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Article

Distribution of *Alexandrium tamarense* in Drake Passage and the Threat of Harmful Algal Blooms in the Antarctic Ocean

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Abstract: While phytoplankton diversity and productivity in the Southern Ocean has been widely studied in recent years, most attention has been given to elucidating environmental factors that affect the dynamics of micro-plankton (mainly diatoms) and nano-plankton (mainly *Phaeocystis antarctica*). Only limited efforts have been given to studying the occurrence and the potential risks associated with the blooming of dinoflagellates in the relevant waters. This study focused on the appearance and toxicological characteristics of a toxic dinoflagellate, *Alexandrium tamarense*, identified and isolated from the Drake Passage in a research cruise from November to December 2001. The appearance of *A. tamarense* in the Southern Ocean indicates the risk of a paralytic shellfish poisoning (PSP) outbreak there and is therefore of scientific concern. Results showed that while the overall quantity of *A. tamarense* in water samples from 30 meters below the sea surface often comprised less than 0.1% of the total population of phytoplankton, the highest concentration of *A. tamarense* (20 cells L^{-1}) was recorded in the portion of the Southern Ocean between the southern end of South America and the Falkland Islands. Waters near the Polar Front contained the second highest concentrations of 10-15 cells L^{-1}. *A. tamarense* was however rarely found in waters near the southern side of the Polar Front, indicating that cold sea temperatures near the Antarctic ice does not favor the growth of this dinoflagellate. One strain of *A. tamarense* from this cruise was isolated and cultured for further study in the laboratory. Experiments showed that this strain of *A. tamarense* has a high tolerance to temperature variations and could survive at temperatures ranging from 5-26°C. This shows the cosmopolitan nature of *A. tamarense*. With regard to the algal toxins produced, this strain of *A. tamarense* produced mainly C-2 toxins but very little saxitoxin and gonyautoxin. The toxicological property of this *A. tamarense* strain coincided with a massive death of penguins in the Falkland Islands in December 2002 to January 2003.

Key words : *Alexandrium tamarense*, harmful algae, toxicity, Antarctic Ocean

1. Introduction

Harmful Algal Bloom (HAB) is the result of the sudden and unexpected growth of phytoplankton, which often results in high algal yield and leads to massive killing of fish due to anoxia and secretion of shellfish poisons by some phytoplankton. Sometimes, seawater is discolored, becoming red or yellowish brown due to these kinds of blooms, which results in the phenomenon commonly known as "red tide".

While HAB has been extensively studied in the temperate and tropical regions, its appearance and potential deteriorative effects in polar waters have not been reported. Su *et al.* (1989) however stated that *Alexandrium tarmarense*, which may cause acute and severe paralytic shellfish poisoning (PSP) in human beings through ingestion of

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toxin-contaminated shellfish, was widely distributed in the world: it had been found in the waters of southern and eastern England, Scotland, along the coast of western Europe from Norway to Portugal, the Atlantic coast of the U.S.A. from Maine to 40°N, the Pacific and Atlantic coasts of Canada, Argentine waters (from the littoral of Buenos Aires to the Gulf of San Jorge in the littoral of Patagonia), Venezuela (Gulf of Cariaco), the coast of Chile, all Japanese waters, Korean waters, Gulf of Thailand, and the northern, eastern and southern coasts of China, including Hong Kong and Taiwan. Yet, the appearance of *A. tamarense* in the Southern Ocean near the Antarctic Peninsula has not been previously documented. This paper describes for the first time an investigation of the population of *A. tamarense* in the Drake Passage, between the southern end of South America and the water near the South Shetland Islands in late November to December 2001. The risk of HAB in that part of the Southern Ocean was evaluated by assessing the toxicological specialties of a strain of *A. tamarense* isolated during the research cruise.

2. Sampling and study methods

This study was carried out as part of the 15th Korean Antarctic Research Program (KARP) during November 2001 to December 2001. Phytoplankton samples were collected during a cruise across the Drake Passage from Punta Arenas to the South Shetland Islands bypassing the southern waters of the Falkland Islands. The distribution of the 23 sampling locations is shown in Fig. 1.

