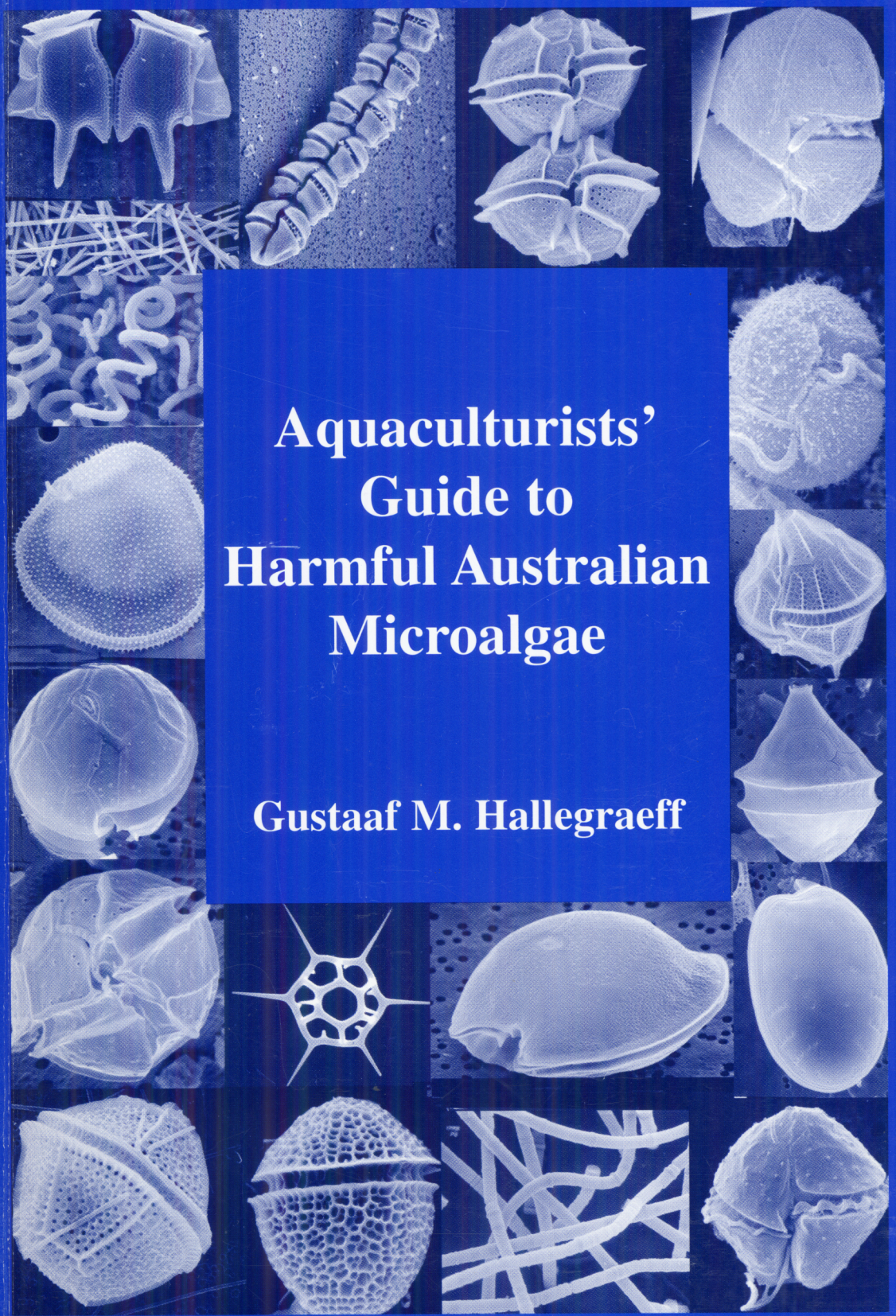

Aquaculturists' Guide to Harmful Australian Microalgae

Gustaaf M. Hallegraeff

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2nd Edition

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1st Edition February 1991
2nd Edition September 2002

Published by and available from:
School of Plant Science,
University of Tasmania,
GPO Box 252-55,
Hobart, Tasmania,
Australia

ISBN 1–86295–049

Printed by The Print Centre, Hobart

Foreword

Within the next decades, the increasing value of aquaculture production in Australia is expected to approach the decreasing value of the total catch of wild fish and shellfish.

The most successful aquaculture products are the edible oysters, pearl oysters, tuna and salmonids (combined farm gate value of about A$600 million), which are all dependent on good water quality. This expansion of production and value will not be without problems and one of the most damaging set-backs — harmful algal blooms — is already occurring. Algal blooms have the potential to wipe out fish farms virtually overnight and contamination of seafoods with algal toxins can poison human consumers of fish and shellfish. During the past two decades, there have been significant increases throughout the world in the economic losses and human health impacts due to harmful algal blooms. Many scientists believe that this increase in algal blooms may be stimulated by pollution from domestic, industrial and agricultural wastes as well as wastes from aquaculture operations themselves ("self-pollution").

The 1st edition of *Aquaculturists' Guide to Harmful Australian Microalgae* published in 1991 described 35 species of potentially harmful algae which had been found in Australian waters, but in this 2nd edition it was necessary to cover some 80 harmful species. Most of these species can be readily recognised using a simple monocular microscope (cost around $800). For the purpose of identification, emphasis has been placed on illustrations (line drawings, light micrographs and in some cases electron micrographs). The written species descriptions are accompanied by a summary of the known distribution of the alga in the Australian region, its toxicology and (where available) suggestions for counter measures. Relevant literature references and Internet access to a directory of Australian experts and institutions have been included.

With the help of this guide, fish and shellfish farmers can more effectively monitor for the presence of the algal species described and take the appropriate counter measures, thereby greatly reducing the hardship caused by algal blooms. This guide will also be valuable to fisheries and public health officials as well as persons involved in environmental water quality assessment.

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INTRODUCTION

1. Introduction

1.1 What are microalgae?

On land, plants are conspicuous everywhere, whereas in the sea the only plants visible to the casual observer are the seaweeds along our rocky shores and the seagrasses of shallow estuaries. The enormous productivity of the oceans, which cover 70% of the Earth's surface (our planet should more appropriately have been called "Water"!), is based on untold millions of unicellular microscopic algae, collectively called *phytoplankton* ('phyto' = plant; 'planktos' = made to wander). These minute plants range in size from 1/1000 of a millimetre to 2 millimetres and live a floating existence in the upper 200 m of the ocean, where sunlight is available for photosynthesis.

