ICES Conference, Aalborg; Theme Session on Ballast Water; CM 1995/O:15

Transport of Toxic Dinoflagellates via Ships' Ballast Water: An interim review

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Abstract

Toxic dinoflagellates are probably the best studied model organism to assess the bioeconomic risks of ballast water introduction of nonindigenous marine pests into Australian waters. A plausible scenario for their successful introduction and establishment includes: (1) ballast water intake during seasonal plankton blooms in Japanese or Korean ports, and to lesser extent via resuspended cysts in sediments; (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; (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 we achieve international agreement and acceptance of a fully effective, safe, economically viable and environmentally friendly ballast water treatment option (heat treatment is actively being explored), 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 should be sited well clear from the ballast water influence of shipping ports.

The evidence

My interest in the problem of transport of toxic dinoflagellates in ship's ballast water was raised by alarming observations of an apparent global increase in the frequency, intensity and geographic distribution of paralytic shellfish poisoning (PSP) (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. While in a strict sense 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. By 1990, PSP was documented throughout the Southern Hemisphere, including South Africa, Australia, New Zealand, India,Thailand, Brunei, Sabah, the Philippines and Papua New Guinea (Fig.1). Similarly, in the Australian region PSP was unknown until the late 1980s when the first outbreaks appeared in the ports of Hobart (caused by *Gymnodinium catenatum*), Melbourne (*Alexandrium catenella*) and Adelaide (*Alexandrium 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 exotic estuarine dinoflagellate species across oceanic boundaries (either via ship's ballast water or translocation of

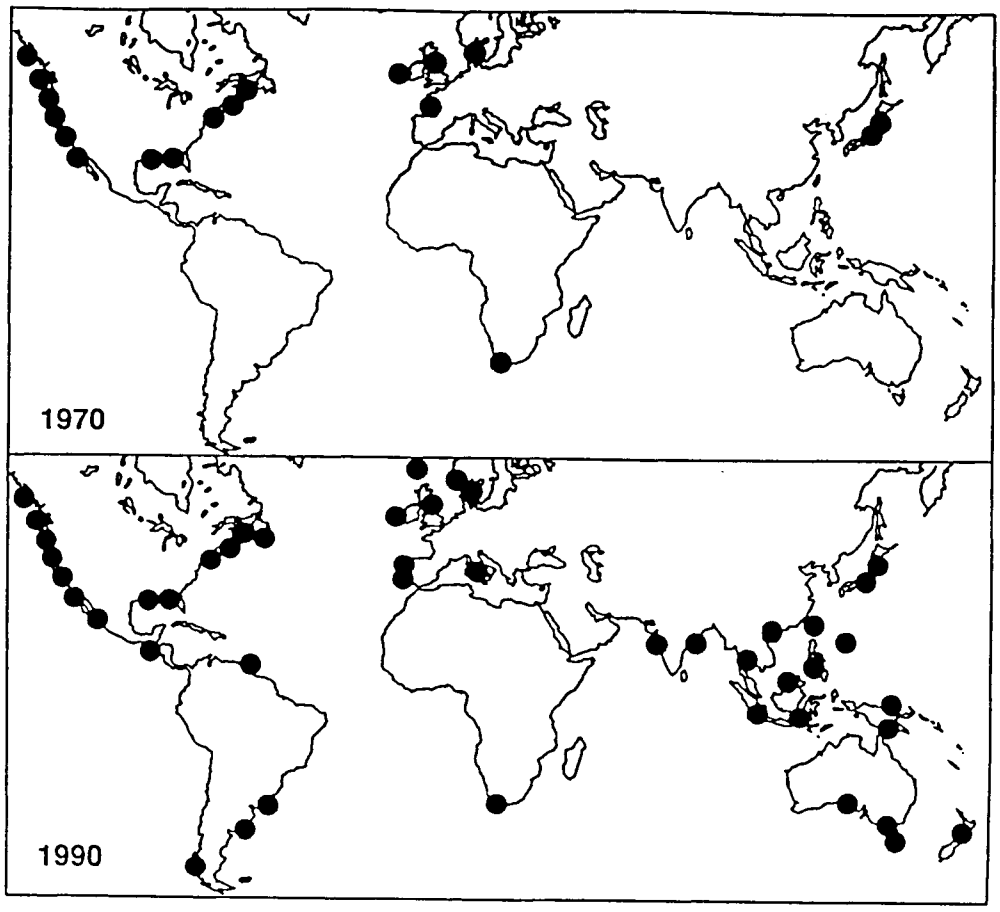

Fig.1. Known global distribution of paralytic shellfish poisoning (PSP) in 1970 and 1990 [From Hallegraeff 1993].