At each of these sampling locations, water temperature and salinity were measured with a Sea-Bird 911 plus CTD. Water samples were collected at 1 m, 5 m, 10 m, 20 m, 30 m and 50 m below the sea surface for further laboratory analyses. For the study of *A. tamarense*, the water samples of 1 m, 5 m, 10 m, 20 m and 30 m depth were divided into two portions: one portion was preserved immediately with a Lugol solution consisting of 1% per volume, whereas the second portion was kept fresh in both indigenous seawater and K-medium for subsequent identification and isolation. During identification and enumeration of dinoflagellates, including *Alexandrium* spp., sample volumes of 100-500 m*l* were concentrated in a 25 mm diameter, with 0.45 μm pore size Millipore filters concentrated to about 5-10 m*l* aliquots. The samples were then examined under inverted microscopes with a Sedgwick Rafter counter. Since dinoflagellates, including *A. tamarense,* were the main focus of the present study, other phytoplankton species were only noted and not reported here. When live dinoflagellates were found, they were specially treated for taxonomic identification, enumeration and possible isolation for cultivation in an environmental chamber. As a result, a strain of *A. tamarense* was identified and isolated. This strain of *A. tamarense* was isolated from water samples and is being kept in the laboratory of the Open University of Hong Kong and the Algal Culture Collection of the University of Hong Kong. The morphological properties of this strain were further examined using a scanning electronic microscope (SEM).

The toxicological characteristics of the isolated *A. tamarense* were studied in-depth with HPLC and mouse assay according to methods specified by Hallegraeff (1995) - with modifications from Oshima (1995 a and b); and the Association of Official Analytical Chemists (AOAC) (1975) - with modifications from Yang *et al.* (2002). Standards for algal toxins were referenced to those of the School of Oceanography at the University of Rhode Island and the School of Fishery Science at Taiwan University.

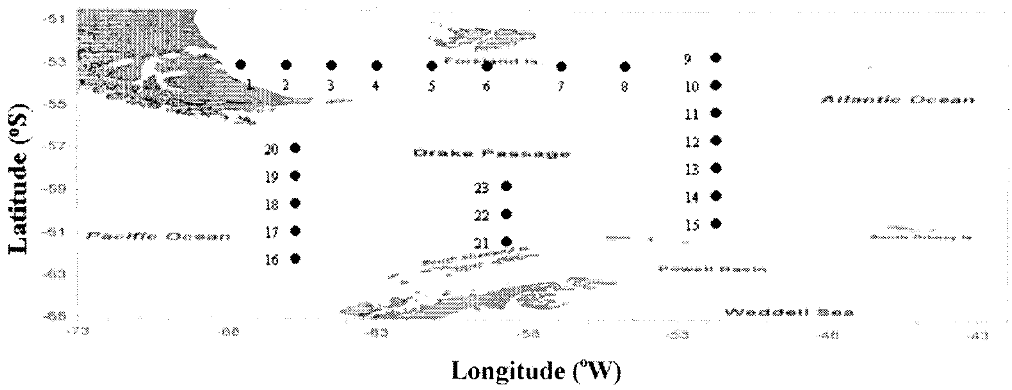

Fig. 1. Sampling locations in the Southern Ocean during late November 2001 to December 2001.

3. Results

Alexandrium tamarense (Plate 1) was found in most of the sampling locations, particularly at water depths of 1-20 m below the sea surface. Fig. 2 shows the spatial distribution of *A. tamarense* in the composite samples of 1 m, 5 m, 10 m and 20 m below the sea surface (no *A. tamarense* was found in water samples >30 m below sea surface). It is interesting to note that *Alexandrium tamarense* was at highest concentration in waters near the southern end of South America (Site 20) and in the southeastern waters of the Falkland Islands (Sites 5 and 6). Nevertheless, we found that the maximum concentration of *A. tamarense* was only 20 cells L^{-1} and this species of dinoflagellate normally comprised less than 0.1% of the total phytoplankton *i.e.* the total micro-phytoplankton plus total nano-phytoplankton.