A single litre of seawater may contain as many as 1 million microscopic plant cells. Unlike plant life on land, which is dominated by a single category of organism (the 'higher plants'), plant life in the oceans includes representatives of as many as thirteen algal divisions. More than 10,000 species have been described. These organisms show an immense diversity of form, pigmentation and cellular structure, which are all adaptations to living in the oceanic environment. Phytoplankton species range from the primitive blue-green algae (more correctly called cyanobacteria), which were among the first living organisms on our planet, through the various golden-brown algal groups such as diatoms and dinoflagellates, to the green flagellates which are thought to have been the immediate precursors of the higher plants on the land. A representative selection of phytoplankton species from Australian waters is illustrated in Figs. 1, 2 and 3.

These minute plants are critical food for bivalve shellfish (oysters, mussels, scallops, clams) as well as the larvae of commercially important crustaceans and fish. All oxygen-breathing creatures, including humans, are indebted to the phytoplankton because through millenia of photosynthesis they have contributed significantly to the oxygen we breathe. Furthermore, much of the oil that we use today probably began millions of years ago when the sun shone on plankton drifting in prehistoric seas and produced, through photosynthesis, minute globules of oil within these cells.

Fig. 1 Representative selection of diatom species from Australian coastal waters; a. *Coscinodiscus*; b. *Thalassiosira*; c. *Planktoniella*; d. *Lauderia*; e. *Skeletonema*; f. *Leptocylindrus* ; g. *Cerataulina*; h. *Leptocylindrus*; i. *Chaetoceros*; j. *Bacteriastrum*; k. *Rhizosolenia sensu lato*; l. *Ditylum*; m. *Eucampia*; n. *Biddulphia* (*Odontella*); o. *Asterionellopsis*; p. *Thalassionema*; q. *Thalassiothrix*; r. *Pseudo-nitzschia*; s. *Nitzschia* (*closterium*); t. *Pleurosigma*; u. *Navicula*; v. *Surirella*; w. *Diploneis*; x. *Cocconeis*; y. *Licmophora* (not to scale)

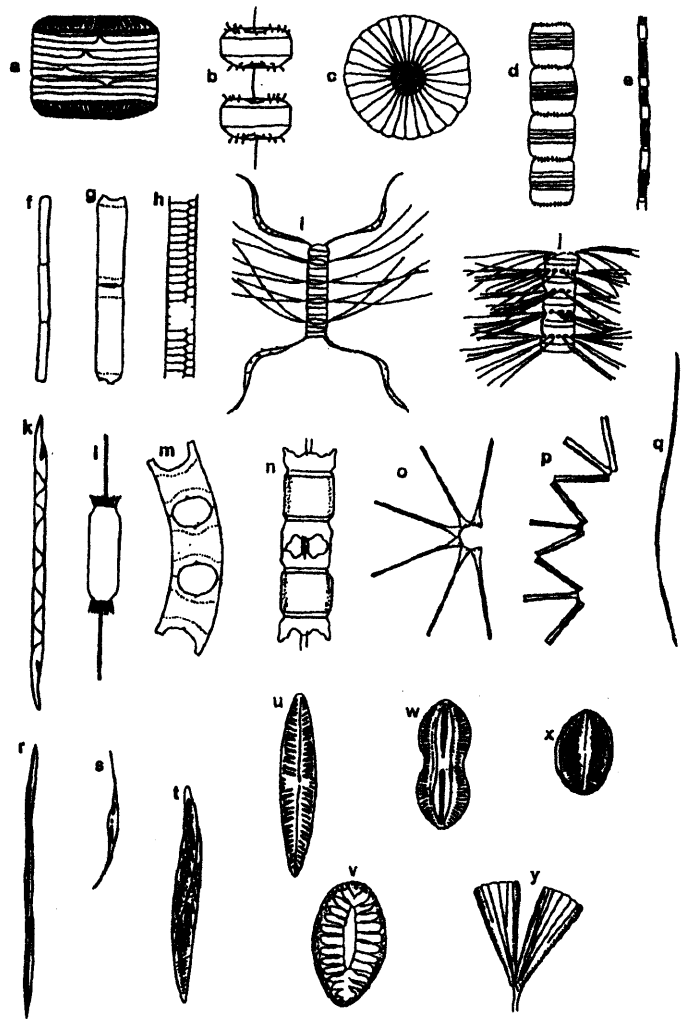


Fig. 2 Representative selection of dinoflagellate species from Australian coastal waters; a. *Prorocentrum*; b. *Dinophysis*; c. *Protoperidinium*; d. *Diplopsalis*; e. *Scrippsiella*; f. *Gonyaulax*; g. *Goniodoma*; h. *Alexandrium (catenella)*; i. *Ceratium*; j. *Gymnodinium*; k. *Gyrodinium*; l. *Cochlodinium*; m. *Amphidinium*; n. *Polykrikos*; o. *Noctiluca* (not to scale)

