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REVIEW

Transport of toxic dinoflagellates via ships' ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies

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ABSTRACT: The results of 10 yr of Australian research efforts on transport of toxic dinoflagellate cysts via ships' ballast water are reviewed, supplemented with the conclusions of similar studies now underway in Europe, Israel, North America, Canada, Japan, China and New Zealand. Toxic dinoflagellates are probably the best studied model organism to assess the bioeconomic risks of ballast water introduction of nonindigenous marine pests. A plausible scenario for their successful introduction and establishment in Australian waters is: (1) ballast water intake during seasonal plankton blooms and to a lesser extent via resuspended cysts in sediments from Japanese or Korean ports; (2) survival as resistant resting cysts during the ballasting process, the voyage in a dark ballast tank, and subsequent ballast water discharge (inoculation); (3) successful germination of cysts, sustained growth and reproduction of plankton cells in an Australian port; and (4) further spreading via coastal currents or domestic shipping, culminating under suitable environmental conditions in harmful algal blooms impacting on aquacultural operations (causative organisms of paralytic shellfish poisoning). Until international agreement and acceptance of a fully effective, practicable, safe, economically viable and environmentally friendly ballast water treatment is achieved (mid-ocean ballast water exchange and heat treatment are the only options offering promise at present), an international warning network for algal blooms in ports appears to be an effective way to minimise risks. It is also recommended that aquaculture operations and marine parks should be sited well clear of the ballast water influence of shipping ports.

KEY WORDS: Ships' ballast water · Toxic dinoflagellate cysts · Ballast water treatment · Ballast water management

INTRODUCTION

My interest in the problem of transport of toxic dinoflagellates via ships' ballast water was raised by alarming observations of an apparent global increase in the frequency, intensity and geographic distribution of paralytic shellfish poisoning (PSP) (Anderson 1989, Smayda 1990, Hallegraeff 1993). This human illness (15% mortality) results from the consumption of shellfish products contaminated with alkaloid toxins from some 11 species of plankton dinoflagellates (Hallegraeff et al. 1995). These species include *Alexandrium catenella* (Whedon and Kofoid) Balech, *A. minutum* Halim, *A. tamarense* (Lebour) Balech, *Gymnodinium catenatum* Graham and *Pyrodinium bahamense* Plate var. *compressum* (Böhm) Steidinger, Tester et Taylor. While in a strict sense this is a completely natural phenomenon well known to native North American Indian tribes, until 1970 poisoning records were confined to temperate waters of Europe, North America and Japan (Hallegraeff 1993). However, by 1990 PSP outbreaks were documented throughout the Southern Hemisphere, including South Africa, Australia, New Zealand and Papua New Guinea and the Northern Hemisphere, including India, Thailand, Brunei, Sabah, and the Philippines (Fig. 1). Similarly, in the Australian

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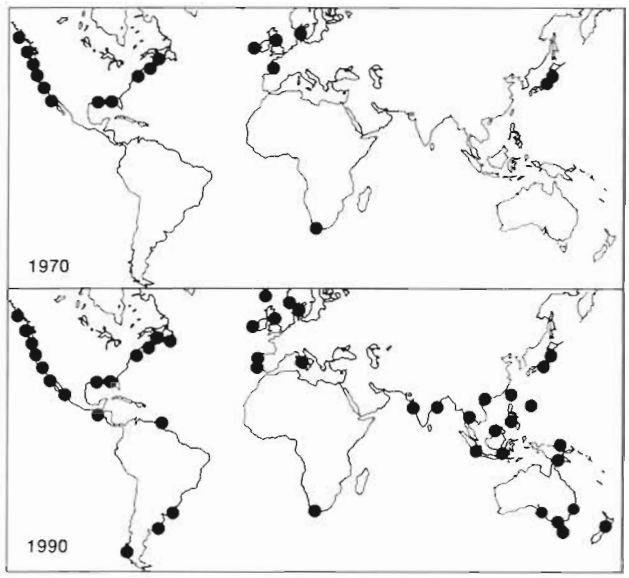

Fig. 1. Apparent global increase in the geographic distribution of paralytic shellfish poisoning (PSP) between 1970 (top) and 1990 (bottom) (after Hallegraeff 1993). Explanations for this increase include increased scientific awareness, stimulation of dinoflagellate blooms by coastal eutrophication, as well as translocation of species via ships' ballast water

region PSP was unknown until the late 1980s when the first toxic dinoflagellate blooms appeared in the ports of Hobart (caused by *G. catenatum*), Melbourne (*A. catenella*) and Adelaide (*A. minutum*) (Hallegraeff et al. 1988). Explanations for this apparent global increase include increased scientific awareness caused by the developing aquaculture industry and stimulation of dinoflagellate blooms by increased coastal eutrophication, but in a limited number of cases translocation of nonindigenous estuarine dinoflagellate species across oceanic boundaries (either via ships' ballast water or translocation of shellfish products) appears more probable (Hallegraeff & Bolch 1991).

The extent to which the extensive blending of coastal waters by the transport of seawater ballast has contributed to the apparent 'cosmopolitanism' of many coastal plankton taxa cannot yet be assessed. While unambiguous evidence for the presence of viable toxic dinoflagellate cysts in ships' ballast water (up to 300 million cysts per ballast tank; both *Alexandrium* and *Gymnodium catenatum*; Hallegraeff & Bolch 1992) as well as associated with shellfish stocks (Scarratt et al. 1993, Rhodes et al. 1994, Honjo et al. 1998) is now available, to prove that a particular dinoflagellate population is nonindigenous is extremely difficult. For *G. catenatum* in Tasmania such evidence has focused on an Australian-wide sediment survey for its distinct fossilisable resting cyst (Bolch & Hallegraeff 1990, McMinn et al. 1998). Fossil cyst records of this species are absent from the whole Australian region, recent cyst beds are confined to southeast Tasmania, and ^{210}Pb-dated sediment cores from the Hobart region unambiguously demonstrate its sudden appearance around 1972 coinciding with the commencement of bulk woodchip export from southern Tasmania via Japanese cargo vessels (Fig. 2). The precise origin of the Tasmanian dinoflagellate population is still unclear and is currently being traced by means of a global population study of toxin signatures (Oshima et al. 1993), sexual mating compatibility (Blackburn et al. 1989) and RAPD-PCR molecular markers (Adachi et al. 1997, Bolch et al. 1998). Early results indicate that *G. catenatum* populations which recently appeared also on the Australian mainland in Victoria (since 1992; Sonneman & Hill 1997), South Australia (1996; Bolch & Hallegraeff unpubl.) and New South Wales (Hallegraeff et al. 1998) are most closely related to the Tasmanian populations (Bolch et al. 1998), which may be the result of recent dispersal of Tasmanian *G. catenatum* via domestic ballast water or domestic translocation of shellfish products. Unfortunately, *Alexandrium* cysts lack resistant sporopollenin walls and hence do not leave a fossil record. In this latter case, the evidence has focused on a comparison of population-specific small subunit rRNA sequences which has

