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Cyst and radionucleotide evidence for the recent introduction of the toxic dinoflagellate *Gymnodinium catenatum* into Tasmanian waters

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ABSTRACT: Cysts of the dinoflagellate *Gymnodinium catenatum* were present only in the top sections of duplicate marine sediment cores from Deep Bay in southern Tasmania, Australia. ^{210}Pb and ^{137}Cs analyses indicate that the appearance of the cyst of this toxic dinoflagellate (one of the causative organisms of paralytic shellfish poisoning) occurred after 1972. This sediment core evidence and the absence of this species from the phytoplankton of most other neighbouring Australian waters suggest that *Gymnodinium catenatum* is not endemic to Tasmania but has been introduced recently. This species was first seen in bloom proportions in Tasmania in 1980, with major blooms having occurred since then in 1986, 1991 and 1993. Several lines of evidence suggest that ballast water discharge from cargo vessels originating from Japan and South Korea, or less likely Europe, is the most probable mechanism of introduction.

KEY WORDS: *Gymnodinium catenatum* · Sediment cysts · Ballast water introduction · Radiometric dating

INTRODUCTION

Blooms of the toxic dinoflagellate *Gymnodinium catenatum* Graham were first recognised in southern Tasmanian waters in late 1985 (Hallegraeff & Sumner 1986, Hallegraeff et al. 1989). Soon after, commercial shellfish from the Derwent and Huon River estuaries proved to be contaminated with high concentrations of paralytic shellfish poisons (Oshima et al. 1987). However, in years when water temperature, rainfall and windstress were unfavourable for blooms of this species, it remained only a minor component of the phytoplankton (Hallegraeff et al. 1995). The question arose as to whether *G. catenatum* was a recent introduction into the area or whether it had occurred there previously in low concentrations without actually having been recorded. If it had been present prior to the events in the past decade, its sudden mass occurrence in the last 20 yr could perhaps be related to a change in environmental conditions favourable for the growth of this dinoflagellate. However, *G. catenatum* is a conspicuous, large (23 to 41 µm long, 27 to 36 µm wide), chain-forming dinoflagellate (up to 64 cells long), which is readily collected by plankton nets and which preserves even when using harsh fixatives such as formaldehyde. A survey of historic plankton samples and data collected by workers at the University of Tasmania (D. P. Thomas), the Electrolytic Zinc Company, Tasmania (W. Ball), and the CSIRO Division of Fisheries & Oceanography (Wood 1964) indicated that there is no evidence that the organism had been in Tasmanian waters in the periods 1945–1950 and 1975–1978, and first appeared in the Derwent River in

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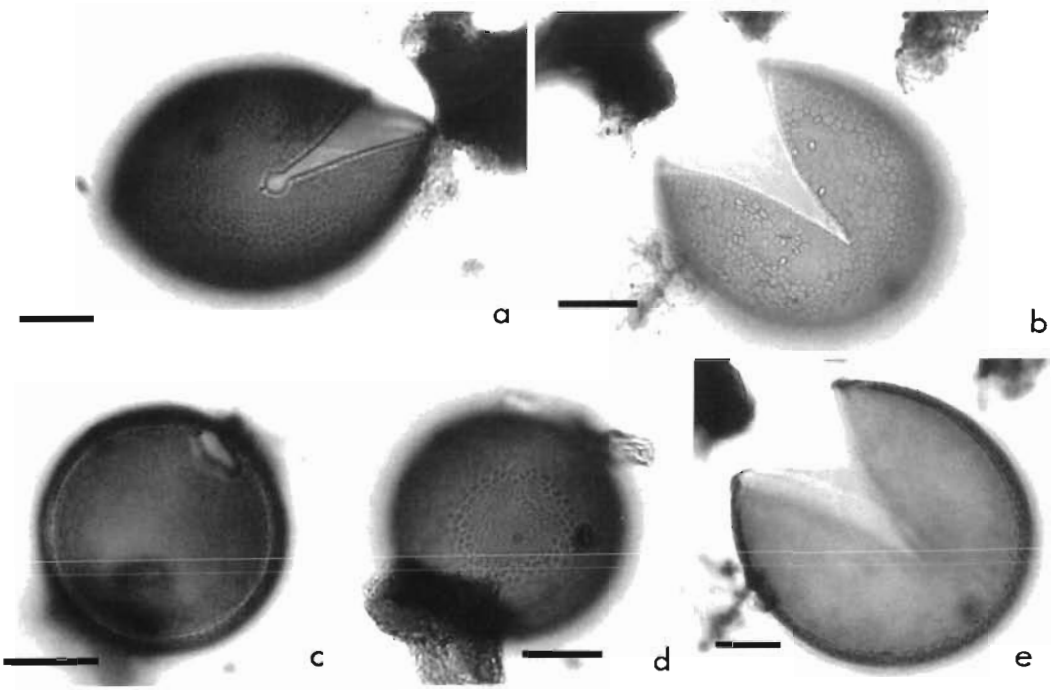