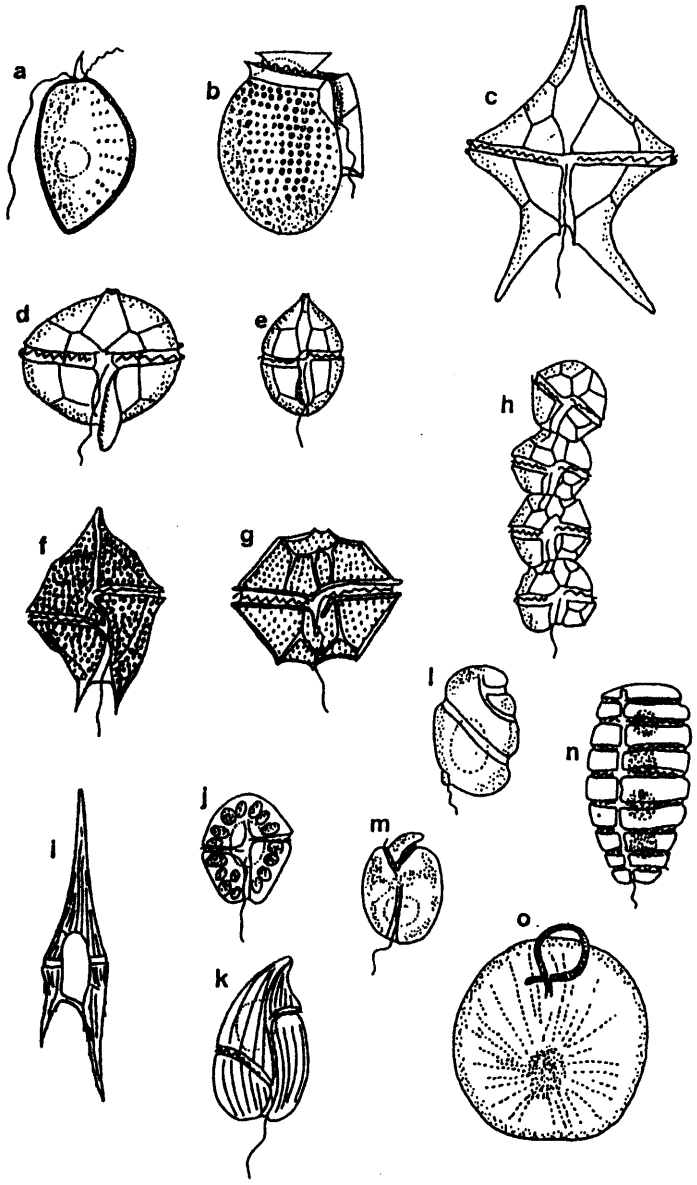
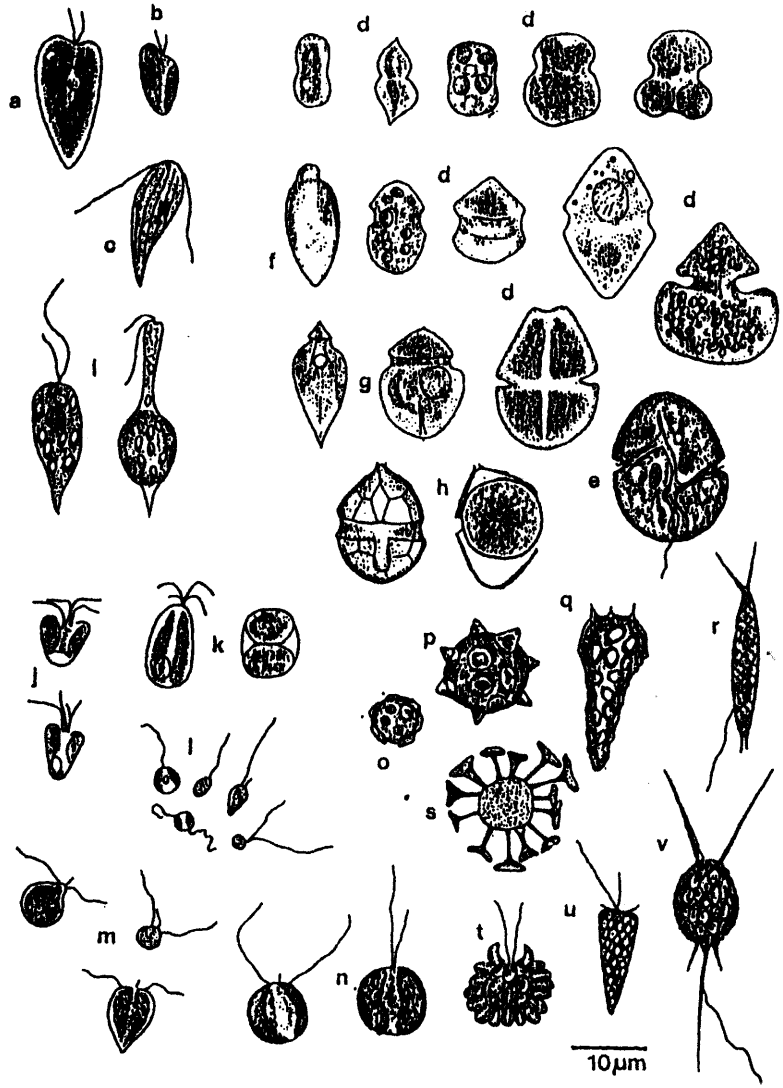


Fig. 3. Representative selection of small flagellate species from Australian coastal waters; a – c. Cryptomonads; d – h. Small dinoflagellates, d, e. gymnodinioids (several species); f. *Amphidinium*; g. *Oxytoxum*; h. *Scrippsiella*; i. Euglenoid *Eutreptiella*; j – l. Green flagellates, j. *Pyramimonas*; k. *Tetraselmis*; l. *Micromonas/ Mantoniella*; m – v. Golden-brown flagellates, m. *Chrysochromulina/ Prymnesium*; n. *Phaeocystis*; o – v. Coccolithophorids; o. *Emiliania*; p. *Gephyrocapsa*; q. *Syracosphaera*; r. *Calciosolenia*; s. *Discosphaera*; t. *Anthosphaera*; u. *Calciopappus*; v. *Acanthoica*; Scale bar = 10 µm.



1.2 Algal Blooms: The good and the bad

The colour of the sea is ever changing. The open sea is blue while nearshore waters are more green, and the water is more transparent in winter than in spring. These differences are caused by the proliferation of microalgae, which just like land plants use dissolved nutrients (nitrate, phosphate), carbon-dioxide and sunlight to grow and reproduce. In most cases algal "blooms" are beneficial for aquaculture because they mean more food for shellfish and larval fish. Sometimes the plankton blooms can become so dense (millions of cells per litre) that they obviously discolour the surface of the sea. It is believed that the first written reference to such a bloom ("red tide") appears in the Bible: "... all the waters that were in the river were turned to blood. And the fish that was in the river died; and the river stank, and the Egyptians could not drink of the water of the river" (Exodus 7: 20–21)

Algal blooms may also appear yellow, brown, green, blue or milky in colour, depending upon the organism involved. Most water discolourations are caused by motile or strongly buoyant species. Their high concentrations are achieved through a combination of high growth rates and vertical (behavioural) or horizontal (physical) aggregation. Dense algal concentrations are most strongly developed under stratified stable conditions, at high temperatures and following high organic input from land run-off after heavy rains.

The majority of these algal blooms appear to be completely harmless events, but under exceptional conditions, non-toxic bloom-formers may become so densely concentrated that they generate anoxic conditions that cause indiscriminate kills of fish and invertebrates in sheltered bays. Oxygen depletion can result from high respiration by the algae (at night or in dim light during the day) but is more commonly caused by bacterial respiration during decay of the algal bloom.

An essentially different phenomenon is the production by certain species (especially dinoflagellates) of potent toxins that can find their way through fish or shellfish to humans. In this case, even low densities of the toxic algae in the water column may be sufficient to cause such illnesses in humans as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), azaspiracid poisoning (AZP) and ciguatera fish poisoning (CFP). PSP can result from eating either bivalve shellfish or planktivorous fish (Fig. 4a), while DSP, NSP, AZP and ASP are caused by eating shellfish, and ciguatera by eating tropical fish (Fig. 4b). The toxins involved evoke a variety of gastro-intestinal and neurological symptoms in humans (Table 1) but rarely affect the nervous systems of fish or shellfish. Another group of toxins (ichthyotoxins) selectively kill fish by inhibiting their respiration.

On a global scale, close to 2,000 cases of human poisoning through fish or shellfish consumption are reported each year, and, if not controlled, the economic damage through reduced local consumption and reduced export of seafood products can be considerable.

Generally speaking, both the number and intensity of algal blooms seem to be on the rise and their geographic extent seems to be spreading. Four explanations for this apparent global increase have been suggested: (1) increased scientific awareness of toxic species; (2) increased utilisation of coastal waters for aquaculture; (3) stimulation of plankton blooms by coastal eutrophication

Fig. 4A Transfer of dinoflagellate toxins via shellfish to humans (Paralytic Shellfish Poisoning).