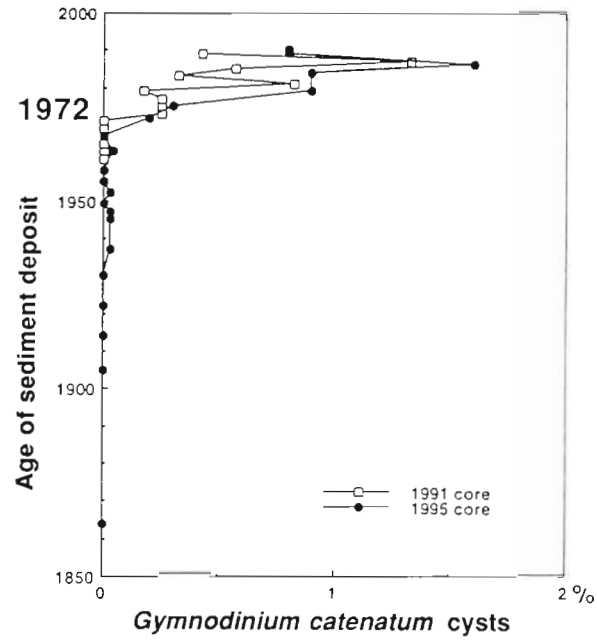

Fig. 2. Micropaleontological evidence for the introduction of the toxic dinoflagellate *Gymnodinium catenatum* into southern Tasmanian waters. Depth distribution of cysts (as % of total dinoflagellate cysts) in 2 undisturbed 40 cm deep sediment cores demonstrate the sudden appearance of this species around 1972 coincident with the opening of a woodchip mill. Sediment dating is based on ^{210}Pb and ^{137}Cs radionuclide analyses (from McMinn et al. 1998)

revealed a remarkable match between Japanese and Australian *A. catenella*, and between European and Australian *A. minutum* (Fig. 3; Scholin et al. 1995) while at the same time *A. catenella* and *A. minutum* strains from neighbouring New Zealand exhibit unique ribotype signatures (C. Scholin pers. comm.). The problem with such molecular evidence is that because of the slow rate of evolution of rDNA and our current inability to date the 'molecular clock', we cannot yet confidently distinguish whether matching molecular fingerprints are the results of thousands of years of natural dispersal along coastlines or anthropogenic translocations within the last 50 yr. The possibility that *Alexandrium* cells and cysts in sediment cores can leave diagnostic biochemical markers such as sterols is being explored as a further line of evidence (compare Hallegraeff et al. 1991).

In conclusion, while the scientific evidence on the magnitude of the problem of toxic dinoflagellate dispersal via ships' ballast water is not yet complete, the risk of ballast water introductions by this and a wide range of other marine organisms (Carlton 1985, Carlton & Geller 1993) has been amply demonstrated and the 'not doing anything' option is no longer acceptable. The Australian Government's position (Jones 1991) has been to classify ballast water as a quarantine problem and to attempt 'minimising quarantine risks' rather than to aim for the near impossible task of complete elimination of the risks from introduced species. In this context, cyst-producing toxic dinoflagellates have proved to be useful model-organisms, based on the premise that any monitoring strategy or ballast water treatment technique capable of dealing with them most likely would also eliminate most of the other target species. In Australia, other target species include the bacterium *Vibrio cholerae*, seaweed *Undaria pinnatifida*, starfish *Asterias amurensis*, crab *Carcinus maenas*, and fan worm *Sabella spallanzanii*. This review summarises 10 yr of Australian research efforts on toxic dinoflagellate cysts in ballast water, supplemented by similar studies now underway in Europe, Israel, North America, Canada, Japan, China and New Zealand.

SCENARIO OF SUCCESSFUL BALLAST WATER INTRODUCTION

The scenario for a successful ballast water introduction comprises up to 8 separate steps (Fig. 4), which for convenience of discussion will be grouped below into 4 broad categories:

(1) Ballast water intake during seasonal plankton blooms in a donor port. In Australia, 85% of ballast water imports (a total 120 million tonnes per year) derive from the Asian region, of which 54% originate in Japanese ports, with a further 34 million t of ballast water transported in association with coastal shipping around Australia (Kerr 1994). Global figures for ballast