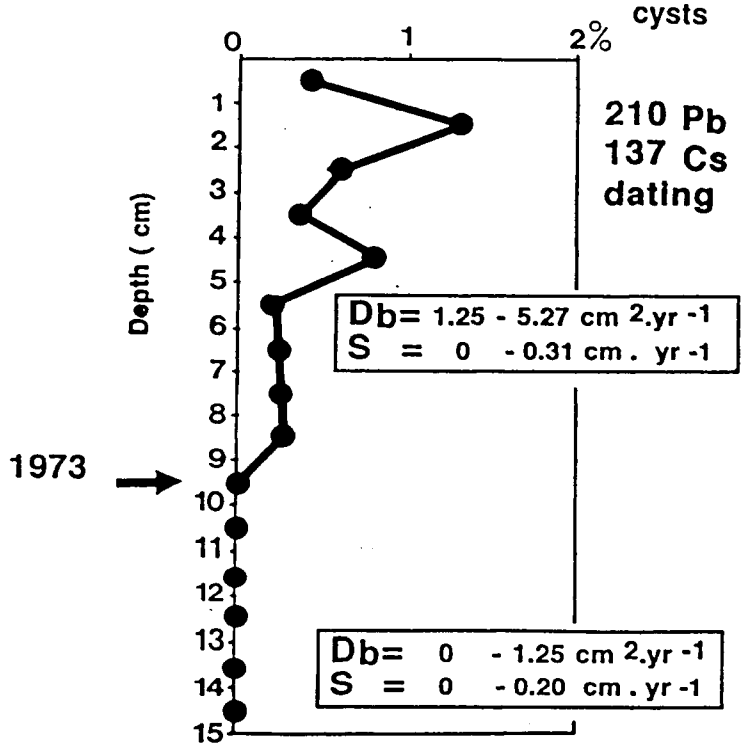

Fig.2. Depth distribution of *Gymnodinium catenatum* cysts in an undisturbed 40 cm deep sediment core from southern Tasmanian waters, showing absence of this species before 1973. Estimates of sediment bioturbation rates Db and sedimentation rates S are based on radionuclide dating using ^{210}Pb and ^{137}Cs [McMinn & Hallegraeff, unpublished data].