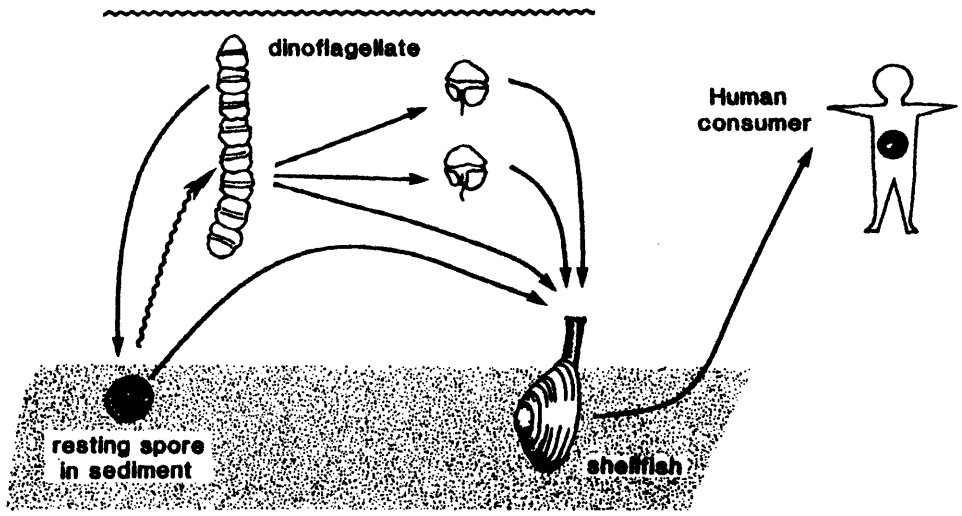
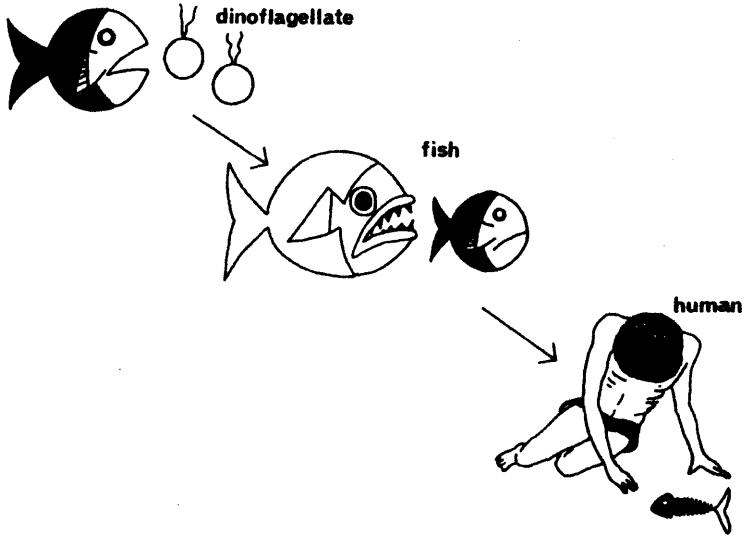


Fig. 4B Transfer of dinoflagellate toxins via tropical coral reef fish to humans (Ciguatera Fish Poisoning).



and/or unusual climatological conditions; and (4) transport of dinoflagellates or their resistant benthic resting cysts either in ships' ballast water or associated with movement of shellfish stocks from one area to another. While toxic plankton blooms appear regularly on a seasonal basis in temperate waters of Europe, North America and Japan, until the 1980s, they were relatively rare in Australasian waters. Tasmania has been the first state in Australia to suffer problems with toxic PSP dinoflagellates contaminating farmed shellfish (1986); New South Wales suffered a significant DSP-like outbreak in 1997; New Zealand had an outbreak of NSP in 1993 ; South Australian tuna farmers suffered major losses associated with algal blooms affecting fishes' gills in 1996, and ciguatera fish poisoning has been a public health risk in the Great Barrier Reef region since it was first reported in 1934.

Nowhere is the need for correct identification of plankton organisms more critical than in the study of the toxic species. As algal blooms are often composed of a single species, correctly identifying that species becomes crucial not only to understanding the bloom event but also when deciding on possible measures for its control. Some dinoflagellates (e.g. *Alexandrium* species) produce benthic cysts that can seed further blooms; the monitoring for these species must therefore also take into account the benthic cyst populations in the sediments.

Most algae have no common name and we have used scientific species names throughout this booklet. Even though the names may appear unpronouncable, they are the same for all countries of the world. Scientific species names are composed of two parts, the first part is the name of the genus and the second part is the name of the species. Algae belonging to the same genus, for example *Alexandrium catenella* and *Alexandrium minutum* are closely related. One problem with scientific names is that they are apt to change as more research is carried out. In general the oldest valid species description has priority, for example, *Gymnodinium mikimotoi* described by Miyake and Kominami in 1935 should be used in preference over *Gymnodinium nagasakiense* used for the same organism by Takayama and Adachi in 1984. The notation *Karenia mikimotoi* (Miyake et Kominami ex Oda) G. Hansen & Moestrup means that this species eventually was recognised to be distinct from *Gymnodinium*, and therefore transferred to a new genus *Karenia* by Hansen & Moestrup in 2000.

Three types of harmful microalgae are described in this guide: species that produce mostly harmless water discolourations (e.g. *Trichodesmium, Noctiluca, Scrippsiella*); species that are non-toxic to humans, but harmful to fish and invertebrates by damaging or clogging their gills (e.g. *Karenia mikimotoi, Heterosigma akashiwo*); and species that produce potent toxins that can find their way through the food chain to humans (e.g. *Gymnodinium catenatum, Alexandrium catenella, Gambierdiscus toxicus*) (Table 2).

1.3 Problems for Shellfish Farms

Poisonous shellfish neither look nor taste different from non-poisonous ones, and cooking and other treatments do not destroy the toxins. Shellfish farming areas infested by toxic dinoflagellate species therefore need to have costly monitoring programs to check for dinoflagellates in the water and, whenever these are present, seafood products must be tested regularly for toxins. The best known type of shellfish poisoning is *Paralytic Shellfish Poisoning* (PSP). One of the first recorded cases happened in 1793, when members of Captain George Vancouver's crew were poisoned by eating contaminated shellfish from British Columbia, Canada. These dinoflagellate toxins are so potent that a pinhead-size quantity (about 500 micrograms), which can easily accumulate in just one 100 gram serving of shellfish, could be fatal to humans. To date, the AOAC mouse bioassay is the only internationally, legally accepted method for PSP toxins. In this bioassay, 100 g of shellfish meat is macerated in a blender, gently boiled for 5 min with 100 ml 0.1 N HCl, and 1 ml of the clarified extract (pH adjusted to 2.0-4.0) injected intraperitoneally into a 20 g test mouse. The toxicity of the solution is established by measuring the time from injection to the mouse's last gasping breath, using a table of dose/death time relationships and correcting for the precise weight of the test animal. Substantial errors can result in estimating dose at long or short death times, and sample extracts therefore need to be diluted by trial and error to achieve death times in the range of 5-7 min. Test results can be expressed as mouse units (MU) or can be calibrated against pure saxitoxin and expressed as µg saxitoxin equivalents per 100 g of shellfish meat. The method is relatively easy to perform and requires no special equipment. The major disadvantage is its poor precision (± 20%) and insensitivity (detection limit is 50 µg saxitoxin /100 g).