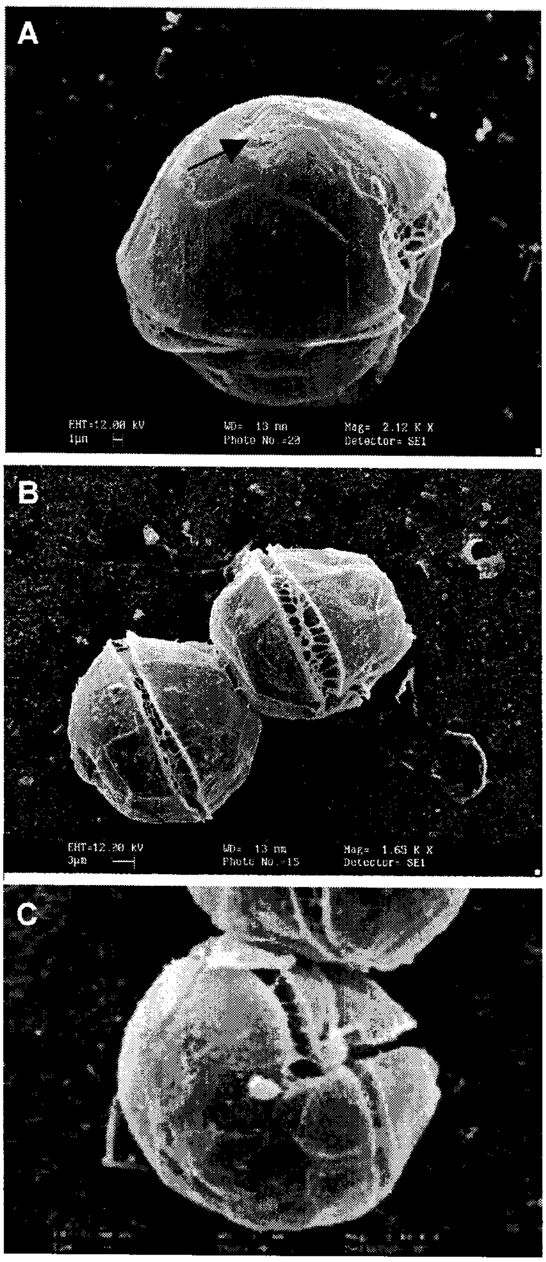

Plate 1. The dorsal view (A), lateral view (B) and griddle view (C) of the strain of *A. tamarense* isolated in the Southern Ocean near the Falkland Islands, by SEM.

A second peak of *A. tamarense* appeared near the polar front (58-61°S), indicating that upwelling waters at the front might have provided the required nutrients (e.g. Total Inorganic Nitrogen (TIN) and Dissolved Inorganic Phosphorus (DIP)) in order to achieve better growth of *A. tamarense* there. However, at the southern side of the Polar front, very few dinoflagellates, including *A. tamarense,* were recorded. Generally, the closer to the waters of the South Shetland Islands and Elephant Island, the less *A. tamarense* were detected. This suggests that the colder water (with temperatures of 0.72 to 2.71°C recorded during the cruise) near the Weddell Sea of Antarctica was generally not favorable for the growth of this strain of *A. tamarense*. The present results may explain why *A. tamarense* was not documented in previous scientific surveys in the Antarctic Ocean, because geographically they were mostly focused on the icy water to the south of the Polar Front and biologically concentrated in the population dynamics of diatoms (the dominant micro-phytoplankton) and the nano-phytoplankton.

The third peak of *A. tamarense* was found in the waters between South America and King George Island. The lower the attitude, the higher the concentrations of *A. tamarense* were found. Interestingly, we observed that *A. tamarense* was able to survive at seawater temperatures ranging from 4.85°C-8.13 °C during the research cruise. This shows the persistence of this species in tolerating temperature variations.