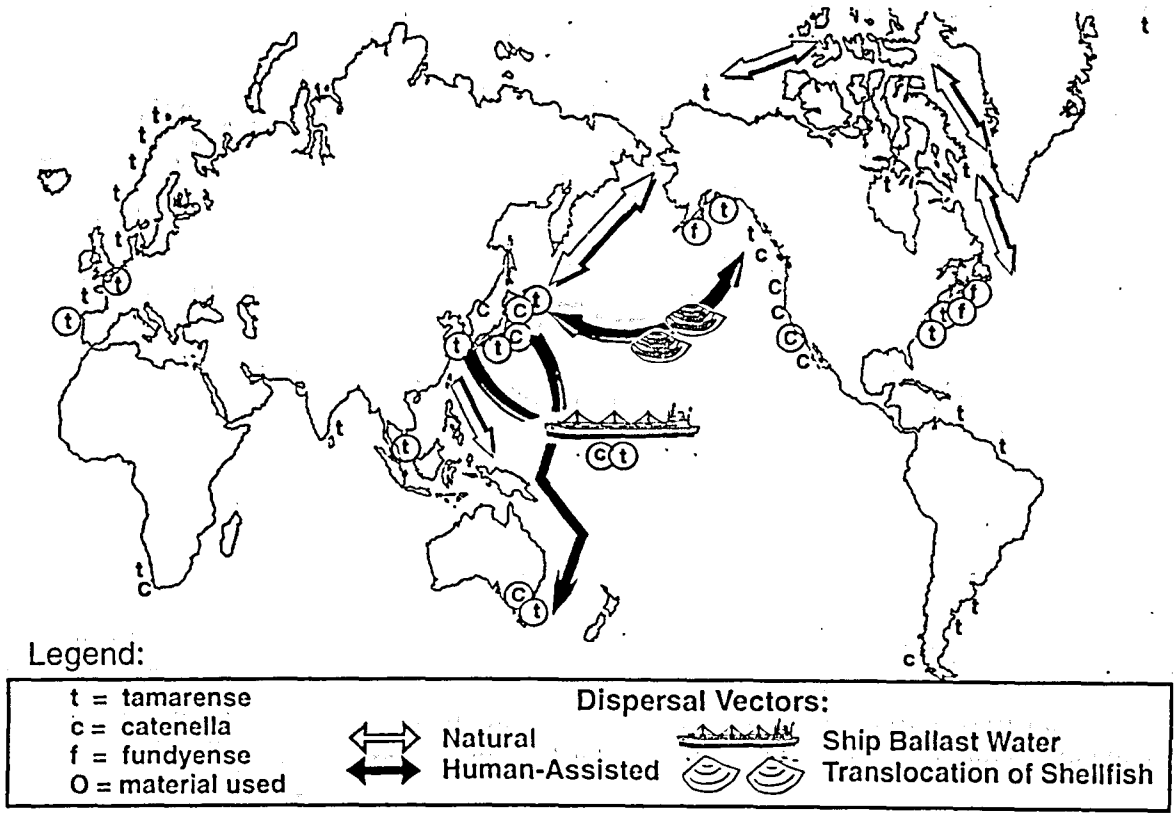

Fig.3. Global map summarizing known distributions of the closely related toxic dinoflagellates *A.tamarense* (t), *A.catenella* (c) and *A.fundyense* (f). Using SSU rDNA sequences, evidence could be obtained that Japan has been on the receiving end of introductions from Europe, the east and west coast of North America, as well as possessing its own indigenous Asian populations. Some of these Japanese molecular fingerprints were caught in the act of being transported via ballast water to Australia, where they have now established reproducing populations [From Scholin *et al.*,1995].

shellfish products) has to be invoked. While unambiguous evidence for the presence of viable toxic dinoflagellate cysts in ship's ballast water (up to 300 million cysts per ballast tank; both *Alexandrium* and *G. catenatum* ; Hallegraeff & Bolch 1992) as well as associated with shellfish stocks (Scarett *et al.*, 1993) is now available, to prove that a particular dinoflagellate population is nonindigenous is extremely difficult. For *Gymnodinium catenatum* in Tasmania such evidence has focused on an Australian -wide sediment survey for the distinct fossilisable resting cyst (Bolch & Hallegraeff 1990; McMinn 1991). Fossil cyst records of this species are absent from the Australian region, recent cyst beds are confined to south-east Tasmania, and Pb^{210} -dated sediment cores from the Hobart region unambiguously demonstrate its sudden appearance around 1973 (Fig.2; McMinn & Hallegraeff, unpublished). The precise origin of the Tasmanian 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 and microsatellite molecular markers (Bolch & Hallegraeff, in progress). Unfortunately, *Alexandrium* cysts lack resistant sporopollenin walls and hence do not leave a fossil record. In this case, the evidence has focused on elucidating population-specific small subunit rRNA sequences which showed a remarkable match between Japanese and Australian *A.catenella* , and between European and Australian *A. minutum* (Fig.3; Scholin *et al.* ,1995). 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 years.