Fig. 1. *Gymnodinium catenatum*. Light micrographs of cysts from Deep Bay, Tasmania, showing paracingular excystment aperture (chasmic archeopyle) and fine reticulate surface ornamentation. Scale bars = 10 µm

1980. A concomitant survey of local hospital records confirmed that suspected human poisonings after shellfish consumption had occurred as early as 1980, but not before that date (Hallegraeff et al. 1988). Furthermore, there is no tradition of human poisonings among Aboriginal tribes that lived in the area prior to European colonisation and which consumed large quantities of shellfish (Jones 1978).

As part of its life cycle, the dinoflagellate *Gymnodinium catenatum* produces a resistant, brown, spherical resting cyst with a fine, microreticulate surface ornamentation (Fig. 1) (Anderson et al. 1988, Blackburn et al. 1989). A Tasmania-wide survey of coastal sediments found abundant, viable *G. catenatum* resting cysts in the Huon and Derwent River estuaries (Bolch & Hallegraeff 1990). Sparse cyst beds were also recorded from the east coast of Tasmania, from Spring Bay (Triabunna) and Georges Bay (St Helens) (Fig. 2), but no motile dinoflagellates or shellfish toxicity have been detected at these locations. This cyst, which can withstand palynological treatment (Anderson et al. 1988, Nordberg & Bergsten 1988), has never been observed in prehistoric Australian sediments (McMinn 1989, 1992a, b). It was also absent in a survey of Recent sediments from the entire east coast of mainland Australia (McMinn 1990, 1991) and from New Zealand (Baldwin 1987). Recently, however, *G. catenatum* cysts have been detected in sediments, from Warrnambool and Lorne, Victoria (Sonneman & Hill 1997), and from Port Lincoln, South Australia (Hallegraef unpubl. data).

MATERIALS AND METHODS

Duplicate sediment cores (15 to 25 cm long, 4.5 cm diameter) were collected in September 1991 from Deep Bay in southern Tasmania, Australia, using a modified Craib corer (Craib 1965). Further cores were collected from the same site in October 1994 (80 cm long, 11 cm diameter) using an Impact corer (Neale & Walker 1996) and in June 1995 [21 cm long, 11 cm diameter using a Glew corer (Glew 1989)]. Deep Bay is an 18 m deep, sheltered embayment near the mouth of the Huon River, Tasmania (Fig 2). The 1991 cores were stored in the dark at 4°C prior to examination. One core was frozen in liquid nitrogen and partitioned into 2 cm intervals for ^{210}Pb dating. Samples from the second core were partitioned into 1 cm intervals and sent to Laola Pty Ltd, Western Australia, for palynological processing. This involved disaggregation in HF, density separation in a $ZnBr_2$ solution (specific gravity 2.1), sieving on an 8 µm filter, and mounting in a permanent mounting medium (Eukitt). For the 1994 core, samples for ^{210}Pb and dinoflagellate analysis were taken from the same core. Samples for