A strain of *A. tamarense* was identified and isolated from live samples collected in waters near the South American coast and the Falkland Islands. Mono-cultures of this strain are now being kept in the Algal Culture Collection of The University of Hong Kong and the Environmental Research Laboratory of The Open University of Hong Kong for further research. SEM pictures of this strain of *A. tamarense* show the cells to be globular, slightly longer in terms of width and pentagonal in shape. A prominent ventral pore (as indicated with an arrow in Plate 1-A) was found near the middle of the

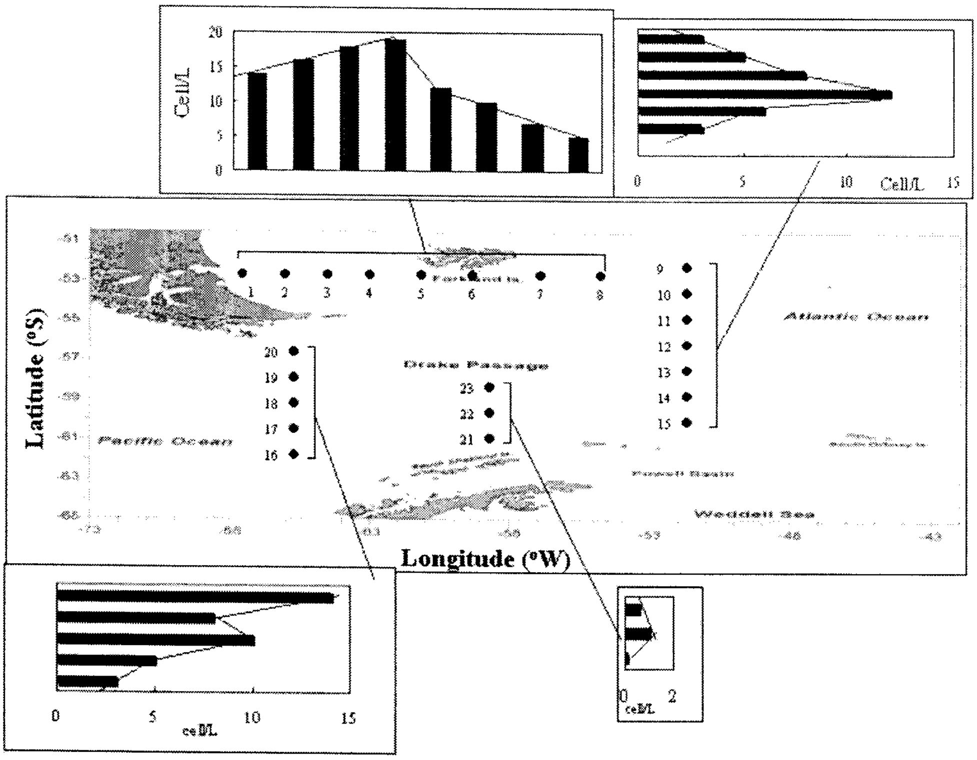

Fig. 2. Concentration of *Alexandrium tamarense* in the composite samples of 1 m, 5 m, 10 m and 20 m at various locations.

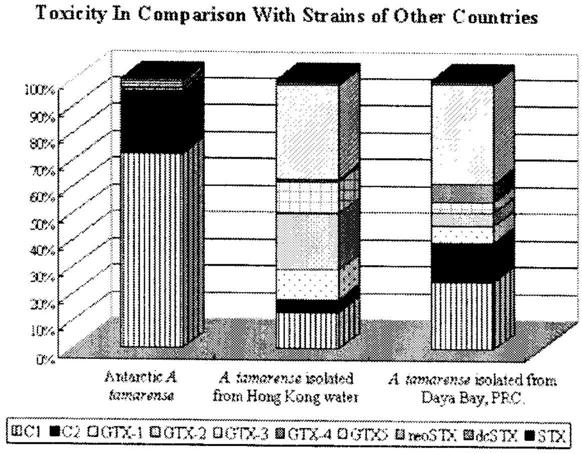

Fig. 3. Toxicological characteristics of the Southern Ocean strain of *Alexandrium tamarense,* which contrasts with strains of the Hong Kong and South China (Daya Bay) water.
In chart above, it should read:
A. tamarense obtained in Hong Kong Water
A. tamarense obtained in Daya Bay, PRC.