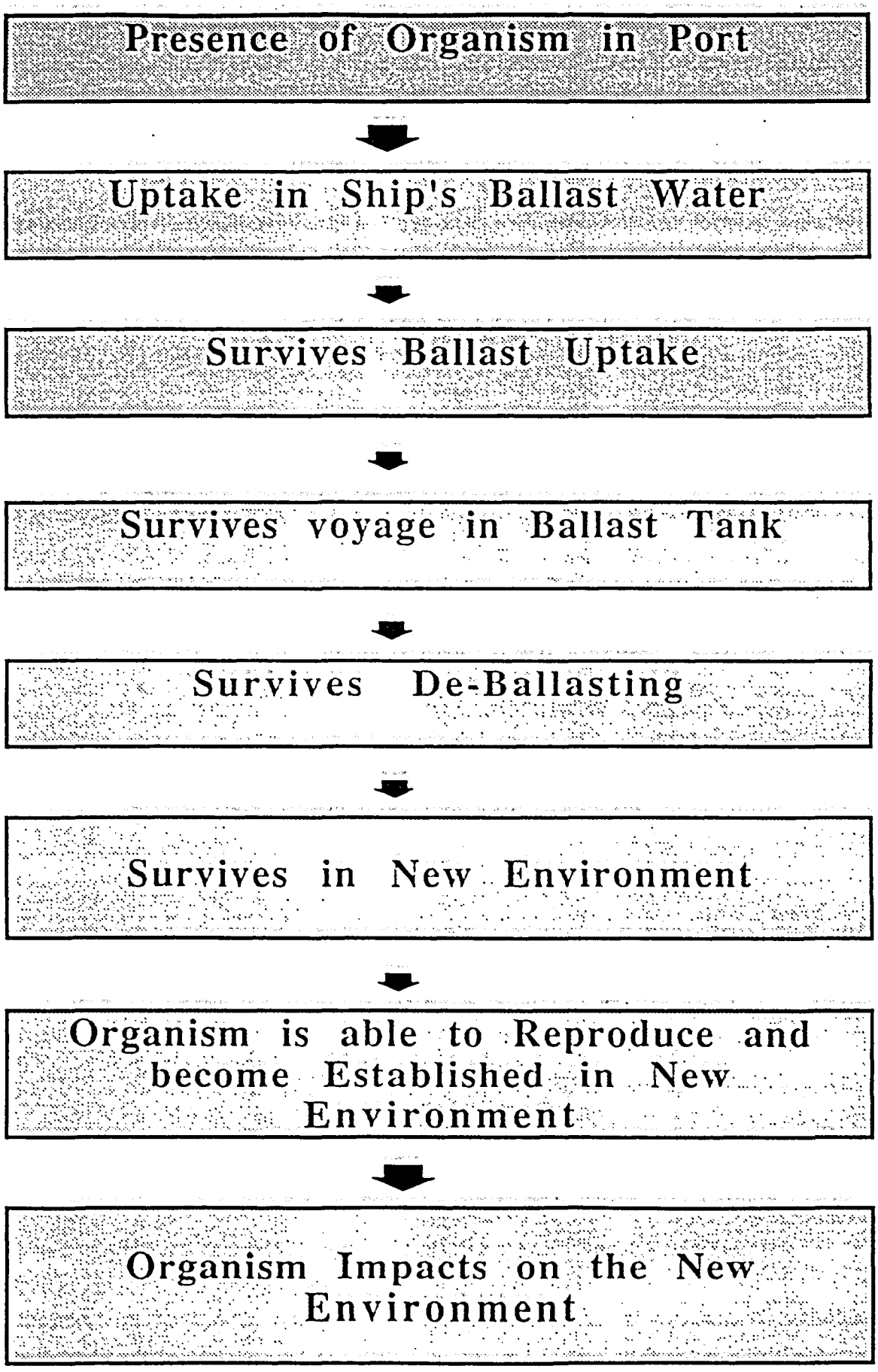

Fig.4. Flow chart summarising the steps necessary for the successful introduction of a marine organism via ship's ballast water. Monitoring of ballast water would be most effective if carried out at the overseas port, before or during ballast water uptake.

In conclusion, while it may still be too early to take the Australian PSP evidence to the "International High Court ", the risk of ballast water introductions has now been amply demonstrated and the "not doing anything" option is no longer acceptable. The Australian Government's position has been to attempt *reducing 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 appeared 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.

Scenario of successful ballast water introduction

There exist many hurdles for a toxic dinoflagellate to be succesfully transported via ship's ballast water (Fig.4):

(1) *ballast water intake during seasonal plankton blooms in a Japanese or Korean port.*

In Australia, 85% of ballast water imports (a total 120 millions tonnes per year) derive from the Asian region, of which 54% originate in Japanese ports, with a further 34 million tonnes of ballast water transported in association with coastal shipping around Australia (Kerr 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 and Fukuyo 1994). *A.tamarense* was first recognised in Korean waters in 1986 (Park, 1991), and our identification of *Gymnodinium catenatum* cysts in Korean ballast water samples represented a new record of this species for this region (Hallegraeff and Bolch 1992), which was subsequently confirmed by cyst surveys in Chinhae Bay.