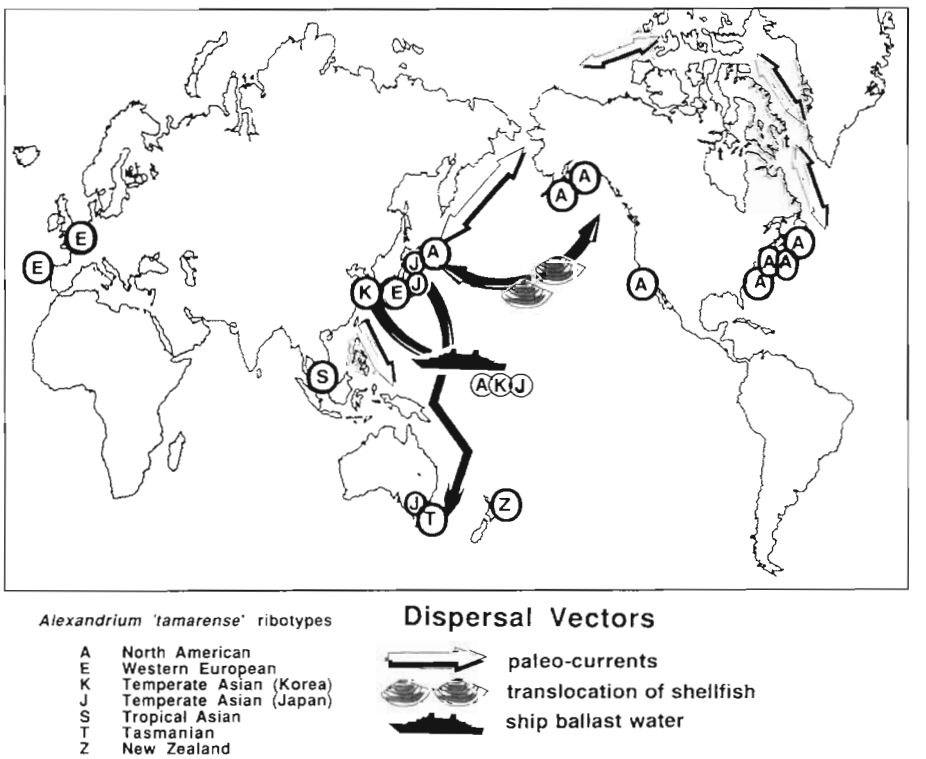

Fig. 3. Global map summarizing known distributions of different ribotypes of the toxic dinoflagellate species complex *Alexandrium tamarense*. Using SSU (small subunit) rDNA sequences, evidence could be obtained that Japan has been on the receiving end of introductions from Europe (E), the east and west coast of North America (A), as well as possessing its own indigenous temperate Asian populations (Japan, J; Korea, K). Some of these Japanese, Korean and American rDNA-types were gained from cultures germinated from ballast water transported from Japan/Korea to Australia. At the same time, indigenous tropical Asian (S), Tasmanian (T) and New Zealand (Z) ribotypes not known from any other region in the world also exist (modified after Scholin et al. 1995)

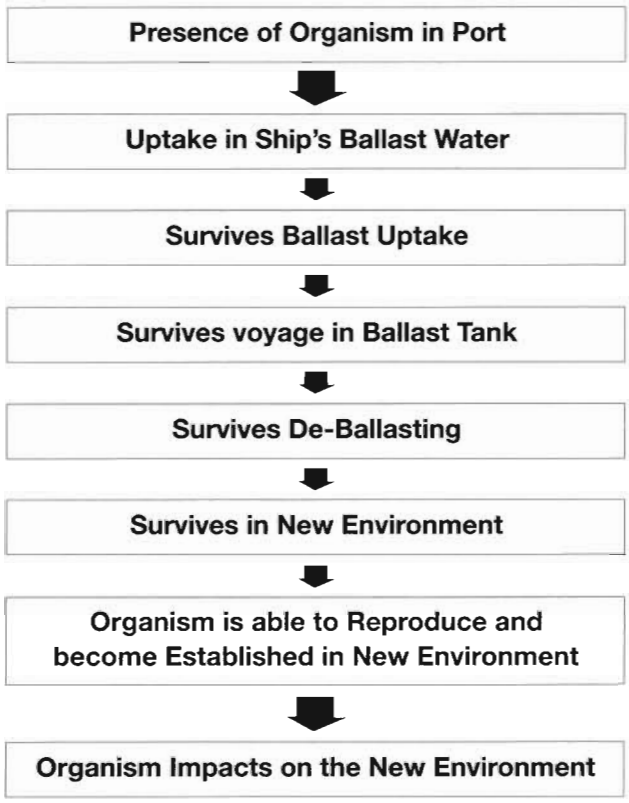

Fig. 4. Flow chart summarising the steps necessary for the successful introduction of a marine organism via ships' ballast water. Monitoring of ballast water would be most effective if carried out at the overseas port, before or during ballast water uptake. Ballast water treatment would be most effective if carried out while the ship is in transit

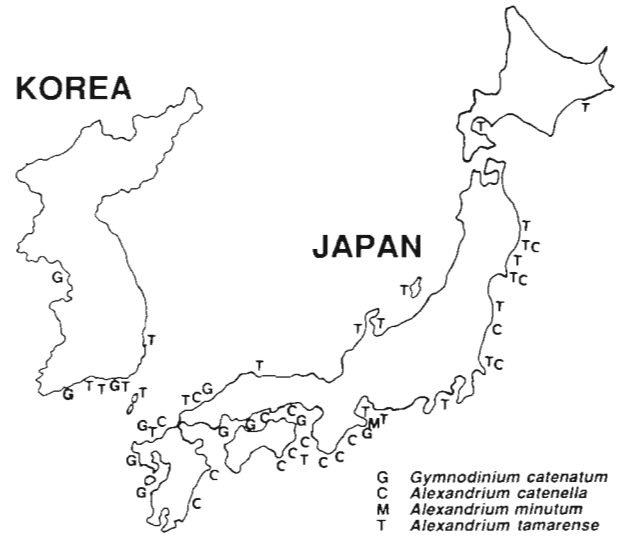

Fig. 5. Known distribution of PSP dinoflagellates in Japanese and Korean ports, which represent the major donor regions for Australian ballast water introductions (based on Fukuyo 1985, Matsuoka & Fukuyo 1994). An international warning network for algal blooms in ports appears to be an effective way to minimise risks

water transport are as high as 10 billion t yr^{-1} (Carlton & Geller 1993) Toxic PSP dinoflagellates occur widespread in Japanese coastal waters: *Alexandrium tamarense* is found mainly in Northern Japan, *A. catenella* is found mainly in Southern Japan, while *Gymnodinium catenatum* occurs in the Seto Inland Sea and Yatsushiro Sea (Fukuyo 1985, Matsuoka & Fukuyo 1994; Fig. 5). *A. tamarense* was first recognised in Korean waters in 1986 (Park 1991), and our identification of *G. catenatum* cysts in Korean ballast water samples represented a new record of this species for that region (Hallegraeff & Bolch 1992), which was subsequently confirmed by cyst surveys in Chinhae Bay (Kim et al. 1995). The probability of ballast water intake of toxic dinoflagellates is strongly dependent upon shipping patterns, seasonality of plankton blooms in ballasting port waters and the presence of local sediment cyst beds. In both Japanese and Korean coastal waters, toxic dinoflagellate blooms tend to occur mainly in early spring to summer (March to June) and again in fall (September to November) (Ogata et al. 1982, Fukuyo 1985). However, bloom events vary considerably in magnitude from year to year, dependent upon water temperature and rainfall. In a survey of 343 cargo vessels, Hallegraeff & Bolch (1992) found that 65% of ships contained ballast tank sediments. Of the sediment-containing samples, 50% contained dinoflagellate cysts and 5% contained toxic dinoflagellate cysts. Significantly, of the 80 known extant dinoflagellate cyst species, 53 taxa were detected in ballast tank samples and 20 species were successfully germinated to produce viable cultures (Hallegraeff & Bolch 1992). Toxic dinoflagellate cysts of *A. catenella*, *A. tamarense* and *G. catenatum* were positively identified in these Australian ballast water surveys. Similarly, Macdonald & Davidson (1997) detected *Alexandrium* cysts (including *A. minutum* and *A. tamarense*) in 17% of ships arriving in Scottish ports after short voyages through regional European seas. Tropical *Pyrodinium bahamense* dinoflagellate cysts have not yet been detected in ballast water samples, but the vegetative cells of this species have been found in New Zealand ballast water monitoring (Hay et al. 1997). Of further concern is the possible ballast water dispersal of cyst stages of the potent toxic dinoflagellate *Pfiesteria piscicida* Steidinger et Burkholder (Burkholder et al. 1998), only known thus far from North Carolina and the Chesapeake Bay areas of North America. The seasonality of ships arriving in Australian ports testing positive for toxic dinoflagellate cysts closely reflects the seasonality of overseas plankton blooms (Fig. 6). Cyst dormancy requirements indicate that most ballast water cysts derive from plankton blooms in the water column