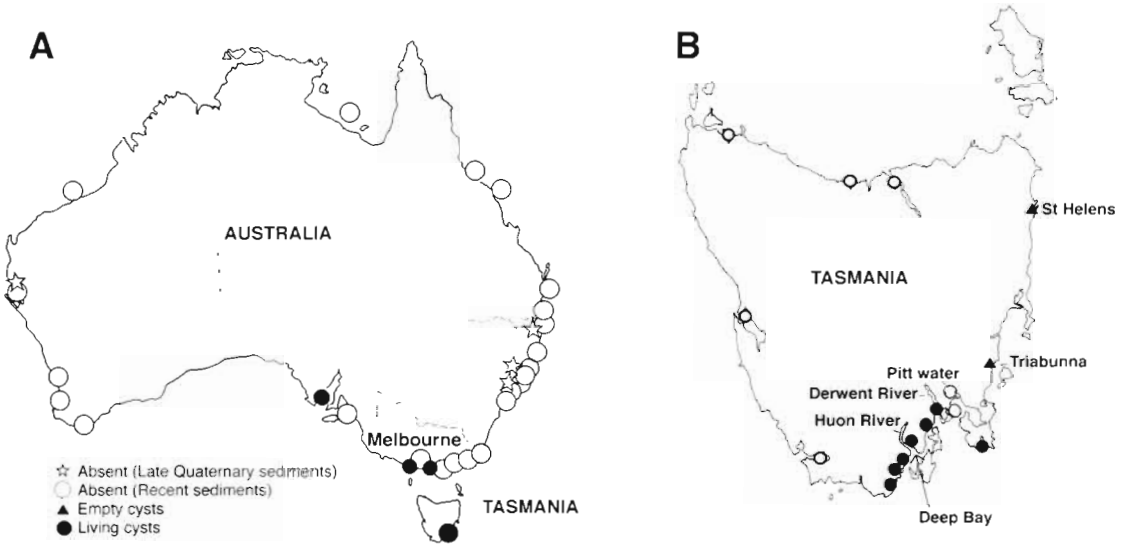

Fig. 2. (A) Australia showing the location of (B) Tasmania and Deep Bay. The presence or absence of cysts of *Gymnodinium catenatum* in sediments has been summarised (based on various sources including Bolch & Hallegraeff 1990, McMinn 1991 and B. Dale pers. comm.)

^{137}Cs analysis were taken from the Glew core collected in 1995. Slides prepared for dinoflagellate cyst analysis were examined with a Zeiss Axioskop microscope and estimates of dinoflagellate cyst abundance were made from 3 replicate counts of 400 specimens (see Table 1).

Samples for ^{210}Pb analysis were dried at 105°C to constant weight and ground to pass through a 150 µm sieve. Approximately 5 g was weighed into a beaker and ^{209}Po and ^{133}Ba yield tracers added. 25 ml of concentrated HNO_3 was added and evaporated until dry in order to eliminate most organic matter. 25 ml of 10% H_2O_2 was then added and the beaker returned to a water bath until effervescence ceased, to oxidise the remaining organic matter. 25 ml of concentrated HCl was then added and the mixture refluxed on the water bath for 4 to 6 h. The mixture was cooled and the residue removed by centrifugation. Excess Fe was removed by diethyl ether extraction and the solution evaporated until dry. The residue was then dissolved in 50 ml of 0.1 M HCl and ^{210}Po (+ ^{209}Po) autodeposited onto a silver disk. 1 g of hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) was used as the reductant and 100 ml 1.0 M citric acid added to complex trace Fe and Cr. The silver disk was then subjected to alpha spectrometry and the spent solution was diluted to 900 ml, and 20 ml of concentrated H_2SO_4 and 100 ml of saturated Na_2SO_4 were added. Radium and barium were coprecipitated with $PbSO_4$ carrier. The supernatant was removed by suction and the Pb/$BaSO_4$ residue washed with 50% ethanol which is then discarded after centrifugation. The precipitate was then dissolved in 5 ml of alkaline DTPA (pH 9.5) and the pH readjusted to >9. The solution was filtered through a 0.45 µm filter and the filter rinsed with 5 ml of 4% Na_2SO_4. Two drops of methyl red and 2 ml of glacial acetic acid were added. Immediately after, 1 ml of a colloidal $BaSO_4$ seeding suspension was added and the solution left for 30 min. The colloidal Ba/$RaSO_4$ precipitate was collected on a Millipore fluoropolymer filter and air-dried. Barium and radium recovery were estimated by submitting the membrane filter to gamma spectrometry on a HPGe gamma detector. ^{226}Ra was estimated by alpha spectrometry of the filter source.