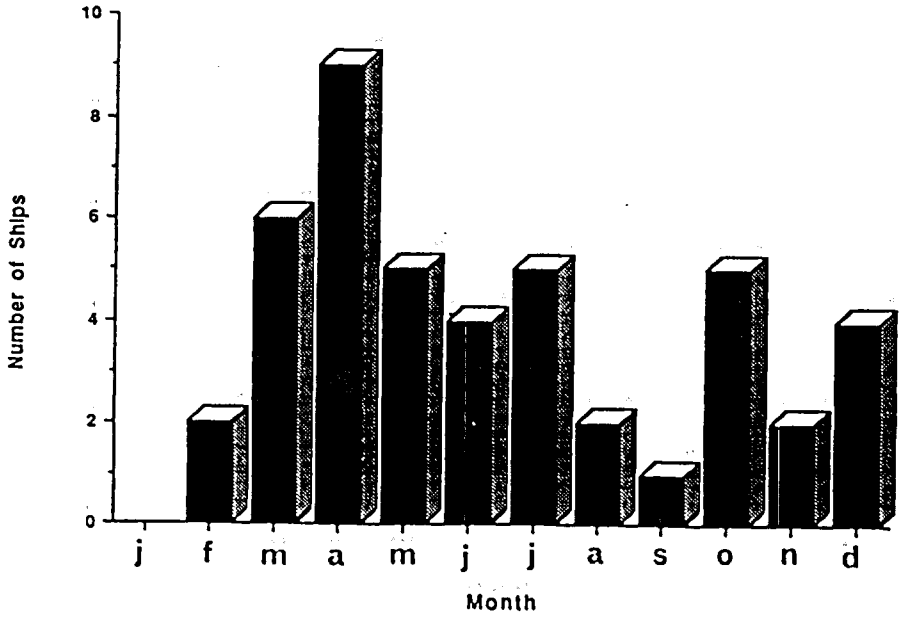

Fig.5. Seasonal occurrence of toxic dinoflagellate cysts in ship ballast water samples, intercepted at 18 different Australian ports during 1987 to 1995 [based on data from Hallegraeff & Bolch 1992 and Australian Government Analytical Laboratories (AGAL), unpublished data].

The probability of ballast water intake of toxic dinoflagellates is strongly dependent upon shipping patterns, seasonality of plankton blooms in overseas 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) . 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. The seasonality of ships testing positive for toxic dinoflagellate cysts closely reflects the seasonality of overseas plankton blooms (Fig.5) .Cyst dormancy requirements also indicate that most ballast water cysts are derived from plankton blooms in the water column (estimated 90% of cases) . Cysts often failed to germinate until about 6 months later, suggesting that they were newly formed cysts undergoing a mandatory dormancy period, 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 (300 million cysts in 25,000 t ballast water of a woodchip carrier) , 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 for example from 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 cases).

(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 and Bolch, 1992) as well as routine ballast water inspections made en -route during two voyages on the "Iron Whyalla" (Rigby and Hallegraeff, 1993) have shown that motile dinoflagellate cells usually do not survive long voyages in ballast tanks where they are exposed to darkness , high zooplankton grazing pressure and changing temperature and nutrient conditions. 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.*, 1995) and Japan and Canada / North America (Kelly 1993, Rigby & Hallegraeff 1994, Yoshida *et al.* 1995). The major risk is posed by the resistant cyst stages (hypnozygotes) of dinoflagellates, such as *Alexandrium catenella, A. minutum, A. tamarense* and *Gymnodinium catenatum* . 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 persistant cysts survive passage through the animals' guts and can be excreted in a viable form within their fecal pellets. Mortality would occur , however, if the cysts germinate and find themselves in the wrong environmental conditions. *Gymnodinium catenatum* cysts , formed in the plankton, will germinate within 2 wks 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 up to 6 months (Anderson 1980) 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 the anoxic ballast tank sediments would guarantee their further survival during the voyage .

