Blooms and gloom

You might think that the answers to such questions as 'how did the Red Sea get its name?', 'what is a dinoflagellate?', and 'when is a good time to eat oysters in Tasmania?' are completely unrelated to each other — and of little practical use to any but the most dedicated players of *Trivial Pursuit*. But you'd be wrong...

A type of microscopic single-celled alga provides the answer to all three questions, directly or indirectly. The tiny marine plants, called dinoflagellates, can make the Red Sea and other bodies of water appear red, brown, or luminous by night, and can cause oysters to be poisonous. They can also sometimes bring about widespread death of fish.

With the right environmental conditions, such as high water temperatures and an input of organic compounds from the land after heavy rains, dinoflagellates can multiply rapidly. The result is a 'bloom' — a vast population of cells in one area. So high is their concentration that the cells can literally discolour the water, turning the sea red.

Usually these plankton blooms look spectacular but are quite harmless; sometimes, though, the vast quantity of algae and the bacteria decomposing them deplete all the available oxygen dissolved in the water, killing fish and most other types of marine life that are unfortunate enough to be in the area. (Of course, this can't happen in the open sea, but may occur in sheltered bays with restricted outflows.) In Australia such fish kills are not common, but one occurred in West Lakes near Adelaide in 1983.

Toxins

A completely different side of dinoflagellate biology — but one of even greater concern to us — involves the production of poisons. Because of this property, the work of plankton specialist Dr Gustaaf Hallegraeff of the CSIRO Division of Fisheries has recently been of considerable importance in Tasmania — of which, more anon.

In order not to besmirch the good name of dinoflagellates in general, Dr Hallegraeff points out that, of the approximately 1500 species floating in the world's oceans, only a mere 20 can make toxins, as far as scientists know. What's more, because they are tiny and produce only minute quantities of toxin, you won't suffer any ill effects from swallowing a few of these algae if you go swimming in the sea. And, for the most part, the same holds true for marine creatures; it's only when we come to eat seafood that itself fed on the dinoflagellates that problems can arise.

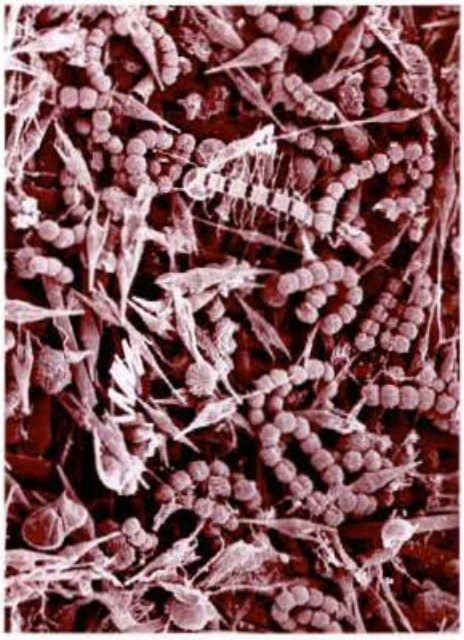
What has been called the process of 'biological magnification' ensures that the concentration of a non-degradable substance — whether it be a pollutant, such as a heavy metal, or a biological toxin — that builds up within an organism increases the higher up the food chain the organism lies.

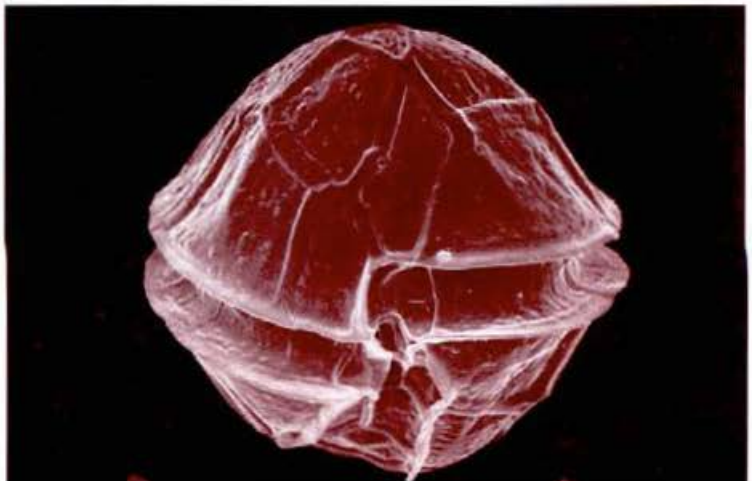
Filter-feeding creatures, such as oysters, mussels, and scallops, extract large quantities of the tiny dinoflagellates from the water continuously passing through their gills. The accumulated toxin, although apparently harmless to the shellfish, renders it contaminated. The gourmet who then feeds on it will suffer from the toxin, the severity of the symptoms ranging from a mild stomach upset with diarrhoea to severe neurological disorders classified as paralytic shellfish poisoning, which can result in death from paralysis of the respiratory muscles. (See the box on page 23 for more information on the nature of these toxins.)