^{137}Cs measurements were made by gamma spectrometry on a compton suppressed HPGe detector using petri dishes for source geometry.

RESULTS

Dinoflagellate cysts

Sediment cores taken from Deep Bay in southern Tasmania contained a sequence of unlaminated black muds with no obvious evidence of either stratification or bioturbation. Relative abundances of dinoflagellate cysts in the 1991 and 1994 cores are summarised in Table 1. Dinoflagellate cyst assemblages in both cores were dominated by the cysts of *Protoceratium reticulatum* (= *Operculodinium centrocarpum*) (89.0 to 97.1% of total cysts, range over both cores), which increased in abundance from 1×10^6 g^{-1} at the surface to 5×10^6 g^{-1} sediment at 80 cm depth. Cysts of *Gonyaulax*

Table 1. Distribution of dinoflagellate cysts with depth in the 1991 and 1994 sediment cores from Deep Bay, Tasmania. All abundances are given as percentage abundance. Names in parentheses are palaeontological names

Species	1991 core													
Depth (cm):	1	2	3	4	5	6	7	8	9	10	11	12	14	15
Gymnodinium catenatum	0.4	1.3	0.6	0.3	0.8	0.2	0.3	0.3	0.3	0	0	0	0	0
Protoceratium reticulatum	90.7	93.0	92.8	92.4	89.0	93.0	91.7	91.3	93.2	94.5	93.0	93.7	93.5	89.5
Polykrikos schwartzii	0.2	0	0	0.1	0.9	0.1	0.7	0.1	0.1	0.1	0.2	0.2	0	0
Protoperidinium spp.	0.7	0.4	0.2	0.7	1.2	0.5	0.2	0.9	0.1	0	0	0.2	0	0
Gonyaulax scrippsae	0.9	1.4	1.9	1.8	1.3	0.5	0.7	0.5	0.4	0.5	1.6	0.7	1.4	4.5
Gonyaulax spinifera (= *S. mirabilis*)	5.4	4.9	4.6	4.6	4.9	4.7	6.0	5.7	6.0	4.7	4.9	3.7	4.7	5.4
Gonyaulax spinifera (= *S. ramosus*)	0.2	0	0	0	1.1	0.5	0.2	0.7	0.2	0.2	0.2	0.98	0	0.2
Gonyaulax spinifera (= *S. membranaceus*)	1.4	0.1	0.2	0.2	0.2	0	0.2	0.7	0	0	0	0.5	0.5	0.7