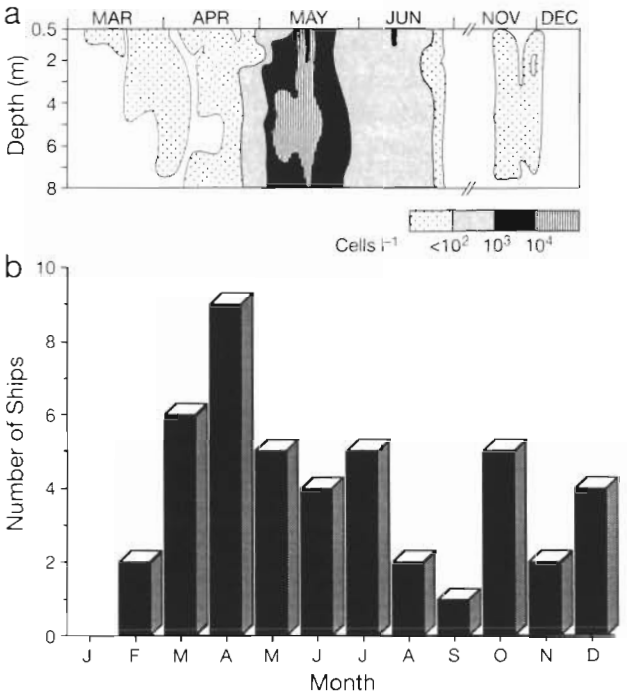

Fig. 6. (a) The seasonal occurrence of toxic dinoflagellate blooms (*Alexandrium*) in Japanese coastal waters (after Ogata et al. 1982) is reflected in (b) the seasonal occurrence of toxic dinoflagellate cysts in ship ballast water samples, intercepted at 18 different Australian ports during 1987 to 1995 (based on data from Hallegraeff & Bolch 1992 and Australian Government Analytical Laboratories (AGAL) unpubl. data)

(estimated 90% of ships). Some cysts failed to germinate until about 6 mo later, suggesting that they were newly formed cysts undergoing a mandatory dormancy period (Anderson 1980), rather than mature cysts resuspended from harbour sediments. Oshima et al. (1992) traced a toxic dinoflagellate bloom from Muroran in Japan (40 000 cells l^{-1}; July 1989) to Eden in Australia, by matching up toxin fingerprints of Japanese dinoflagellate plankton, of ballast water cysts and of the germinated dinoflagellate cultures. In a limited number of cases, ships originating from, for example, Kure in Japan were found to carry toxic dinoflagellate cysts throughout the year which suggests that resuspended sediment cysts can be an additional source for contaminated ballast water (estimated 10% of ships) or that material entrained in ballast tanks can be retained for several voyages.

(2) Survival as resistant resting cysts during the ballasting process, the voyage in a dark ballast tank, and subsequent ballast water discharge. Examination of ballast water samples from ships arriving in 18 Australian ports (Hallegraeff & Bolch 1992) as well as routine ballast water inspections made en-route during 3 voyages on the 'Iron Whyalla' (Rigby & Hallegraeff 1994, Rigby et al. 1997) have shown that motile, photosynthetic dinoflagellate cells usually do not survive long voyages in ballast tanks where massive phytoplankton mortalities are incurred 1 to 3 d after ballasting. Many dinoflagellate cells are sensitive, first to the turbulence imposed during ballast pumping (Thomas & Gibson 1990), followed by unfavourable conditions in ballast tanks of darkness, changing temperature and nutrient conditions, as well as increased vulnerability to zooplankton grazing. The only motile dinoflagellate species that tend to survive are members of mixotrophic and heterotrophic genera such as *Protoperidinium*, *Phalacroma* and *Diplopsalopsis* which primarily feed on detritus (see also Galil & Hülsman 1997). Such on-board or end-of-voyage phytoplankton ballast tank observations are now available for ships travelling between Japan and Australia (Rigby & Hallegraeff 1994, Yoshida et al. 1996), Japan and North America (Kelly 1993), Japan and Canada (Rigby & Hallegraeff 1994, Yoshida et al. 1996) and voyages within Europe (Macdonald & Davidson 1998). Unfortunately, numerous phytoplankton ballast tank surveys exclusively utilised preserved samples (e.g. Subba Rao et al. 1994, Zhang 1997) and hence were unable to determine whether species were being discharged in a viable condition. Overall, the major risk is posed by the resistant cyst stages (hypnozygotes) of dinoflagellates, such as *Alexandrium catenella*, *A. minutum*, *A. tamarense* and *Gymnodinium catenatum* (Fig. 7). No cyst mortality can be expected to result from the ballasting process itself, and during the voyage grazing by zooplankton will not affect survival as the persistent cysts survive passage through the animals' guts and can be excreted in a viable form within their fecal pellets (Scarratt et al. 1993). Mortality would occur, however, if the cysts were to germinate and find themselves in the wrong environmental conditions. *G. catenatum* cysts, once produced in the plankton and unless inhibited by anoxic conditions in sediments, will usually germinate within 2 wk after formation (Blackburn et al. 1989) and thus could suffer major mortality. In contrast, *Alexandrium* cysts, newly formed in the plankton, require a mandatory dormancy period of 1 up to 6 mo (Anderson 1980, Anderson et al. 1987) and hence are not exposed to this risk. Mature *Gymnodinium* or *Alexandrium* cysts, buried in harbour sediments where they are prevented from germination by anoxic conditions, would probably try to germinate when resuspended during ship ballast water intake. Rapid burial within ballast tank sediments would enhance their further survival during the voyage.