Blooms of toxic dinoflagellates occur in many parts of the Northern Hemisphere on a seasonal basis. They can result in poisonous shellfish that appear, and taste, quite normal. Once people start getting sick from eating the seafood, then economic disaster can strike a region's oyster-farmers and may possibly even affect its tourism industry, if it has one.

World-wide, about 2000 cases of human poisoning through eating toxin-contaminated fish or shellfish occur each year. Many shellfish-farming areas have to carry out regular testing of the sea water for dinoflagellates, and if these are present must test seafood products for their toxins. This is an expensive business.

Fortunately, in Australia blooms of toxic dinoflagellates have, until recently, been virtually unknown and so we had no need of monitoring programs. The only problem we had was ciguatera poisoning from eating tropical reef fish. Captain Cook described this poisoning, which he suffered when





Toxic dinoflagellates in close-up. The electron micrograph on the right shows plankton from Tasmanian estuaries dominated by the chain-forming *G. catenatum*. Those below show the cause of ciguatera poisoning (left) and a toxic alga found near Adelaide.

visiting New Caledonia in 1774. Although well known since then, it was not until 1978 that scientists identified the cause as a dinoflagellate, called *Gambierdiscus toxicus*, that can only live in tropical waters.

This species grows on the bottom of shallow reefs, attached to seaweed or coral rubble. Small fish graze it, and the nerve toxin passes on when larger fish make a meal of them. Predatory fish at the top of the food chain — such as barracuda, Moray eel, Spanish mackerel, red bass, or coral trout — accumulate enough toxin to poison humans, although the fish seem to be unaffected.

Victims suffer the usual gastro-intestinal disturbances, but the neurological effects include a strange reversal of the perception of hot and cold. Cool sea-spray landing on the skin may burn painfully, while a warm bath can seem ice-cold. Symptoms can recur without warning years later, and the toxin can accumulate in the human body; thus, one meal of contaminated fish may have no effect, but a second, even though eaten months later, may precipitate a full attack. In extreme cases, death may result from respiratory failure.

Nearly 500 cases of ciguatera poisoning (including one fatality) have been reported from Queensland, and a few cases have occurred in north-western Australia. Unfortunately, we have no adequate treatment yet.

Tasmanian blooms

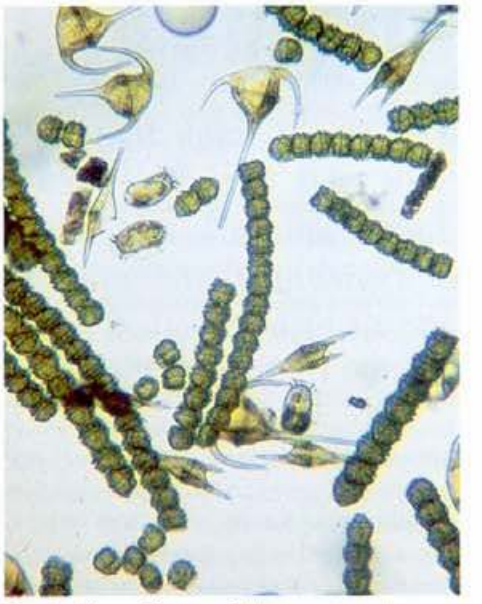
It was only in 1986 that, as part of a general plankton survey, Dr Hallegraeff (using funds from the Fishing Industry Research Trust Account) identified the toxic dinoflagellate *Gymnodinium catenatum* occurring in dense blooms of up to 100 000 cells per litre around the Huon and Derwent estuaries in Tasmania. Scientists overseas had already reported that *G. catenatum* contamination had caused illness in Spain and Mexico.