	1994 core													
Depth (cm):	1	2	3	7	9	11	13	15	17	19	21	23	25	27
Gymnodinium catenatum	0.8	0.8	1.6	0.9	0.9	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Protoceratium reticulatum	93.0	93.7	93.7	91.6	94.3	95.8	92.2	95.8	95.7	96.3	95.4	95.8	95.4	96.1
Polykrikos schwartzii	0.2	0.2	0.3	0.1	0.2	0.2	0.1	0.2	0.1	0.3	0.1	0.2	0.2	0.3
Protoperidinium spp.	0.4	0.6	0.6	0.3	0.3	0.2	0.4	0.4	0.3	0.2	0.2	0.2	0.3	0.3
Gonyaulax scrippsae	0.5	0.6	0.8	0.5	0.8	0.6	0.3	0.2	0.3	0.6	0.1	0.1	0.0	0.2
Gonyaulax spinifera (= *S. mirabilis*)	3.2	2.8	0.3	5.2	3.2	2.4	6.4	3.3	3.5	2.7	3.8	3.5	4.1	3.1
Gonyaulax spinifera (= *S. ramosus*)	1.8	1.2	0.8	1.2	0.3	0.4	0.4	0.0	0.0	0.1	0.3	0.1	0.0	0.0
Gonyaulax spinifera (= *S. membranaceus*)	0.2	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0

Depth, continued (cm):	29	31	33	35	37	39	45	51	55	60	65	70	75	80
Gymnodinium catenatum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Protoceratium reticulatum	96.0	96.4	94.8	95.0	94.0	96.2	95.7	96.3	96.2	9.6	95.6	96.0	95.8	97.1
Polykrikos schwartzii	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.0	0.1	0.2
Protoperidinium spp.	0.5	0.2	0.1	0.2	0.5	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.3	0.3
Gonyaulax scrippsae	0.2	0.2	0.3	0.2	0.3	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.0
Gonyaulax spinifera (= *S. mirabilis*)	2.8	2.7	4.2	3.8	4.2	2.7	3.1	2.7	2.9	2.9	3.7	3.3	3.2	0.3
Gonyaulax spinifera (= *S. ramosus*)	0.2	0.2	0.4	0.5	0.7	0.3	0.3	0.2	0.4	0.3	0.2	0.2	0.4	1.9
Gonyaulax spinifera (= *S. membranaceus*)	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.2

spinifera (= *Spiniferites mirabilis*) were common (0.3 to 6.4%, over both cores), while other species such as *Polykrikos schwartzii*, *Protoperidinium conicum*, *Protoperidinium oblongum*, *Gonyaulax scrippsae* (= *Spiniferites bulloideus*) and *Gonyaulax spinifera* (= *Spiniferites membranaceus* and *Spiniferites ramosus*) were rare. The cysts of *Gymnodinium catenatum* were a rare (0 to 1.6% of total cysts, range for both cores) but consistent element in all cyst assemblages from the surface down to a depth equivalent to 1972 (i.e. 9 cm in the 1991 core and 13 cm in the 1994 core). They were virtually absent, however, from assemblages deposited prior to that time (Fig 3). There is a trailing, low frequency of *G. catenatum* cysts down to 31 cm (equivalent to 1937 ± 6 yr) in the 1994 core but this almost certainly represents local bioturbation at the time of introduction (i.e. minor bioturbation to a depth of 18 cm at the time when sediment at 13 cm in the core, or 1972 ± 2 yr, was being deposited). There are no cysts older than 1972 ± 2 yr in the 1991 core or older than 1937 ± 6 yr in the 1994 core (Fig. 3).

Sediment isotope studies

The sediment cores from Deep Bay were analysed using 2 ^{210}Pb dating models. The more simple CIC (i.e. Constant Initial Concentration) model assumes a constant sedimentation rate as well as a constant flux of excess ^{210}Pb. Therefore, a plot of the log of unsupported ^{210}Pb (i.e. excess ^{210}Pb originating from the atmosphere) against depth should yield a straight line (Fig. 4). The slope of this line is a measure of the sedimentation rate. The CRS (Contant Rate of Supply) model assumes a constant rate of supply of ^{210}Pb to the surface and sedimentation rates are calculated at each point in time. This method allows for changes in sedimentation rate within a profile with a constant supply of ^{210}Pb (Oldfield & Appleby 1984). The 2 models predict similar ages for the top 33 cm of the core; below this a deviation is visible for the CIC model due to uncertainties in the average sedimentation rate (Fig. 4).