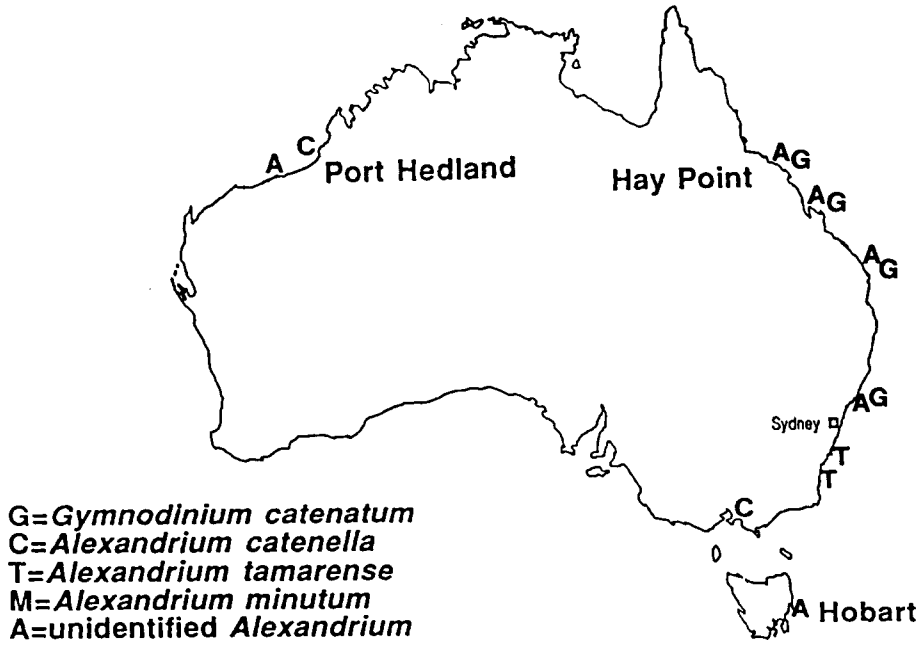

Fig.6. Toxic dinoflagellate cysts detected in ballast water from ships entering Australian ports during 1987 to 1995.

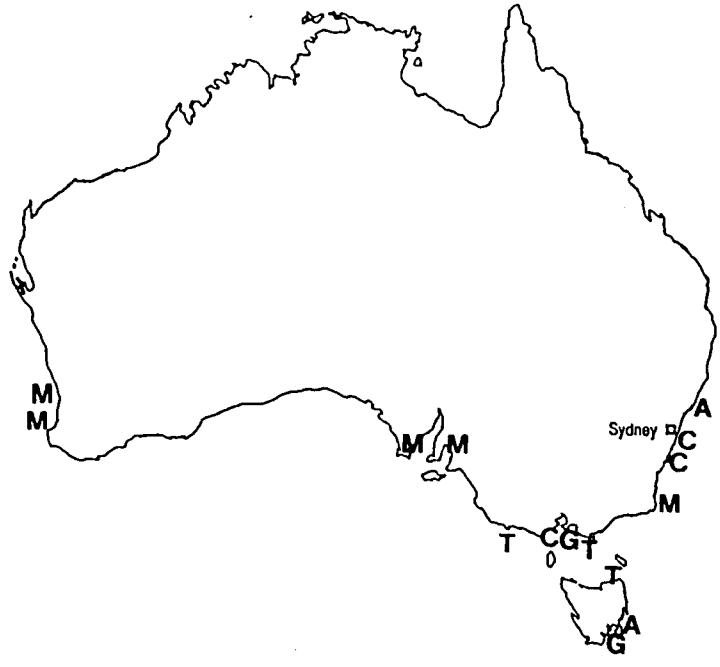

Fig.7. Australian ports where toxic dinoflagellate populations, both indigenous and introduced strains, have become established.

(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 indicator. A viable inoculum could consist of approximately 1000 cells , and the frequency of ships' visits 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 1993) . Ballast water movements are most significant to the first three ports, but the climate match of ships entering Port Hedland (17%) 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. Figure 6 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 will attempt recurrent germination attempts over the next 10- 20 years. When successful this will result in dinoflagellate blooms in the Australian port. Once it 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 1973 (soon after the opening of a new woodchip mill in 1971), it took another 8 years for the first bloom events to develop in its new environment in 1980.