The Tasmanian blooms occurred in December 1985–February 1986 and again in April–June 1986. A great deal of rain fell in the middle of both these periods, and quite possibly this stimulated the burst of dinoflagellate reproduction by bringing a large amount of nutrients from the land.

Dr Susan Blackburn, another researcher in the Division, decided to culture the species in the laboratory. She found that growth occurred best at temperatures between 15 and 20°C, but stopped completely below 12°C. The algae could tolerate a fairly wide range of salinities — from 23 to 34 parts per thousand — but would stop growing below 20 p.p.t. (The salinity in the open ocean around Tasmania is 34–35 p.p.t., and in estuaries can range from 28 to 34.)

The cultures needed organic growth factors as well as the usual inorganic nutrients such as nitrates and phosphates. To provide the necessary 'goodies' Dr Blackburn added a soil extract, which did the trick. Exactly what these organic factors are nobody knows for sure, but the fact that, in the sea, blooms often occur after heavy rainfall suggests that they may indeed originate in the soil.

Following monitoring of the toxins in shellfish by the Tasmanian Department of Sea Fisheries, 15 oyster- and mussel-farms had to close in 1986, some for as long as 6 months. Dr Hallegraeff's plankton sampling showed that the dinoflagellates had disappeared from the water by the beginning of July 1986, and 3 weeks later the toxins had gone from most shellfish, although some remained toxic for a further 3 months.



Toxins from *Gymnodinium catenatum* — the chain-forming alga in this light-micrograph, forced Tasmanian oyster- and mussel-farm closures in 1986.

Fortunately, 1987 was less severe, with only five farms affected, and then only for a shorter period. The rainfall beforehand was not unusually heavy — a fact that may be significant. So far, 1988 has been toxin-free for the shellfish and has also been very dry.

Elsewhere in Australia, in October 1987 toxins appeared in wild mussels near Adelaide, and in January 1988 Port Phillip Bay near Melbourne also suffered a small bloom that led to some contamination of wild shellfish, although commercial farms — possibly because of their location further out to sea and away from the discharge plume of a nutrient-rich river — remained unaffected. Dr Hallegraeff has identified the two dinoflagellates involved as two species of the genus *Alexandrium*, which are therefore different from the Tasmanian culprit. Both species have been reported occurring overseas. They are now also being cultured in the Division and they can produce toxins. Fortunately, neither bloom gave rise to any human sickness as far as we know.

Dr Hallegraeff's work on the 1986 Tasmanian outbreak showed that oysters grown on long lines were more toxic than those cultured near the shore. This is because the shallow-cultured ones, on racks, spend less time in the water, being exposed at low tide — of course, they therefore grow more slowly — whereas the

An intertidal oyster-farm in the d'Entrecasteaux Channel, near Hobart, Tasmania.

others are attached to lines dangling from floating structures that may be right in the middle of a bloom.

The scientists also found that mussels, oysters, and scallops from the affected area contained high levels of the toxins causing paralytic shellfish poisoning. (Mussels were often the most contaminated, as their feeding behaviour is less selective than that of oysters.) Two human poisonings showing the expected symptoms occurred in February 1986. The patients developed numbness of the lips, fingertips, and toes and mild respiratory problems after each eating more than three dozen wild shellfish from Port Cygnet.

As his next step, Dr Hallegraeff decided to investigate the precise chemical nature of these toxins. He therefore sought the help of specialists in their analysis at Tohoku University in Japan.

A dramatic example of a red tide in Lake Macquarie, New South Wales, caused by the non-toxic dinoflagellate *Noctiluca scintillans*.

