited in Hallegraeff 1998). However, its high cost of application may render it unsuitable as a routine tool, and one would only consider its use in emergency situations with highly contaminated ships. It is expected that the costs of chemical biocides would be significantly reduced when mass production is undertaken to feed the global demand for ballast water treatment. Strict approval processes are currently worked out by IMO to safeguard against any potential side effects of the release of billions of tons of treated water into the environment.

29.5 Conclusions

The potential for transport of non-indigenous marine microalgae via ships' ballast water and by translocation of shellfish has been amply demonstrated. Molecular approaches are increasingly suggesting that global microalgal diversity has been underestimated, and as a result human-mediated translocations are likely to have been seriously underestimated. The broader environmental impacts from microalgal invasions causing altered food webs have not yet been assessed. The dogma of phytoplankton cosmopolitanism has led to false complacency, and more than 100 years after this environmental problem was first raised in the scientific literature, a general consensus has now been reached that not doing anything is no longer an option. Minimizing the risk of ballast water introductions by microalgae and their cysts represents a very significant scientific and technological challenge, which cannot yet be adequately achieved with best currently available technologies and will be high on the research and development agenda in the decade to come.

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