(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). 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 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.7) , 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*, seaweed *Undaria pinnatifida*, starfish *Asterias amurensis* etc.). This illustrates the importance of matching port conditions in the successful establishment of introduced species. Domestic transport of viable *Alexandrium* and *Dinophysis* dinoflagellate populations has been documented by Gosselin *et al.* (1995) for short (<36 hr) voyages in the Gulf of St Lawrence region.

Ballast Water Management

Until we achieve international acceptance of a fully effective, safe, financially viable and environmentally friendly ballast water treatment option , prevention is better than to cure and a monitoring network warning about the possible contamination of ship's 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 precise depths from which ballast water intake occurs.

(2) *"en-route"* during the 10-20 day voyage, focusing on toxic dinoflagellate cysts in ship's 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 on-board monitoring therefore have implications for the management of ballast water in situations where only one port may be contaminated. Various ballast water treatment options including reballasting at sea , a continuous ballast exchange (flushing) , sterilisation with hydrogen peroxide (Ichikawa *et al.*, 1992, Bolch & Hallegraeff 1993) ,heat treatment 40- 45 ^{o}C (Bolch & Hallegraeff 1993, Yoshida *et al.* ,1995) and electric shock (Montani *et al*, 1995) are best carried out at this stage (Table 1; see also Rigby & Taylor, this session).

Table 1. Treatment options for toxic dinoflagellate cysts

CHEMICAL	**treatment**	**species**	**reference**
chlorine	500 ppm, 24 hrs	*G. catenatum* cysts	Bolch & Hallegraeff 1993
hydrogen peroxide	100 ppm, 96 hrs	*A.catenella* cysts	Ichikawa *et al.*, 1993
	150 ppm, 48 hrs	*Alexandrium* cysts	Montani *et al.*, 1995
	5000 ppm, 24 hrs	*G. catenatum* cysts	Bolch & Hallegraeff 1993
PHYSICAL			
electric shock	100 V, 5 secs	*Alexandrium* cysts	Montani *et al.*, 1995
heat	35 ^{o}C, 30 min	*G. catenatum* motile cells	Marshall & Hallegraeff, unpublished
	35-37.5^{o}C, 1-2 hrs	*G. catenatum* cysts	Marshall & Hallegraeff, unpublished
	40-45^{o}C, 30-90 secs	*G. catenatum* cysts	Bolch & Hallegraeff 1993
	45^{o}C, 3 mins	*Alexandrium* cysts	Montani *et al.*, 1995
flushing	42 hrs (90-95% exchange)	natural plankton	Rigby & Hallegraeff 1994
reballasting	28 hrs (not 100% effective)	natural plankton	Hallegraeff & Bolch 1992

(3) *Upon arrival at the receiving Australian port,* and before commencement of deballasting. Unfortunately, the routine monitoring of toxic dinoflagellate cysts in ship's ballast water and sediments is at present severely hampered by the lack of a sensitive , rapid diagnostic test which can be used by untrained personnel. A significant recent breakthrough has been the development of a suitable fluorescent staining protocol (primuline) for *Alexandrium* dinoflagellate cysts (Yamaguchi *et al.*, 1995). The feasibility of 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.* (1994). Until we achieve international agreement on a suitable ballast water treatment option , 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 from the ballast water influence of shipping ports.

Acknowledgements

Mr Chris Bolch (CSIRO Division of Fisheries, Hobart) assisted in the early stages of the routine dinoflagellate cyst monitoring (1987-1990), which was subsequently continued by Mr Neil Blain and Mr Dean Clarke of the Australian Government Analytical Laboratories (AGAL, Melbourne). I have benefitted from valuable collaboration with Dr Geoff Rigby and Mr A. Taylor (BHP Research Laboratories) who taught me about shipping operations, and am grateful to Dr Meryl Williams (previously at Bureau of Rural Sciences) and Mr Dennis Patterson (Australian Quarantine and Inspection Service) for instructing me in the art of government policies.

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