They found that the toxins in the Tasmanian shellfish are related to paralytic toxins known overseas. They are all based on the same parent compound called saxitoxin (first described from a clam called *Saxidomus*). However, the analyses showed that 'our' toxins contained sulfamate groups rather than carbamate ones. The sulfamate derivatives are less toxic, which seems like good news until you realise that under certain conditions — for example, acidity — that may occur during preparation, storage, or digestion, the sulfamates could possibly be converted to the far more toxic carbamates. Further research on this is under way.

Why have we been bothered only recently by blooms of the toxic *Gymnodinium catenatum*? Was it simply not recognised or is this alga a new arrival to our shores? Regular plankton sampling in Australia has been going on since 1945, and Dr Hallegraeff has been examining the stored specimens. He believes that *G. catenatum* has only been around Tasmania since 1980.

The resting stage of the alga — a tough spore — has provided further clues. These spores remain in the bottom sediment for many years, and Dr Hallegraeff, with the help of Mr Christopher Bolch, has identified them only in sediment collected around the port of Hobart. He suspects that quite possibly the alga arrived in our waters on ships, probably in the ballast tanks that are cleaned out at ports (see the box).

Now that we have this unwelcome unicell in some of our waters we need to ensure it

Microscopic stowaways

Here in Australia, we don't need reminding that humans have frequently taken living things from one part of the globe to another, intentionally or otherwise — and often with disastrous results. Now that we are wiser, we spray insecticide inside aircraft arriving here, and are scrupulous in trying to prevent the accidental importation of biological material that could affect our agriculture.

But Australian ports regularly play host to ships that have the potential to introduce alien species that at best are unwanted, and at worst could develop into dangerous pests. We remain generally unaware of this because the creatures are sea-dwelling and sometimes microscopic.

Many ships use sea water as ballast when they are not carrying cargo, and discharge the water when they load up. Dr Pat Hutchings of the Australian Museum has calculated that ships (more than one-third of them Japanese in origin) discharge a total of 60 million tonnes of ballast water into Australian ports each year. This staggering amount is equivalent to half the volume of Sydney harbour.

Dr Hallegraeff and Mr Bolch, of the Division, in collaboration with Mr Brian Koerbin and Mr John Bryan, from the Department of Primary Industries and Energy in Hobart, examined ballast-tank water from Japanese woodchip vessels arriving in Tasmania.

These ships take on up to 25 000 tonnes of water in Japanese ports when the Australian woodchips are unloaded and then sail to Tasmania, where they empty the water from the tanks. Floating plant and animal plankton species and fish larvae could be transported to Australia. And, if weather conditions in Japan stir up the fine bottom sediment, so too could dinoflagellate spores.

In support of these ideas, the researchers' surveys showed that, in three of the six ships tested, the mud from the bottom of the ballast tanks — normally shovelled up and dumped over the side — contained viable dinoflagellate spores, some of which were suspected of belonging to a toxin-producing species.

Dr Hallegraeff believes that harbour authorities must become as vigilant in preventing the entry of foreign marine organisms as their colleagues at airports are with terrestrial creatures, although at present no international, Commonwealth, or State laws exist to prevent discharge of ballast water into Australian ports, provided the water is not polluted.

To help solve the problem of the arrival of toxic dinoflagellates, the Division of Fisheries is studying the conditions that affect the viability of the spores in the hope of finding ways of killing them that could possibly be applied to ballast holds.

Dinoflagellate spores in the ballast water of ships. G. Hallegraeff, C. Bolch, B. Koerbin, and J. Bryan. *Australian Fisheries*, 1988, **47** (in press).