The ^{137}Cs data (Fig. 5) show a maximum input around 7 cm depth, or 1981 However, only 1 to 2% of

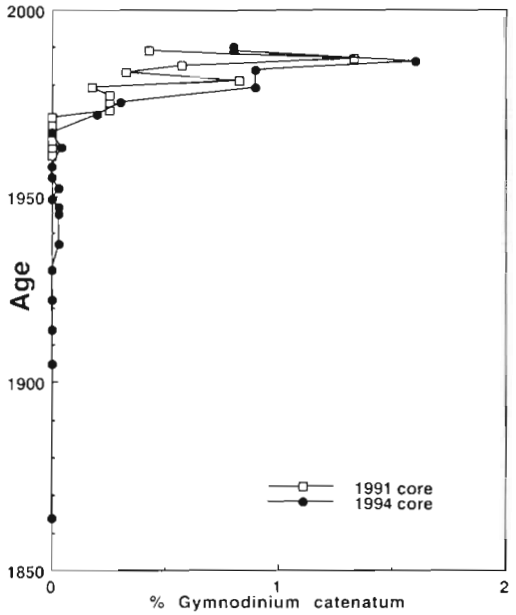

Fig. 3. *Gymnodinium catenatum*. Abundance of cysts (expressed as percentage of total dinoflagellate cysts) with date for 2 sediment cores from Deep Bay, Tasmania

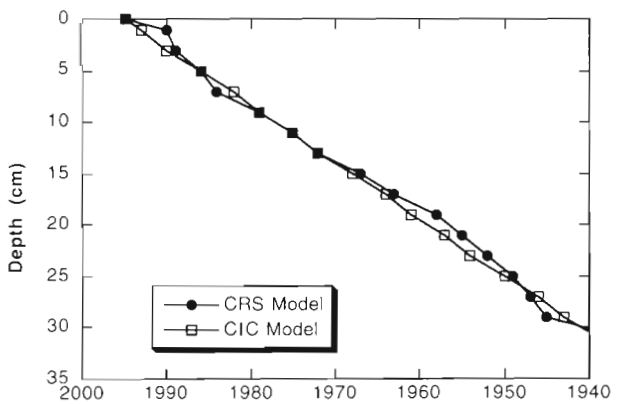

Fig. 4. Age interpretation with depth in the 1994 core. The interpretations are based on the Constant Rate of Supply (CRS) and Constant Initial Concentration (CIC) models

the ^{137}Cs in the sediments at the Deep Bay site would have resulted from direct atmospheric fallout at the site. Most of the ^{137}Cs is likely to have been transported from the catchment area by attachment to clay minerals (Eakins et al. 1984). Landuse changes in the catchment, such as changing logging practices, lead to a pulse release of ^{137}Cs into local streams and thence into an estuarine depositional site (Eakins et al. 1984, Zuo 1992). The pulse of ^{137}Cs in the Deep Bay core cannot be attributed to the normal peak in atmospheric fallout as a results of atmospheric testing, which occurred in

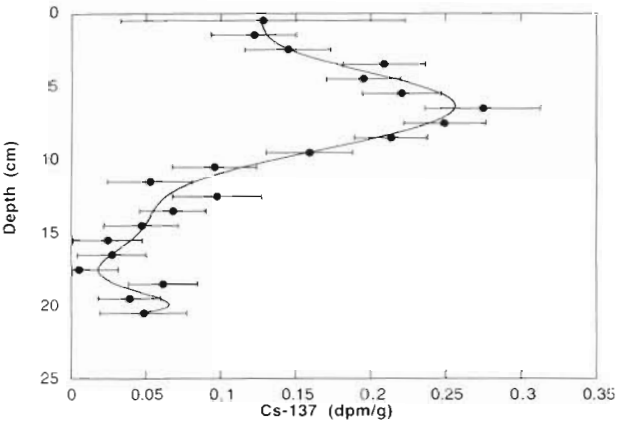

Fig. 5. Distribution of ^{137}Cs with depth in the 1995 core

the early 1960s, but to changed landuse patterns (probably increased logging in the Huon catchment during the 1970–80s). Therefore the peak in ^{137}Cs in the Huon cores can be used to investigate the extent of sediment mixing. The pulsed profile indicates that sediment mixing has not been significant and so the ^{210}Pb sedimentation rate estimates are reliable.