(3) Successful germination of cysts, sustained growth and reproduction of plankton cells in an Australian port. For toxic dinoflagellates, which tend to occur in comparatively high concentrations, the volume of ballast water transported is not necessarily the best risk

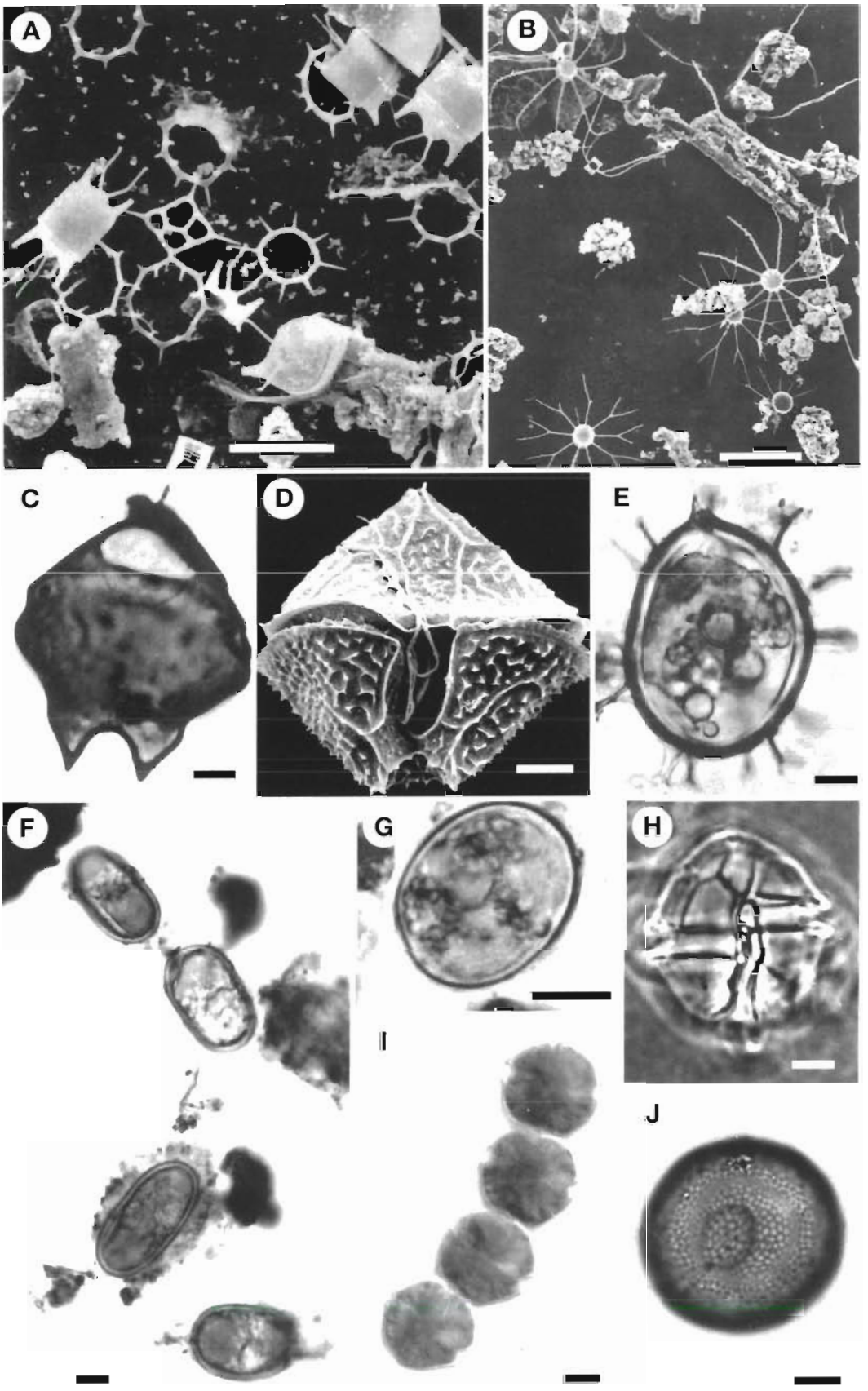
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Fig. 7. Representative micrographs of diatom plankton cells and dinoflagellate resting cysts from ballast water samples arriving in Australian ports. SEM = scanning electron micrograph; LM = light micrograph. (A) SEM. Diatom *Odontella aurita* and silicoflagellate *Dictyocha speculum* from a woodchip vessel arriving in Triabunna, Tasmania, from Yatsushiro, Japan. (B) SEM. Warm-water diatom *Bacteriastrum furcatum* from a woodchip vessel arriving in Triabunna from Yura, Japan. (C) LM. Dinoflagellate cyst of *Protoperidinium leonis* from a vessel arriving in Triabunna from Yatsushiro, Japan. (D) SEM. Ventral view of motile dinoflagellate cell germinated from the cyst shown in C. (E) LM. Live dinoflagellate cyst of *Gonyaulax digitale* showing cell contents, from a vessel arriving in Port Hedland from Pohang, Korea. (F) Live toxic dinoflagellate cysts of *Alexandrium tamarense* from a vessel arriving in Eden from Muroran, Japan. (G) LM. Live toxic dinoflagellate cyst of *A. catenella* from a vessel arriving in Port Hedland from Kashima, Japan. (H) LM. Motile toxic dinoflagellate cell of *A. tamarense* cultured from the cysts in F. (I) Motile toxic dinoflagellate chain of *A. catenella* cultured from the cyst in G. (J) round, brown toxic dinoflagellate cyst of *Gymnodinium catenatum* with microreticulate ornamentation and horse-shoe shaped apical groove; from a vessel arriving in Newcastle from Kohong, South Korea (after Hallegraeff & Bolch 1992). All scale bars = 10 µm

indicator. A viable inoculum could consist of as little as a single cell, and frequency of ships' visits (Fig. 8) therefore more adequately reflects the risk of repeated ballast water discharges during different seasons. During 1991, the Australian Port Hedland received 407 visits, Hay Point 403, Newcastle 500, Sydney 171 and Hobart 26 (Kerr 1994). Ballast water movements are most significant to the first 3 ports, but the climate match of ships entering the tropical ports of Port Hedland (17% match) and Hay Point (4%) is poor. In contrast, Newcastle (90% of ships coming from similar climates), Sydney (56%) and to lesser extent Hobart (85% match, but limited traffic) are exposed to more significant risks (Rigby & Hallegraeff 1996). Fig. 9a summarises Australian ports where toxic dinoflagellates have been detected in ship ballast water samples.