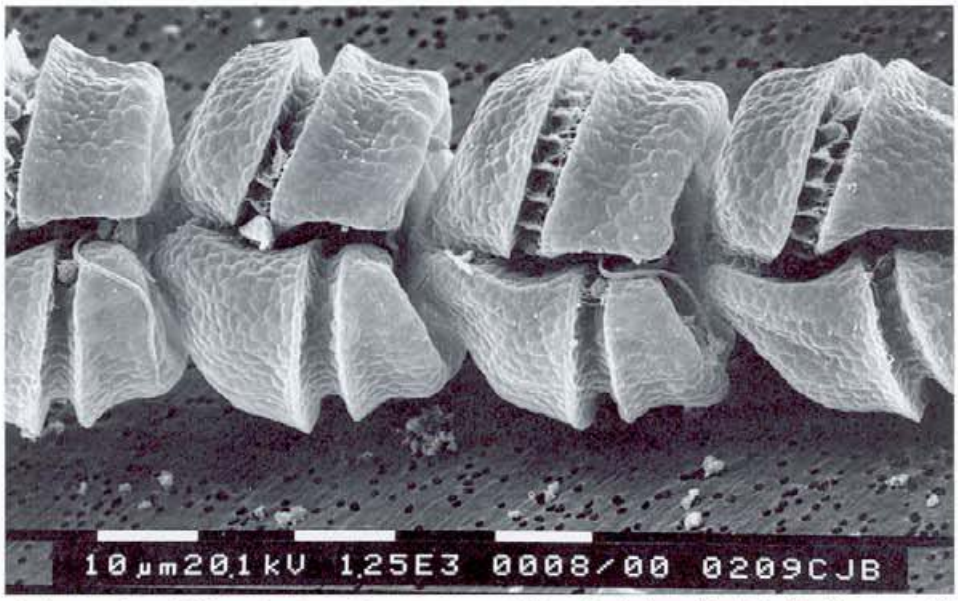
Guidelines for the conduct of surveys for detecting introductions of non-indigenous marine species by ballast water and other vectors — and a review of marine introductions to Australia. P.A. Hutchings, J.T. Van der Velde, and S.J. Keable. *Occasional Reports of the Australian Museum*, No. 3, 1987.

doesn't spread further. In the 1986 outbreak, the Tasmanian Department of Sea Fisheries imposed a temporary ban on transferring shellfish stocks from one area to another. A necessary procedure with many shellfish is cleansing or 'depuration', designed to rid the delicacies of bacterial contamination or of any toxins — be they biological or from industrial activity — that they might have acquired during their lifetime. Growers attempting to carry out depuration by putting their stock in 'clean' areas could easily spread the dinoflagellate. So the process must be carried out in tanks that do not discharge back into the sea.

Unfortunately, the depuration time for this toxin, which scientists from the State Department of Sea Fisheries have established, is of the order of a month, during which time the shellfish can lose their condition.

Any answers?

What can we do about the problem? The short answer to that question is 'not much'. We cannot get the dinoflagellates out of the water, and even if we could their resting spores would remain in the sediment, ready to germinate as soon as temperature and nutrient levels are right. (Of course, this is what keeps the species going in between blooms, when the active stage disappears from the water.) Naturally, it seems sensible to try to prevent any further spread of the toxic algae, and the scientists have already alerted the responsible authorities.



A closer view of *G. catenatum*, the unwanted dinoflagellate. The central groove is clear on all the cells, and two have a flagellum visible. The scale bar here represents 10 μm, which is one-hundredth of a millimetre.

Equally, there is little that oyster-farmers can do. A visible bloom may be caused by harmless algae, and so may not require any action. On the other hand, dangerous levels of *G. catenatum* may not be easily visible. To help with this, the farmers will be issued with plankton samplers, and will learn to identify the species and monitor it themselves, as happens in Japan. It's hoped that this will enable them to take early note of any population increases that suggest a full-scale bloom may be imminent, and harvest their shellfish then.

Regular toxin-testing of the shellfish will continue and, where necessary, temporary bans will be imposed by the Tasmanian Department of Sea Fisheries so that consumers can be quite sure of the safety of these products. Our best hope is further

What is a dinoflagellate?

Dinoflagellates are a type of alga. Once considered plants because they carry out photosynthesis, algae are now accorded the status of their own kingdom — neither plant nor animal but 'Protist'. This group also includes non-photosynthetic unicellular creatures, such as the well-known amoeba, that are not algae.

Living on land, we seldom see algae, except perhaps as a blue-green scum on stagnant pond water, or green coatings on trees in wet areas. These manifestations are caused by single-celled green and blue-green algae; but the sea contains algae of other colours as well — red, brown, and golden-brown, for example — some of which, such as kelp, are large and multicellular. These giant seaweeds have more in common with many of the tiny single-celled creatures in pond water, gyrating under a microscope lens, than they do with the tall plants of the land.