DISCUSSION

The monitoring of recent environmental change in coastal waters and estuaries by examining the changing proportions of dinoflagellates cysts in sediment cores is a comparatively new field of study (Dale 1989). McMinn (1990, 1991) showed that dinoflagellate cyst assemblages from estuaries in eastern Australia reflected impacts of urbanisation and agricultural runoff. The unvarying relative abundance of the dominant dinoflagellate species *Protoceratium reticulatum* (= *Operculodinium centrocarpum*) and *Gonyaulax spinifera* (= *Spiniferites mirabilis*) throughout the Deep Bay sediment cores suggests that little major environmental change has occurred in this estuary over the last 60 yr. The appearance of cysts of *Gymnodinium catenatum* at 9 cm in the 1991 core and at 13 cm in the 1994 core therefore, in all likelihood, does not reflect a response to changing environmental conditions but a recent introduction event. ^{210}Pb and ^{137}Cs data provide a date of approximately 1972 for this introduction.

It is significant that the cyst-forming dinoflagellate species accompanying *Gymnodinium catenatum* in Deep Bay (Table 1) are significantly different from the species accompanying *G. catenatum* in Japan (Matsuoka & Fukuyo 1994). This evidence, combined with the very restricted distribution of *G. catenatum* in Tasmania (Fig. 2) and much wider distribution in warm temperate waters of southern Japan (Yat-

sushiro Sea to Seto Inland Sea, and Wakasa Bay), lends further support to the suggestion that this species is exotic to Tasmania. The absence in the Tasmanian cyst assemblages of the warm-water species *Operculodinium israelianum* (microfossil name as there is no known biological equivalent), *Pyrophacus vancampoae* and *Lingulodinium hemicystum* (microfossil name as there is no known biological equivalent) agrees with the climatological gradients proposed by McMinn (1990).

Cysts of the dinoflagellate *Alexandrium tamarense* have a half life in anoxic sediments of approximately 5 yr (Keafer et al. 1992) but there is no similar information available for the more fragile *Gymnodinium catenatum*, although it is likely to be similar. Empty dinoflagellate cysts can survive in sediments for extremely long periods of time. The oldest known dinoflagellate cyst is approximately 420 million yr old (Sarjeant 1974) and cysts of Triassic age (approximately 200 to 250 million yr) and younger are common (Evitt 1985). The oldest cysts of *G. catenatum* are approximately 6000 yr old (Nordberg & Bergsten 1988), demonstrating that these cysts can survive for long periods of time without degrading. It also confirms that the disappearance of *G. catenatum* cysts in the Huon River cores is due to an introduction event and is not a result of progressive degradation over time.