suture between apical 1' and 4', which coincided with the taxonomic guide of Fukuyo *et al.* (1990).

Results (Fig. 3) of toxin analysis showed that this strain of *A. tamarense* mainly produced C-2 toxins, which is very different from the common strains of *A. tamarense* isolated in the waters of Hong Kong and Daya Bay of Guangdong Province, China. For the dominant strains of *A. tamarense* in Hong Kong and Daya Bay, they mainly produce GTXs and STXs but rarely C-toxins. The property of the Southern Ocean strain of *A. tamarense* was that it exhibited tremendous uniqueness in terms of in its toxicological characteristics. Further phylogenetic and biogeographic analyses are highly recommended. It is believed that such studies will clarify the global distribution of *A. tamarense* and the dispersal mechanism of toxic marine algae in the world.

4. Discussion

Boyd (2002) stated that although relatively comprehensive groups of stations were sampled in the Weddell Sea and around the Peninsula (sampled as early as 1930), insufficient temporal or spatial resolution had been provided to establish comprehensive maps of phytoplankton stocks there. He also mentioned that in addition to studying the variations in chlorophyll-*a* by means of various oceanographic and satellite tools, shipboard surveys played a key role in investigating the relationship between environmental factors and spatial and temporal distribution of phytoplankton. He further pointed out that the shipboard surveys, from Hendey (1937) to Kang *et al.* (2001), had provided an inventory of algal species in the Southern Ocean and had reported a greater diversity of pico- and nano-plankton than previously thought.

On the other hand, Knox (1994) stated that diatoms are the abundant constituents in all Antarctic marine environments. Therefore, amongst the various micro- and nano-phytoplankton species in the Southern Ocean, diversity of diatoms has been better documented than that of dinoflagellates (Abbott 2000; Ahn and Kang 1991; Ahn *et al.* 1997; Kang and Kang 1998; Kang *et al.* 1999; Krebs 1983; Watanabe 1988; Watanabe *et al.* 1990; Whitaker & Richardson 1980). For this reason, the present study (which focused on a dinoflagellate species, *Alexandrium tamarense*) is able to fill the gap in the scientific records in these relevant areas. As shown in the present study, although the total dinoflagellate concentrations were often less than 0.1% of the overall phytoplankton population, the appearance of *A. tamarense* indicated the possible harmful effects of algal blooms in the Southern Ocean.

Ho *et al.* (2000, 2002) stated that the harmful effects of algal blooms in temperate, subtropical, and tropical waters have been studied in depth, but the risks of HABs in polar waters has frequently been overlooked. The present study, which focused on the geographical distribution and toxicological characteristics of indigenous *A. tamarense,* is able to give preliminary assessment of the possible outbreak of *Alexandrium*-caused HABs to the north of the Polar Front of the Southern Ocean.

Alexandrium species are closely related to Paralytic Shellfish Poisoning (PSP). Although blooms of the dinoflagellate genus *Alexandrium* are often referred to as "red tides", for many of the PSP events caused by this species, the concentration of the dominant dinoflagellate was too low to discolor the water or to be observed by the naked eye. Therefore, although dense blooms of *A. tamarense* have not yet been observed in the Southern Ocean, the possible ecological and toxicological impacts of *Alexandrium* in the future should not be overlooked. In particular, the possible risk of *Alexandrium*-caused HAB to the south of Chile and the area of water from the base of South America to the Falkland Islands should be further studied and evaluated.