During and after the deballasting process in the Australian port, the cyst stages may be readily buried below the sediment surface from which they are gradually resuspended into the water column. These cysts may undergo recurrent germination attempts over the next 10 to 20 yr. When successful, this can result in dinoflagellate blooms in the Australian port. Once the organism produces new cyst stages, it will have effectively colonised a new water body from which it cannot be eradicated. Accepting the sediment cyst evidence that indicates that *G. catenatum* was introduced into Tasmania around 1972 (McMinn et al. 1998), it took another 8 yr for the first bloom events to develop in the new environment in 1980 (Hallegraeff et al. 1988). While the 'most damage has already been done' attitude is prevalent among many developed countries,

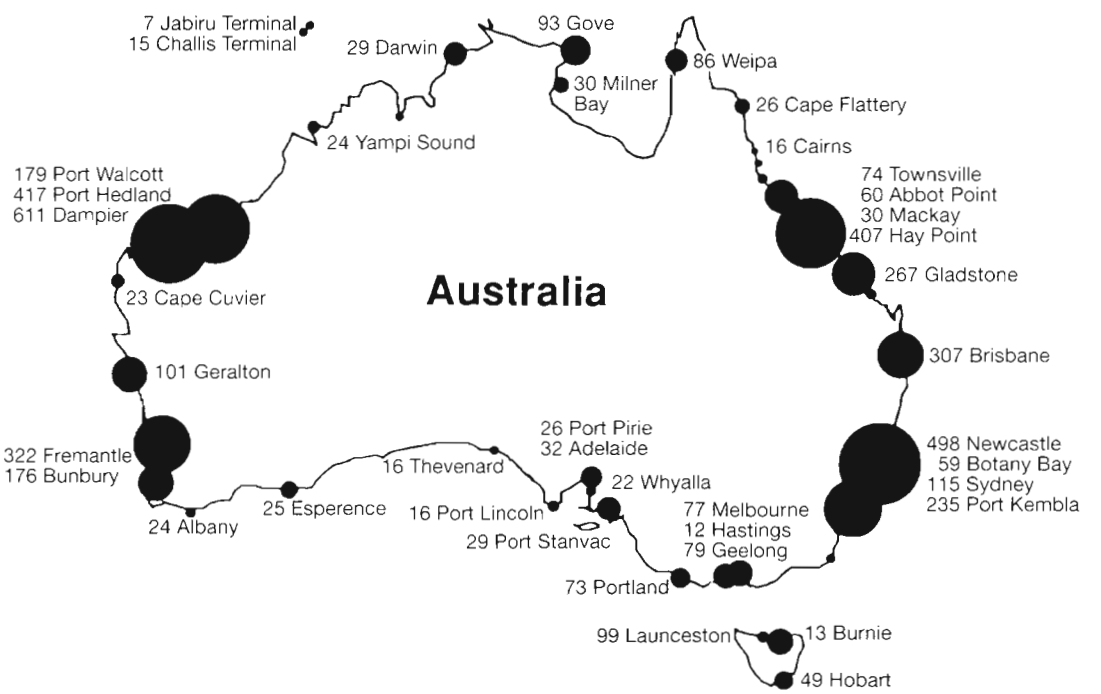

Fig. 8. Frequency of ship visits during 1991 to Australian ports receiving ballast water from bulk carriers (from Kerr 1994)

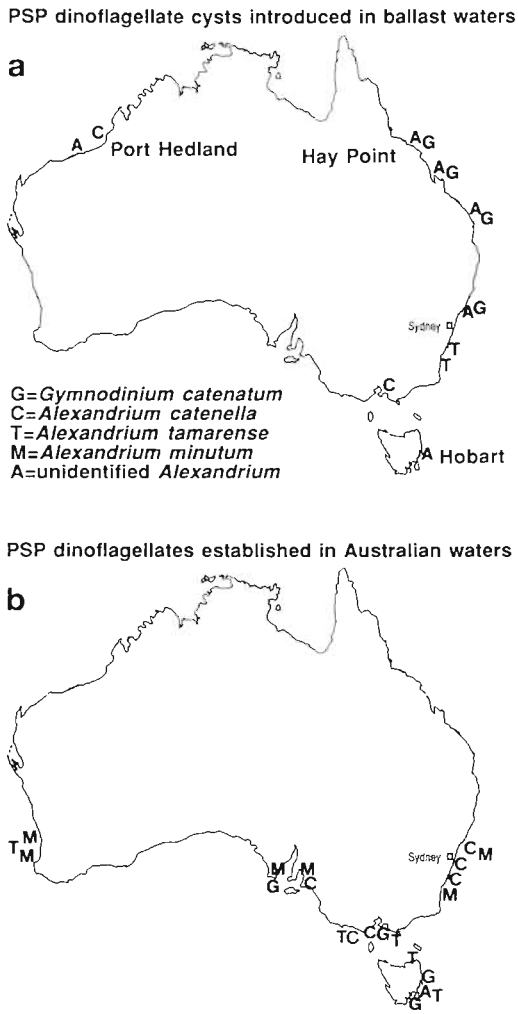

Fig. 9. Illustration of the importance of matching of donor port and receiving port environments. (a) Toxic dinoflagellate cysts detected in ballast water from ships entering Australian ports during 1987 to 1995 (based on Hallegraeff & Bolch 1992). (b) Australian ports where toxic dinoflagellate populations, both indigenous and introduced strains, have become established (based on Hallegraeff et al. 1988, 1991, McMinn et al. 1998, Hallegraeff & Bolch unpubl.)

evidence is accumulating that numerous damaging ballast water invasions (e.g. European zebra mussel into Laurentian Great Lakes; Japanese starfish and *Undaria pinnatifida* seaweed into Tasmania; American Atlantic coast comb jelly into the Black Sea) did not occur until the early 1980s. This suggests that the ballast water problem is increasing, in response to increasing global ballast water movements but also perhaps to environmental factors such as coastal pollution enhancing the invasibility of receiving ports (Carlton & Geller 1993, and references therein).