In the past decade, stricter ethical standards in some countries for animal experiments and drawbacks inherent in the mouse bioassay have led to the development of alternative chemical assays. The most successful methods involve the alkaline oxidation of PSP toxins to fluorescent derivatives using periodic acid in sodium phosphate buffer, their separation by high performance liquid chromatography (HPLC) and detection by fluorometry. The HPLC methods developed by Sullivan and Oshima have found widespread following. The first method uses a polymer PRP column and gradient elution to separate the 10 most common PSP toxins in a single 20 min run. The second method uses a C8 bonded silica gel column and isocratic elution to separate all known 20 or so PSP toxin fractions in three separate chromatographic runs for sulphamate toxins, gonyautoxins and saxitoxins, respectively. Shellfish with simple toxin profiles (e.g. from *A. minutum*) can be adequately analysed with the Sullivan method, whereas complex toxin profiles (e.g. from *G. catenatum*) can be resolved only with the Oshima method. HPLC methods offer increased sensitivity (10-20 µg /100 g) and increased precision (5-10%) compared to mouse bioassays, and during red tide outbreaks can operate continuously with automated injection systems. The use of mass spectrometry (MS) as a detection system is desirable for confirmatory analysis of samples containing PSP toxins. However, mobile phase components, such as phosphate, ion-pair formers, etc., as well as periodic acid from the post-column derivatization unit prevents effective LC/MS coupling. Recently, modification of the HPLC/ FLD (fluorescence detection)/ MS (mass spectrometric detection) method by adding a combination of strong anion- and cation-exchangers enabled ion chromatographic separation of all PSP toxins relevant to seafood regulation within a single chromatographic run. These chemical methods still require extensive calibration against mouse bioassays before they can become legally accepted. At the

Tasmanian Department of Health and Community Services phytoplankton surveys are used for routine monitoring purposes, which then can trigger mouse bioassays on the basis of which closures of shellfish farms can be instigated.

A mouse neuroblastoma cell bioassay kit (MIST™ kit) for PSP (limit of sensitivity is 2 µg / 100 g) was developed by Jellett Biotek Ltd. However, the limited shelf life of such cell-based assays (1-3 weeks) and false positive results due to interfering substances can pose problems and this kit is no longer in production. An ELISA test kit for PSP is marketed as RIDASCREEN® by R-Biopharm, Darmstadt, Germany, while a SAXITOXIN TEST® kit is no longer in commercial production by Institut Armand-Frappier, Quebec, Canada. Jellett Biotek has recently developed a rapid semi quantitative immuno testkit for PSP toxins (MIST Alert™) which is currently undergoing validation trials. Radioreceptor binding assays for PSP and a method based on the saxitoxin binding protein saxiphilin have also been developed. The Asia Pacific Economic Cooperation (APEC) has established the principle of performance based criteria for regulatory purposes. That is, the ability to reliably determine whether the total of PSP toxins are present below or above the regulatory level of 80 µg /100 g is the ultimate criterion for choice of a particular analytical method.

Another, less severe, type of shellfish poisoning called *Diarrhetic Shellfish Poisoning* (DSP) was first documented in 1976 from Japan. Unlike PSP, no human fatalities have been reported and patients usually recover within three days. However, some of the dinoflagellate toxins involved may promote stomach tumours and thus produce chronic problems in shellfish consumers. The clinical symptoms of DSP may often have been mistaken for those of bacterial gastric infections (see section 10) and the problem may be much more widespread and serious than previously thought. Between 1976 and 1982 some 1300 DSP cases were reported in Japan, in 1981 more than 5000 cases were reported in Spain, and in 1983 some 3300 cases were reported in France. In Australia in late 1997/ early 1998 a DSP-like outbreak was caused by the consumption of pipis from Ballina, NSW, contaminated with pectenotoxin-2 seco acids derived from *Dinophysis* dinoflagellates.

An intraperitoneal mouse bioassay procedure developed by the Japanese Ministry of Health and Welfare has proved to be susceptible to producing false positive results due to the presence of contaminating free fatty acids in shellfish digestive glands at certain times of the year. This method has now been generally abandoned. A feeding method with white rats has been used successfully in the Netherlands . Shellfish digestive glands (*not* the complete shellfish meat as in PSP) are mixed with normal rat feed and offered to rats that have been starved for 24 h. After a 16 h observation period, an estimate of DSP toxicity is made on the basis of signs of diarrhea, the consistency of the faeces, and feed refusal. While the intraperitoneal mouse bioassay estimates total toxicity due to a range of lipophilic compounds, the oral dosage rat bioassay evaluates the diarrhetic effect of only certain DSP toxins. An HPLC method developed for separating DSP toxins by Lee has greatly advanced this field of research. Shellfish digestive glands are homogenised and extracted with 80% methanol in water, the DSP toxins esterified to fluorescent esters with 9-anthryldiazomethane (ADAM), and the toxins then separated by HPLC and monitored by fluorometry. Problems with the implementation of this method on a routine basis have been noted, resulting from the poor stability of the ADAM reagent and the presence of co-extractives in shellfish tissues, necessitating a silica column cleanup following the derivatization step. Wild Tasmanian mussels, contaminated by mixed blooms of *D. acuminata* and *D.fortii*, thus proved to contain okadaic acid, dinophysis toxin-1 and an uncharacterised fraction. Semi-quantitative immunoassay test kits for quick detection of OA and / or DTX1 are available from UBE Industries, Tokyo, Japan (DSP Check™ kit), and Rougier Bio-Tech., Montreal. While these chemical and immunological techniques represent an important step forward, they are still not sufficiently reliable as a routine method to accurately detect the full range of toxins involved. OA and DTX1 have been identified as inhibitors of protein phosphatases, and this property is now being explored in radioactive and colorimetric assays for their detection. The primary toxins produced by the dinoflagellates are water soluble DTX4 or derivatives, which cannot be detected by ADAM LC procedures but probably do not contribute significantly to DSP shellfish contamination.. Furthermore, the discovery of an esterase in the outer wall of dinoflagellates led to the recognition that hydrolysis during handling and extraction of shellfish and plankton can lead to erratic results. Liquid chromatography-Mass Spectrometry (LC-MS) is probably the only efficient and comprehensive option for analysis of lipophilic DSP toxins, but which to date is still limited by the lack of a universal extraction solvent and cleanup procedure. To date, no routine DSP assays are being carried out in Australia but LC-MS testing facilities are available at the Queensland Health Scientific Services, Queensland (Geoff Eaglesham). In New Zealand, positive mouse bioassays (death in 6 hrs) are followed by ELISA tests for okadaic acid and derivatives, and dilutions of the acetone extract are further examined for the confounding presence of NSP toxins (mouse deaths < 6 hrs).