Two transport vectors could have played a role in the introduction of *Gymnodinium catenatum* into Tasmanian waters: (1) associated with the introduction of Japanese shellfish stocks or (2) discharge via ships' ballast water. The Pacific oyster *Crassostrea gigas* was introduced into Tasmania in 1947 and 1948 (Thomson 1952). Seed oysters from Sendai, Kummamoto and Hiroshima in Japan (the latter is a known source of *G. catenatum*; Matsuoka & Fukuyo 1994) were acclimatised in Pittwater (shallow, 1 to 2 m depth, tidal inlet) which today is the most productive oyster farming area in Tasmania. The environmental conditions in Pittwater are not conducive to *G. catenatum* growth, however, and neither cyst stages nor motile plankton cells have ever been detected in this area. While a number of Japanese pests (e.g. a *Balanus* barnacle species) were inadvertently introduced at that time, the probability of this dinoflagellate having been successfully introduced at this early date (1947–48) as opposed to after 1972 is low. In contrast, the probability of introduction of viable *G. catenatum* resting cysts via ships' ballast water is high. Approximately 120 million t of foreign ballast water are discharged in Australian ports every year, with approximately half the amount originating from Japan. A number of toxic dinoflagellate strains have been successfully cultured from this source (Hallegraeff & Bolch 1991) and *G. catenatum* cysts have been confirmed from 4 ships entering Australian ports from both Japan and South Korea (Hallegraeff & Bolch 1992). Ports at either Port Huon or Triabunna could have served as point sources of introduction into Tasmanian waters (Fig. 2). During the 1950s and terminating in 1960, ships which ballasted in England and other European ports regularly visited Port Huon, Tasmania, to load fruit. However, none of these ports of origin, except for infrequent arrivals from Spain and Portugal, is known to harbour *G. catenatum*. A small pulp mill in the Huon River, which started in 1962 and temporarily closed in 1982, attracted ships mainly from mainland Australia and New Zealand, but has also been serviced by a few South Korean vessels. Considering the small volumes of ballast water discharged and their major ports of origin (that is, long voyage times from Europe), the probability of a successful ballast water introduction directly into the Huon estuary is low. In contrast, a woodchip mill at Triabunna on the east coast of Tasmania started to operate in 1970, attracting ships from a number of Japanese ports including Ishinomaki, Kushiro, Yura and Yatsushiro (the latter is a known source of *G. catenatum*; Matsuoka & Fukuyo 1994). These cargo vessels, each carrying 25000 t of ballast water, arrived after short, direct, 16 to 21 d voyages from very similar climatic zones, thus increasing the probability of viable species transfer. A possible scenario therefore is the introduction of *G. catenatum* cysts near Triabunna soon after 1970, the transport of viable cysts by currents from this site along the coast and into the Derwent and Huon River estuaries, and eventually the successful establishment of this species in these humus-laden estuaries. At its original point of introduction on the east coast of Tasmania, *G. catenatum* only blooms under exceptional climatological conditions (very long periods of calm stable weather) as were encountered in 1993 (Hallegraeff et al. 1995). Very similar introduction and spreading scenarios have been demonstrated for the Japanese seaweed *Undaria pinnatifida* (Sanderson 1990) and one has also been suggested for the Japanese starfish *Asterias amurensis* (Byrne et al. 1997). In June 1992 cysts and motile cells of *G. catenatum* were also detected off the coast of Melbourne (Sonneman & Hill 1997) and in 1996 off Port Lincoln in South Australia (Hallegraeff unpubl. data). The results of molecular fingerprinting using RAPD-PCR indicate that the Tasmanian and South Australian populations are genetically identical (Bolch et al. in press). The possibility that the dinoflagellate populations on the Australian mainland could have been derived from the Hobart populations via ballast water transfer from coastal shipping is now being investigated. In a strict sense a mainland Australian origin for the Tasmanian population cannot be excluded, but in spite of extensive searches only small populations with limited via-

bility have thus far been detected. Comparative molecular genetic fingerprinting is also in progress on Tasmanian and overseas dinoflagellate populations (notably focusing on Japan, Korea, Spain, Portugal; Bolch et al. in press) and ultimately this may be the only way to conclusively trace the precise source population.

Until the present investigation, an endemic Tasmanian origin for *Gymnodinium catenatum* could not be excluded. The sudden appearance of the cyst of this species in sediments after 1972 (or even after 1937 if the deepest cysts are not thought to be bioturbated) implies an introduction at or after this date from elsewhere. This result validates observational data of the absence of the motile dinoflagellate stage in Tasmanian waters prior to 1980. The most likely method of introduction appears to have been via ballast water discharge either from northeastern Asian ports or less likely European ports. The methods described herein have wide application in addressing the problem of an apparent global increase and spread of harmful algal blooms (Anderson 1989, Smayda 1990, Hallegraeff 1993).

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