(4) Further spreading via coastal currents or domestic shipping, culminating under suitable environmental conditions in harmful algal blooms impacting on aquacultural operations. Estuarine dinoflagellates are sensitive to water temperature, to lesser extent salinity, and often show associations with river plumes and rainfall events (contributing micronutrients and/or chelating humic substances; Hallegraeff et al. 1995, Wells et al. 1991). The most meaningful approach to estimate the probability that introduced dinoflagellates can establish themselves in Australian ports would be to look at dinoflagellate cyst assemblages in various Australian ports with particular attention to the presence of species which in other parts of the world are associated with *Alexandrium* or *Gymnodinium catenatum*. A further approach would be to compare seasonal temperature and tidal current/water column stability regimes in Australian and overseas ports. Of interest is the absence of toxic dinoflagellate populations in the Australian Ports of Hay Point and Port Hedland (Fig. 9b), despite the fact that cyst species of *G. catenatum* and *Alexandrium* have been repeatedly detected in ballast water samples discharged in these areas. In contrast, the port of Hobart which has insignificant shipping traffic has a disproportionally high number of introduced species [dinoflagellate *G. catenatum*, but also seaweed *Undaria pinnatifida* (Sanderson 1990) and starfish *Asterias amurensis* (Byrne et al. 1997)]. This illustrates the importance of matching port environments in the successful establishment of introduced species. Domestic transport of ballast water obviously poses a much greater risk for translocation of organisms than international shipping. Domestic transport of viable *Alexandrium* and *Dinophysis* dinoflagellate populations has been documented by Gosselin et al. (1995) for short (<36 h) voyages in the Gulf of St Lawrence region. Macdonald & Davidson (1998) detected *Dinophysis* and *Pseudo-nitzschia* (toxigenic diatom genus) in 11 and 37% of ships, respectively, arriving in Scottish ports but their viability was not confirmed. Even though the diatom *Pseudo-nitzschia* does not produce resting spores, this organism is able to survive conditions of darkness in some form of physiological resting stage. Viable cultures of *Pseudo-nitzschia* species have been obtained from ballast tanks sampled at the end of domestic Australian voyages as well as international Japanese voyages (E. Forbes & G. Hallegraeff unpubl.). The toxigenic diatom species *P. multiseries* was positively identified from preserved ballast water arriving in Hong Kong (Zhang 1997).

BALLAST WATER MANAGEMENT

Until we achieve international acceptance of a fully effective, safe, practicable, financially viable and environmentally friendly ballast water treatment option, risk minimisation is the only option. A monitoring net-

work warning about the possible contamination of ships' ballast water appears to be an effective way to minimise risks. Monitoring could be carried out:

(1) At the overseas ballasting port, focusing on both the presence of toxic dinoflagellate cells and especially suspended cyst stages in port waters, at the depths from which ballast water intake occurs. In general, sampling marine organisms in overseas port waters is more effective than sampling ballast tanks from which it is extremely difficult to make representative collections. This applies especially to patchy zooplankton distributions in ballast tanks (Rigby et al. 1997).

(2) 'En-route' during the 10 to 20 d voyage, focusing on toxic dinoflagellate cysts in ships' ballast tanks. Dependent upon cargo loading patterns, different tanks may contain water from different ports or mixtures from more than one port. The results of this onboard monitoring therefore have implications for the management of ballast water in situations where only one port may be contaminated. The recent development of fluorescent staining techniques for *Alexandrium* cysts (primuline; Yamaguchi et al. 1995; not effective for *G. catenatum*, however) would enhance detection. The feasibility of developing a rapid diagnostic test, either based on immunological recognition of species-specific cell surface proteins or using DNA probes to detect species-specific DNA/RNA sequences inside the target cells, has been evaluated by Scholin et al. (1996).

Various ballast water treatment options are also best carried out at this stage, especially on international voyages while domestic voyages offer much reduced scope for ballast water treatment. In many instances, however, ships may not have to carry out any ballast water control or management options as there may be no or minimal risk of introducing harmful organisms. To make such decisions, Australia is currently developing a Decision Support System (DSS) consisting of a risk assessment model (Hayes 1997) into which vessel and voyage details can be entered and estimates of risk can be calculated. If a risk is identified, a 'tool box' of options is available. Treatment options that have been trialled on toxic dinoflagellate cysts include reballasting at sea (i.e. complete emptying and refilling of ballast tanks) or continuous flow-through ballast water exchange (flushing) (Rigby & Hallegraeff 1994), sterilisation with hydrogen peroxide (Ichikawa et al. 1992, Bolch & Hallegraeff 1993), heat treatment (Bolch & Hallegraeff 1993, Yoshida et al. 1995) and electric shock (Montani et al. 1995). A summary of the results obtained is provided in Table 1. Of these options, ballast water exchange (in recommended depths greater than 2000 m) is only partially effective, since it usually does not completely remove cyst-containing sediments

Table 1. Summary of treatment options capable of killing and/or removing toxic dinoflagellate cysts in ships' ballast water

	Treatment	Species	Source
Chemical			
Chlorine	500 ppm, 24 h	*Gymnodinium catenatum*	Bolch & Hallegraeff (1993)
Hydrogen peroxide	100 ppm, 96 h[a]	*Alexandrium catenella*	Ichikawa et al. (1993)
	150 ppm, 48 h[a]	*Alexandrium* sp.	Montani et al. (1995)
	2500 ppm, 24 h	*A. catenella*	Hallegraeff et al. (1997)
	2500–5000 ppm, 24 h	*G. catenatum*	Bolch & Hallegraeff (1993); Hallegraeff et al. (1997)
Physical			
Electric shock	100 V, 5 s	*Alexandrium* sp.	Montani et al. (1995)
	7.5 V/cm^2, 5 s		
	10% viability[b]	*G. catenatum*	Hallegraeff et al. (1997)
	5 V/cm^2, 5 s	*A. catenella*	Hallegraeff et al. (1997)
Heat	35–37.5°C, 1–2 h	*G. catenatum*	Hallegraeff et al. (1997)
	40–45°C, 30–90 s	*G. catenatum*	Bolch & Hallegraeff (1993)
	45°C, 3 min	*Alexandrium* sp.	Montani et al. (1995)
	38°C, 4.5 h	*A. catenella*	Hallegraeff et al. (1997)
Ballast exchange	42 h (3 tank exchanges = 90–95% reduction)	Natural plankton	Rigby & Hallegraeff (1994)
Reballasting	28 h (not 100% effective)	Natural plankton	Hallegraeff & Bolch (1992)

[a]The discrepancy between the lower hydrogen peroxide concentration needed to kill *Alexandrium* cysts in Japanese experiments (Ishikawa et al. 1993) compared to Australian experiments (Bolch & Hallegraeff 1993, Hallegraeff et al. 1997) has not yet been satisfactorily explained, but may be due to dinoflagellate strain or methodological differences (notably organic loading of seawater base used)

[b]The killing of cysts by electric shock has been demonstrated to be due to the generation of chlorine and possibly other oxidants, rather than an electrical effect (Hallegraeff et al. 1997)

that have accumulated on the bottom of ballast tanks (Hallegraeff & Bolch 1992). Currently, there exist no appropriate methods for monitoring the efficacy of ballast water exchange protocols and verification of compliance by the shipping industry largely relies on inspection of ship log books. Useful biological indicators in this respect are the incidence of coastal versus oceanic, and coldwater versus warm-water phytoplankton species (Fig. 7a, b), while the presence of abundant viable phytoplankton in ballast tanks tends to reflect recent ocean exchange (Hallegraeff & Bolch 1992, Hay et al. 1997, Macdonald & Davidson 1998).