Dinoflagellates fit within the single-celled algae, and about half of them can perform photosynthesis and carry a variety of pigments, making them range in colour from golden-brown to red. (Others, however, are colourless and will catch and eat their food, or are parasitic.)

The cells can range in length from 20 to 200 μm, and may stay together after dividing, so forming long chains.

All dinoflagellates have two flagella — the thin whip-like projections that cells can use to propel themselves. One hangs down vertically and pushes the cell forwards, while the other lies in a groove that encircles the middle of the cell, and causes rotational movements.

Cells that stick together to form chains can swim faster than those that stay solo, which is possibly why chain-forming is popular among the species that bloom. Many dinoflagellates can migrate up and down in the sea, seeking the best conditions of light, temperature, nutrients, and salinity. They will then cluster in their preferred region, which in toxin-producers can cause parts of the sea to become toxic hot-spots — small areas that are far more dangerous for shellfish than a neighbouring apparently identical patch of water.

The dinoflagellates are divided into two groups: those armoured with a casing of cellulose, often intricately sculpted, and those that lack this and remain as naked cells. In general, only the armoured ones produce toxins, but *Gymnodinium catenatum* is the exception, being the first unarmoured form that scientists have discovered producing a toxin.

Like many unicells, dinoflagellates can reproduce simply by splitting in two. This enables them to multiply when conditions are good. But *Gymnodinium catenatum* also has a type of sexual reproduction, in which two cells of the same species but opposite mating types — rather like male and female — come together. The procedure is usually induced by unfavourable conditions and the result of the sexual fusion is a tough, encased 'spore' or resting cyst that can last out hard times by staying dormant in the sediment at the bottom of shallow areas of the sea. These cysts can remain viable for 20–30 years, germinating into the usual swimming forms when conditions improve.

More on the toxins

Dr Hallegraeff, with his Japanese collaborators, found that shellfish taken from areas affected by the 1986 Tasmanian dinoflagellate bloom contained toxins at a concentration as high as 8 mg per 100 g, which is a hundred times higher than the quarantine level allowed by the United States Food and Drug Administration, and could certainly give somebody a bad case of paralytic shellfish poisoning.

But not all toxins are equal, so a figure for concentration doesn't help us assess the danger unless we know the biological effectiveness of the substance. Therefore the Japanese team separated out the toxins from the shellfish samples and from the dinoflagellates themselves and analysed them. They also tested them for their potency by injection into mice.

Dinoflagellates from the Huon and Derwent estuaries yielded very similar toxins, even though collected from two localities 50 km apart and from two different bloom events separated by 2 months. But dinoflagellates cultured in the laboratory had slightly different toxic components, probably because the conditions and nutrient concentrations in culture differed from those in the natural environment.

Shellfish types varied significantly in the concentrations of the toxin but not in its chemical composition. Mussels generally contained more toxin than oysters because of their different mode of feeding.

However, the Japanese scientists discovered that the toxin recovered from shellfish differed slightly from that found in the dinoflagellates taken from the sea, probably due to biochemical changes wrought by the shellfish during its digestion and storage.

Most dinoflagellate toxins wreak their havoc on nerves. Specifically, they stop or impair the conduction of the nervous impulse, and this they do by blocking a channel in the nerve-cell membrane that allows sodium ions to enter.

In the normal course of events, following a signal, a sudden inrush of positively charged sodium ions from the fluid outside the nerve cell reverses the polarity of the membrane. This change moves along from one nerve cell to the next and constitutes a nervous impulse. Afterwards, all the sodium ions have to be pumped out again, which uses energy and takes a few milliseconds, during which time the cell cannot conduct another impulse.