As a result of the diversity of toxins involved and the methodological complication described, compared with other shellfish toxins such as PSP and ASP, the action level for DSP toxins set by regulatory authorities varies considerably from country to country. Japan uses 0.05 MU /g = 5MU / 100g edible tissue (1 MU= 4 µg OA), taking into consideration the weight that the digestive gland represents of total shellfish tissue, while Canada uses 1µg OA equivalent per g digestive gland but this does not take into consideration the variation in percentage of the weight of the digestive gland. At present satisfactory regulatory levels are only known for the OA series (including OA, DTX1,2 and the DTX3 complex) which are all phosphatase inhibitors and diarrhetic in action. Insufficient information is available to define regulatory levels for PTX, YTX and KT3.The Asia Pacific Economic Cooperation (APEC) has established the principle of performance based criteria for regulatory purposes. That is, the ability to reliably determine whether the OA series of DSP toxins are present below or above the regulatory level of 20 µg OA equivalent /100 g is the ultimate criterion for choice of a particular analytical method, and this action level has also been recommended for adoption by the Australia New Zealand Food Authority (ANZFA).

A third type of shellfish poisoning, called *Amnesic Shellfish Poisoning* (ASP), was first recognised in 1987 in Prince Edward Island, Canada. Most unexpectedly the causative toxin (domoic acid) is produced by diatoms (*Pseudo-nitzschia australis, P. multiseries*) and not by a dinoflagellate. While some of the causative organisms are known from Australian waters, no human health problems have been associated with them. During the early days of the Canadian crisis, domoic acid was extracted from shellfish using the standardized extraction procedure for mouse bioassay of PSP toxins but with longer observation times (up to 4 hrs). At toxin levels >40 µg/g domoic acid, mice exhibit characteristic scratching symptoms but this bioassay method is now generally considered not sensitive enough to accurately estimate the proposed quarantine level in Canada

of 20 µg/g tissue. HPLC is now the preferred analytical technique for the determination of domoic acid in shellfish. A very sensitive procedure, based on reaction with 9-fluorenylmethylchloroformate to form the fluorenylmethoxycarbonyl (FMOC) derivative and HPLC analysis with fluorescence detection, has been developed for monitoring of domoic acid in marine matrices such as seawater and phytoplankton. The detection limit is as low as 15 pg/mL for domoic acid in seawater. This procedure has recently been adapted to shellfish tissue. Domoic acid is extracted from shellfish tissues by homogenization with methanol-water (1:1, v/v). The concentration of domoic acid is determined by HPLC with ultraviolet absorbance detection. Sample extracts are injected following dilution and filtration of the crude extract or after cleanup on strong anion exchange (SAX) solid phase extraction (SPE) cartridges. The latter provides selective isolation of domoic acid and related compounds from interfering substances such as tryptophan, as well as preconcentration to facilitate analysis of trace levels. A photodiode array detector can be used to examine UV spectra in order to confirm domoic acid, but this option may not always be available. The detection limit is 20-30 ng/g (ppb). A receptor assay for ASP is based on binding to the kainate-glutamate receptor in rat brain synaptosomes, using ^{3}H-kainic acid as a standard. For the moment the proposed regulatory level in Canada of 20 µg/g tissue has been adopted by other countries screening for the toxin, and has also since been incorporated into the Australian Food standards code by ANZFA (expressed as 20 mg domoic acid / kg). This is based on the observation of an effect on certain consumers at an estimated domoic acid concentration of 200 µg. g^{-1} wet wt, with a factor of 0.1 applied for safety reasons (Health and Welfare, Canada). It is recognised that isomers of domoic acid also exist and possibly can contribute to toxicity.

While gymnodinioid dinoflagellates (e.g. *Karenia brevis*) responsible for *Neurotoxic Shellfish Poisoning* (NSP) are known from Australia, no human poisonings have been associated with them. The currently accepted method for the determination of NSP toxins is the American Public Health Association procedure based on diethyl-ether extraction of shellfish tissue followed by mouse bioassay. The APHA protocol is widely used in the United States, where the problem of NSP is most acute. After the detection of NSP in New Zealand in 1993, the MAF Regulatory Authority improved the sample preparation method by utilising acetone extraction of lipophilic components, followed by partitioning into dichloromethane. Sample extracts are prepared for mouse injection, and the bioassay results are calculated in mouse units.The above procedure is very effective in extracting unknown lipid-soluble toxins from shellfish containing NSP toxins, and the method presents certain advantages compared with the APHA protocol. However, the discovery of a novel bioactive compound (gymnodimine), produced by the dinoflagellate *Karenia mikimotoi*, a common species in New Zealand waters during neurotoxic events, has led the local health authorities to return to the APHA diethyl-ether extraction procedure. Gymnodimine is not extractable by diethyl-ether, but it causes very rapid mouse deaths when the dichloromethane procedure is used, while this compound is not considered to present a risk to human health. No testing facilities for NSP are available in Australia, but considerable expertise is available at the Institute of Environmental Science and Research and at AgResearch in New Zealand. A sensitive radioreceptor assay for brevetoxin is based on binding to site 5 on the voltage dependent sodium channel in rat brain synaptosomes, using ^{3}H- $PbTx_3$ for quantification. Neuroblastoma and ELISA assays for NSP are also under development. HPLC-MS is likely to become the method of choice in the future. Brevetoxins and their derivatives exert their toxic effect by specific binding to site-5 of voltage-sensitive sodium channels. In Florida and North Carolina, shellfish harvesting is suspended when cell concentrations of *K. brevis* exceed 5,000 cells/L or seafood toxins exceed 20 MU /100g. This latter regulatory level has also been adopted by New Zealand. Respiratory problems in humans occur at about 10^5-10^6 cells /L, while fish kills only happen at > 10^6 cells / L. Levels of NSP during the 1993 New Zealand shellfish poisoning outbreak reached 592 MU / 100 g. In January 1994, mussels from Tamboon Inlet on the Gippsland coast of Victoria contained 27.5 MU / 100 g in association with a *Karenia* type bloom (analyses by Medvet Science Pty Ltd using the Hannah method). At present there exists no regulatory limit for NSP in the Australian Food Standards code.