Fig. 4 summarizes the documented events of HAB in the world caused by *A. tamarense* since the 1970s (Anderson *et al.* 1985; Anderson *et al.* 1998; Brenner *et al.* 2000; de Salas *et al.* 2000; Doblin *et al.* 2000; Gayoso 2001; Graneli *et al.* 1990; Hallegraeff *et al.* 2002; Ho and Hodgkiss 1993; Ho *et al.* 2000; Ho *et al.* 2002; Lassua *et al.* 1995; Okaichi *et al.* 1989; Reguera *et al.* 1998; Smayda and Shimizu 1993; Spector 1984; Taylor and Seliger 1979; Yan *et al.* 2003; Yasumoto *et al.* 1996). As shown in the figure, *Alexandrium*-related PSP events rarely happened in southern Chilean waters, Argentinean waters, southern Australia or the South Pacific before the 1990s. However, an increasing trend of *Alexandrium*-related PSP events was observed after the 1990s, with a particularly obvious proliferation trend after 1999. Ho *et al.* (2002) reported that in 1993-94, trace amounts of PSP toxins were found in shellfish samples collected at King George Island of Antarctica. However, at that time, it was not clear whether the toxins were related to blooms of *Alexandrium* spp. Hence, the present results support previous findings that the area of water in Drake Passage of the Southern Ocean was susceptible to risks of *Alexandrium*-related PSP and there is the potential for a significant outbreak in the coming years. At the time of writing this paper, thousands of penguins were reported dead in the Falkland Islands (Falklands Conservation 2003). It is believed that the event was a consequence of blooms of *Alexandrium tamarense* and/or *A. catenella* in late 2002 to February 2003 (Nic Huin, per. comm.) This disaster to penguins as well as other marine organisms proved that *Alexandrium*-related PSP in the Southern Ocean is highly possible and should be monitored in the

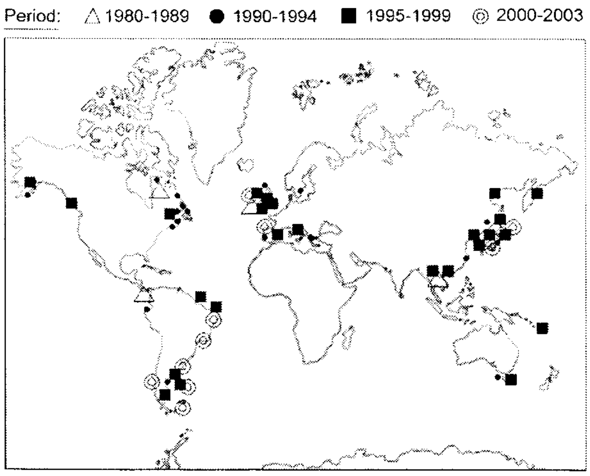

Fig. 4. Global distribution of *Alexandrium tamarenses*-related shellfish toxicity from the 1980s to 2003.

coming years. The present study, which is based on reporting the geographic distribution and toxicological characteristics of *A. tamarense* in the Southern Ocean, forms the basis of further research.

The strain of *A. tamarense* obtained in the Southern Ocean was cultivated in mono-culture in the Algal Culture Laboratory of the University of Hong Kong. As recorded in the laboratory, the *A. tamarense* strain isolated was able to survive at a water temperature as high as 25°C in an environmental chamber. This coincides with the results of Ho and Hodgkiss (1992) who found that HAB caused by *Alexandrium* sp. was globally distributed and could be found in most subtropical waters including China, the Philippines, Venezuela and northern Chile. The present result further shows that *A. tamarense* is a cosmopolitan dinoflagellate, as supported by other researchers like Dodge (1982), Fukuyo *et al.* (1990), Hallegraeff (1991), Spector (1984) and Taylor and Fukuyo (1998). This monoculture of *Alexandrium tamarense* would be a valuable asset for future study of the phylogenic characteristics of *Alexandrium* species.

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