Most forms of chemical treatment (e.g. chlorine) would lead to unacceptable environmental problems in the port waters in which they eventually would be discharged. Chemical treatment with hydrogen peroxide (5000 ppm required to kill *Alexandrium catenella* or *Gymnodium catenatum* cysts; Bolch & Hallegraeff 1993) which would degrade to innocuous water and oxygen has also been ruled out as a treatment option since it would be prohibitively expensive (A$ 500 000 per ship per trip; Rigby et al. 1993). Similarly, electric shock treatment, envisaged to be applied to ballast water outlets (Montani et al. 1995), has been discarded as a treatment option once it became clear that it is not electricity but local generation of heat and/or free chlorine that was responsible for mortality observed in laboratory experiments (Hallegraeff et al. 1997). Efforts to kill ballast water organisms by deoxygenation of ballast tanks (e.g. Mountfort 1997) would not kill dinoflagellate cysts nor many benthic marine invertebrates, while research to apply high speed microfiltration to ballast water (Cangelosi 1997) may not be successful for dinoflagellates or their cysts because of limitations imposed by the 50 and 100 µm screens used (trials with 20 µm screens suffer from rapid clogging). Ozonation and ultraviolet treatment have also proved unsuccessful in killing dinoflagellate cysts when these were contained in turbid ballast water with high organic loading (D. Oemcke pers. comm., J. Valentine unpubl.).

In contrast, heating of ballast water may provide an effective, environmentally friendly solution to the global problem of ballast water transport of unwanted marine organisms. If practicable, it would involve a once-only cost in terms of modifying ship engineering designs, would indiscriminately eliminate a wide range of marine organisms (without the need for monitoring of target organisms in ballast water), and would not pose any environmental hazards as associated with chemical treatment, nor create any ship's safety hazards as posed by mid-ocean ballast water exchange (Rigby et al. 1993). This approach to ballast water treatment is analogous to pasteurization of milk (30 min at 65°C) and other food products, in that it aims to kill certain unwanted pathogenic organisms but it is not synonymous with sterilisation (typically 15 min at 121°C) since not all organisms (i.e. bacterial endospores) can be expected to be killed. Crucial questions remain to be answered, however, as to the amount of heat that can be generated on board ship (Rigby & Taylor 1993). In laboratory studies, toxic dinoflagellates cysts could be killed after 2 h at 35°C (*Gymnodium catenatum*) or 4.5 h at 38°C (*Alexandrium catenella*) (Hallegraeff et al. 1997). While early studies (Bolch & Hallegraeff 1993) employed short exposures (30 to 90 s) to temperatures of 40 to 45°C, it has now become clear that longer exposures (hours to days) to temperatures of 36 to 38°C is more effective, especially for killing zooplankton (Rigby et al. 1997). A careful assessment of various waste heat sources on the BHP bulk carrier 'Iron Whyalla' has confirmed the practicability of this approach, and a pilot heat treatment plant was successfully trialled on-board ship in April 1997, achieving temperatures of 37 to 38.4°C after 24 to 30 h (Rigby et al. 1997). Early indications are that other ballast water target organisms, such as the starfish *Asterias amurensis* (bipinnaria larvae die at >28°C; Sutton & Bruce 1996), seaweed *Undaria pinnatifida* (gametophyte dies at >25°C, sporophyte >30°C; Sanderson 1990) and the mollusc *Dreissena polymorpha* (2 h at 33 to 36°C; Jenner & Janssen-Mommen 1993) can also be killed in this temperature range, but not, for example, the pathogenic bacterium *Vibrio cholerae* (McCarthy & Khambaty 1994). The precise physiological mechanism underlying the effectiveness of this comparatively mild heat treatment is not completely understood, but mortality most likely is due to a loss of cellular organisation rather than simple protein inactivation or disruption of membrane integrity (Brock & Madigan 1994). Concerns that such mild heating of ballast tank seawater could stimulate the growth of pathogenic bacteria such as *V. cholerae* have not been substantiated by simulated laboratory culture experiments (Desmarchelier & Wong 1996).

(3) Upon arrival at the receiving Australian port, and before commencement of deballasting. The routine monitoring of toxic dinoflagellate cysts in ships' ballast water and sediments is hampered by the lack of a sensitive, rapid diagnostic test which can be used by untrained personnel. Until we achieve international agreement on a suitable ballast water treatment option, an international warning network for algal blooms in overseas ports appears to be an effective way to minimise risks. It is also recommended that aquaculture operations and marine parks should be sited well clear from the ballast water influence of shipping ports. For too long, the shipping industry has had a virtually free ride on the world's oceans with grave environmental consequences for our precious

coastal environments. Bulk cargo shipping ought to be subject to strict regulations, analogous to the limitations that apply to coastal or aquaculture developments.

The increasing interest in the ballast water problem is reflected in the establishment in 1997 of a joint study group on Ballast Water and Sediments by the International Council for the Exploration of the Sea (ICES), the Intergovernmental Oceanographic Commission (IOC) of UNESCO, and the International Maritime Organisation (IMO). In 1995, Australia created a new Centre for Research on Introduced Marine Pests (CRIMP) under the auspices of the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Finally, the Australian Quarantine and Inspection Service (AQIS) currently chairs the Marine Environment Protection Committee (MEPC) of the International Maritime Organisation, in which Australia has proposed and argued for international regulations of ballast water control (partly based on voluntary guidelines introduced into Australia in February 1990) to be drafted as Annex VII to the MARPOL 73/78 Convention (International Convention for Preventing Pollution from Ships). IMO (resolution A.868) has now endorsed for such an Annex on mandatory ballast water guidelines to be implemented in the year 2000. The significance of this development is that ships arriving in any port of the signatory member countries would then need to demonstrate that they have done at least something to minimise the risk of ballast water transport of non-indigenous marine organisms, whether it be evidence of mid-ocean exchange, heat treatment or by presenting a certificate of freedom from harmful organisms in the port of origin. The scientific and political challenges to solve the global environmental problem of ballast water introductions compare with the magnitude of the problems faced when addressing global climate change. It is hoped that this ballast water review, focusing on toxic dinoflagellate cysts as a model organism, will contribute to clarifying the bio-economic risk factors involved and to identifying possible management strategies.

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