Many toxic dinoflagellates produce resistant, benthic resting stages (cysts) to survive unfavourable conditions. Accordingly, once an area has been infested by these dinoflagellates it appears impossible to eradicate them. Extreme caution should therefore be used when transferring shellfish from one area to another and when discharging ship's ballast water in sensitive aquaculture areas, because microscopic cysts could easily be transferred with them. When toxins are found in seafood products, control measures may include elimination of the toxin-accumulating hepatopancreas and gonads from scallops (not feasible with oysters or mussels) or avoidance of certain seafood species (e.g. mussels can become much more toxic than oysters). Depuration of toxic shellfish in laboratory tanks has met with limited success due to the long retention times (6–10 weeks) of most toxins. In many situations, therefore, there is no alternative to temporarily closing the shellfish farms but efficient toxin monitoring programs can reduce greatly the economic hardships for the farmers involved. Sensitive and accurate immunological test kits are now being developed ("colour stick test") that will, in future, make it possible to assay seafood for toxins at the farm site or even at the dinner table.

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Table 1. Clinical symptoms of various types of fish and shellfish poisoning

Paralytic Shellfish Poisoning (PSP)	Diarrhetic Shellfish Poisoning (DSP)	Amnesic Shellfish Poisoning (ASP)	Neurotoxic Shellfish Poisoning (NSP)	Ciguatera
Causative organism				
Alexandrium catenella;	*Dinophysis acuminata;*	*Pseudo-nitzschia multiseries; Pseudo-nitzschia pungens* **(some strains)**	*Karenia brevis (Florida): K. papilionacea, K. selliformis, K. bidigitata* **(New Zealand)**	*Gambierdiscus toxicus;*
Alexandrium minutum;	*Dinophysis acuta;*	*Pseudo-nitzschia pseudodelicatissima;*		*? Ostreopsis siamensis*
Alexandrium tamarense;	*Dinophysis fortii;*	*Pseudo-nitzschia australis*		*? Coolia monotis*
Gymnodinium catenatum;	*Dinophysis norvegica*	*Pseudo-nitzschia seriata*		
Pyrodinium bahamense var.compressum	*Prorocentrum lima*	*Pseudo-nitzschia delicatula*		
Symptoms				
Mild Case Within 30 min: tingling sensation or numbness around lips, gradually spreading to face and neck; prickly sensation in fingertips and toes; headache, dizziness, nausea, vomiting, diarrhoea.	After 30 min to a few hrs (seldom more than 12 hrs): diarrhoea, nausea, vomiting, abdominal pain.	After 3–5 hrs: nausea, vomiting, diarrhoea, abdominal cramps.	After 3-6 hrs: chills, headache, diarrhoea; muscle weakness, muscle and joint pain; nausea and vomiting	Symptoms develop within 12–24 hrs of eating fish. Gastro-intestinal symptoms: diarrhoea, abdominal pain, nausea, vomiting.
Extreme Case Muscular paralysis; pronounced respiratory difficulty; choking sensation; death through respiratory paralysis may occur within 2–24 hrs after ingestion.	Chronic exposure may promote tumor formation in the digestive system.	Decreased reaction to deep pain; dizziness, hallucinations; confusion; short-term memory loss; seizures.	Paraesthesia; altered perception of hot and cold; difficulty in breathing, double vision;trouble in talking and swallowing	Neurological symptoms: numbness and tingling of hands and feet; cold objects feel hot to touch; difficulty in balance; low heart rate and blood pressure; rashes. In extreme cases, death through respiratory failure.
Treatment Patient has stomach pumped and is given artificial respiration. No lasting effects.	Recovery after 3 days, irrespective of medical treatment.			No antitoxin or specific treatment is available. Neurological symptoms may last for months and years. Calcium and mannitol may help relieve symptoms.

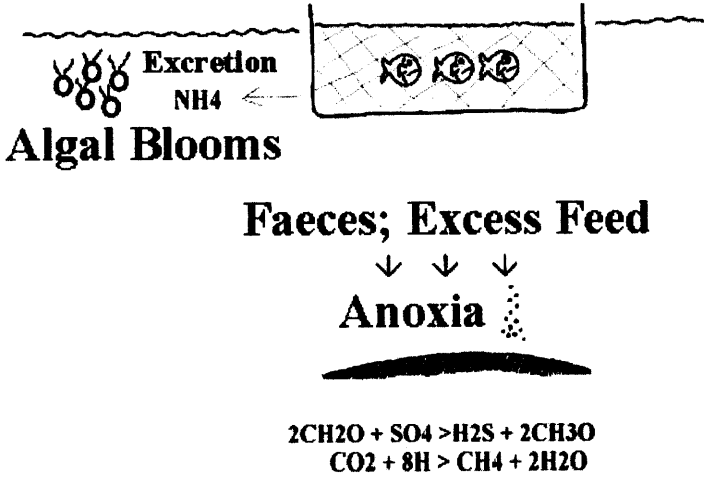
Table 2. Summary of Species Illustrated and their Impacts on Aquaculture, Human Health and the Environment