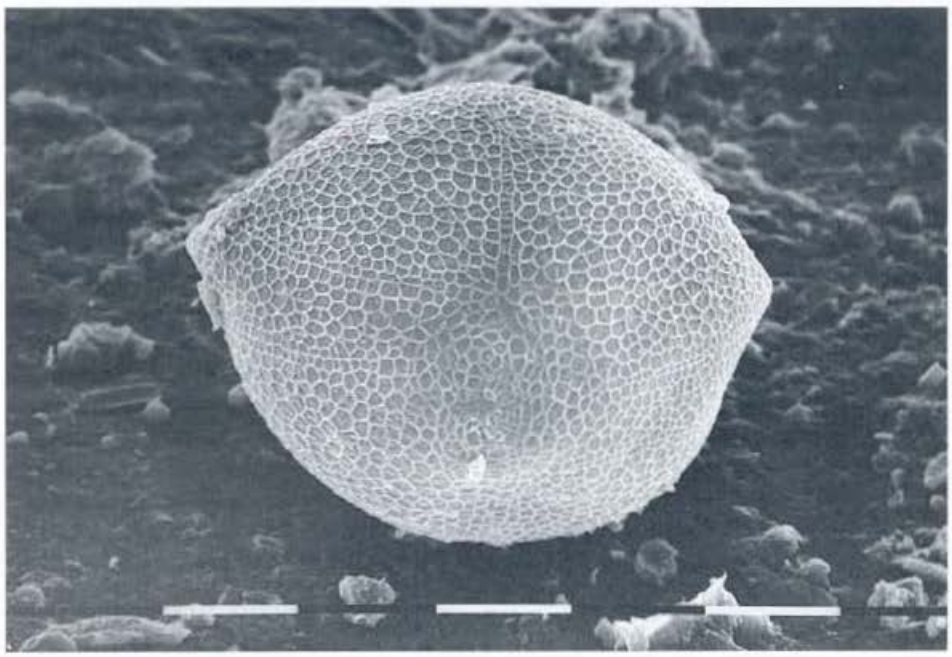
Blocking the channel through which the hordes of positively charged sodium ions re-enter effectively stops the flow of current along a nerve. The end result is paralysis, although vomiting and diarrhoea, tingling in the extremities, and problems with speech could all appear before that stage. We currently have no antidote to these dinoflagellate toxins, the only possible action being to pump the victim's stomach and provide artificial respiration. Unlike ciguatera, PSP poisoning at least has no after-effects.

For some reason the shellfish remain more or less unaffected by the toxins they accumulate, although their filtration behaviour may change slightly. But fish, presumably because their nerves are more similar to ours, can be affected. In sufficient quantity, the toxins can even kill them. Unlike indiscriminate fish kills brought about by oxygen depletion, deaths of this type will affect only those species that feed on other creatures that have eaten dinoflagellates.

Recent reports have suggested that some whale deaths may also be due to these toxins. At the end of 1987 and early 1988, 16 dead whales found along the coast of Massachusetts died after eating Atlantic mackerel with small traces of dinoflagellate toxin. This occurred at a time of year when no algal blooms were present in the area, raising the possibility that the mackerel had been carrying the toxin at relatively low levels — hence without dying — for some time.

Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish. Y. Oshima, M. Hasegawa, T. Yasumoto, G. Hallegraeff, and S. Blackburn. *Toxicon*, 1987, **25**, 1105–11.

When *G. catenatum* disappears from the water, its resting spore, shown here, may remain in the bottom sediment — ready to germinate when the necessary conditions present themselves. The scale bar is 10 μm.



research to provide a better understanding of the conditions for toxin production and for growth of the algae concerned. Then, if scientists can clearly correlate the blooms with weather patterns and nutrient levels, it may be possible in the future to issue definite warnings of danger times — thus enabling the farmers to know which years will be the best ones for ensuring a good product.

Roger Beckmann

More about the topic

Red tides in the Australasian region. G. M. Hallegraeff. *CSIRO Marine Laboratories Report* No. 187, 1987.

Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. G.M. Hallegraeff, D.A. Steffensen, and R. Wetherbee. *Journal of Plankton Research*, 1988, **10** (in press).

Gymnodinium catenatum blooms and shellfish toxicity in southern Tasmania, Australia. G.M. Hallegraeff, S.O. Stanley, C.J. Bolch, and S.I. Blackburn. *Proceedings of the International Symposium on Red Tides, Takamatsu, Japan*, 1988 (in press).

Toxic plankton blooms affect shellfish farms. G. Hallegraeff and C. Sumner. *Australian Fisheries*, December 1986.