Species	Fig.	Impact
Dinoflagellates		
Gymnodinium catenatum	7	Paralytic Shellfish Poisoning
Gymnodinium aureolum	8A	Non-toxic
Gymnodinium pulchellum	8C,D,9B	Fish killer
Karenia brevis	9A	Fish killer; Neurotoxic Shellfish Poisoning
Karenia brevisulcata	9D	Fish killer
Karenia mikimotoi	9C	Fish killer; ?Neurotoxic Shellfish Poisoning
Karenia longicanalis	9E	Non-toxic?
Karlodinium micrum	8D, 9F	Fish killer
Gymnodinium uncatenum	10A	Water discoloration
Akashiwo sanguinea	10B	Water discoloration
Cochlodinium sp.	10C	Fish killer (some species)
Lingulodinium polyedrum	10D	Homoyessotoxin; unknown human potency
Gonyaulax polygramma	10E	Water discoloration
Protoceratium reticulatum	10F	Yessotoxin; unknown human potency
Alexandrium minutum	11A-C, 13A	Paralytic Shellfish Poisoning
Alexandrium catenella	11E,13 B	Paralytic Shellfish Poisoning
Alexandrium tamarense	11D, 12A-C	Paralytic Shellfish Poisoning (some strains)
Alexandrium ostenfeldii	13E	Paralytic Shellfish Poisoning (some strains)
Pyrodinium bahamense	14A-E	Paralytic Shellfish Poisoning (tropical)
Gambierdiscus toxicus	15A-D	Ciguatera Fish Poisoning (tropical)
Ostreopsis siamensis	15E, F	Palytoxin analogues
Coolia monotis	15G	Yessotoxin analogues
Scrippsiella trochoidea	16A	Water discoloration; Oxygen depletion
Noctiluca scintillans	16B	Water discoloration; Ammonia irritation
Pfiesteria shumwayae	16C,D	Fish kills
Kryptoperidinium foliaceum	16E	Water discoloration
Protoperidinium crassipes	16F	Azaspiracid Shellfish Poisoning (?some strains)
Prorocentrum cordatum=minimum	17A,B	Shellfish mortality/ mouse toxicity (?some strains)
Prorocentrum rhathymum	17C-E	Fast acting toxins
Prorocentrum lima	17G	Diarrhetic Shellfish Poisoning
Dinophysis fortii	18A,E	Diarrhetic Shellfish Poisoning
Dinophysis acuminata	18A,D	Diarrhetic Shellfish Poisoning
Phalacroma rotundatum	18F	Diarrhetic Shellfish Poisoning
Diatoms		
Pseudo-nitzschia pungens	19G	Amnesic Shellfish Poisoning (some strains)
Pseudo-nitzschia multiseries	19E	Amnesic Shellfish Poisoning
Pseudo-nitzschia pseudodelicatissima	19F	Amnesic Shellfish Poisoning (some strains)
Pseudo-nitzschia australis	19D	Amnesic Shellfish Poisoning
Thalassiosira mala	20A-D	Shellfish mortality; Clogging of fishing nets
Rhizosolenia cf. chunii	20E-H	Shellfish mortality; Bitter taste
Chaetoceros concavicornis / convolutus	21A-D	Finfish farm mortalities
Prymnesiophyceae		
Prymnesium parvum	22A-D	Finfish farm mortalities
Chrysochromulina polylepis	22E-G	Fish killer
Chrysochromulina leadbeateri	22H	Fish killer (?some strains)
Phaeocystis globosa	23A-E	Foam producer; clogging of fishing nets; ? Finfish farm mortalities
Chrysophyta		
Aureococcus anophagefferens /Aureoumbra lagunensis	23F,G	Shellfish and eelgrass mortality
Heterosigma akashiwo	23I-K, 24A	Finfish farm mortalities
Fibrocapsa japonica	23, 24K	Fish killer
Chattonella marina	23H,24C	Finfish farm mortalities
Chattonella globosa	24D	Fish killer
cf. Haramonas	23M	Fish killer
Dictyocha octonaria/ speculum	25A,C	Fish killer (under some conditions)

continued next page

Species	Fig.	Impact
Miscellaneous		
Mesodinium rubrum	25D,E	Water discoloration
Cyanobacteria		
Anabaena circinalis	26A,C	Paralytic Shellfish Poisoning (freshwater)
Cylindrospermopsis raciborskii	26B	Hepatotoxic (cylindrospermopsin) (freshwater)
Microcystis aeruginosa	26D	Hepatotoxic (microcystins) (freshwater)
Nodularia spumigena	26E,F	Hepatotoxic (nodularin) (brackish)
Trichodesmium erythraeum	26G	Water discoloration; ? skin irritation
Lyngbya majuscula	26H	Swimmers itch

Fig. 5 Problems for fish farms resulting from self-pollution. Excretory products from the fish (especially ammonia nitrogen, NH_4) can stimulate algal blooms, which reduce the fishes' appetite and reduce the oxygen concentration in the water. The build-up of faeces and waste feed underneath the cages can result in anaerobic micro- biological communities that release the toxic gases H_2S (hydrogen sulphide) and CH_4 (methane).



1.4. Problems for finfish farms

Finfish in intensive aquaculture systems are much more vulnerable to environmental perturbations and noxious algal blooms than wild stocks, since the latter have the freedom to swim away from problem areas. Rapid changes in temperature and salinity cause physiological stress related to osmoregulation, whereas an excess of sediment and algal particles in the water affects the delicate gill tissues and thus reduces the fishes' oxygen uptake. Virtually all algal blooms (even of non-toxic species) reduce the fishes' appetite and stressed fish show reduced growth rates and are more vulnerable to diseases (such as furunculosis which is a major bacterial problem in the northern hemisphere). In addition, algal blooms cause reduced oxygen concentrations . Other algal species can seriously damage fish gills, either mechanically (diatom *Chaetoceros convolutus*) or through producing haemolytic substances, neurotoxins and reactive oxygen species (dinoflagellates *Karenia mikimotoi*, raphidophytes *Chattonella marina*, *Heterosigma akashiwo*).

Unfortunately, the effluent from fish farms has the potential to stimulate algal blooms. In seawater, ammonia excreted by the fish is a key nutrient, whereas in freshwater phosphorus from excessive feeding can bring about algal blooms ("self-pollution"). Environmental gill disease which is causing problems for Tasmanian fish farms, can be brought about by toxic levels of free ammonia due to excessive stocking densities. Site selection thus becomes crucial to the success of intensive finfish aquaculture operations and sheltered inshore sites with limited circulation need to be avoided. Once established, regular diver checks are required to monitor the build up of faeces and uneaten food underneath fish cages. The biological oxygen demand of these waste products can result in anaerobic microbiological communities which release the toxic gases hydrogen sulphide and methane (Fig. 5). Where necessary, a rotation system of regularly moving fish cages (for example, every 3 months) may need to be introduced or alternatively giant propellors can be installed to move great volumes of quality seawater through the farm (in some European sites).

Monitoring of algal biomass (either by measuring chlorophyll concentration or recording water visibility with optical sensors) can give advance warning of algal bloom problems. If known problem species are involved, contingency plans need to be in place to allow towing cages away from bloom affected areas (e.g. during the 1988 algal blooms on the Norwegian coast more than 26,000 tons of fish in 1800 cages were moved from their permanent site into inland fjords). Fish losses in cages can also be reduced by stopping to feed fish since this attracts them to the surface and, in some cases, immediate harvesting of marketable fish before they can be killed by algal blooms may also be an option. The recent increase in fish farming worldwide is likely to lead to more reports of algal blooms but also to increased sophistication in bloom monitoring (e.g. the MARINET system in Norway which uses buoys with fibre optical sensors and transfers data by satellites).

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1.5 How to sample microalgae?

Two sampling approaches can be used:

a) *Qualitative* sampling — to determine which species are present (for example by using a plankton net).

b) *Quantitative* sampling — if a dangerous algal species is present, to determine whether it is increasing or decreasing in abundance and whether it is so abundant as to cause a problem. This requires the collection of "water bottle" samples (1 litre) from different depths.

As algal blooms can develop very quickly, sampling should be carried out at least at weekly intervals. Algal blooms can often accumulate at discrete depths within the water column, so vertical net hauls (from surface to bottom) are the best general purpose sampling device. The best plankton nets are made of monofilament nylon with mesh spacings of 20 micrometres. A suitable conical net (23 cm mouth diameter, 40 cm length, 4.5 cm codend diameter) is available from Swiss Screens for about $250. At the end of the net (the cod end) there is a container (100 ml) to retrieve the retained organisms (Fig. 6a).

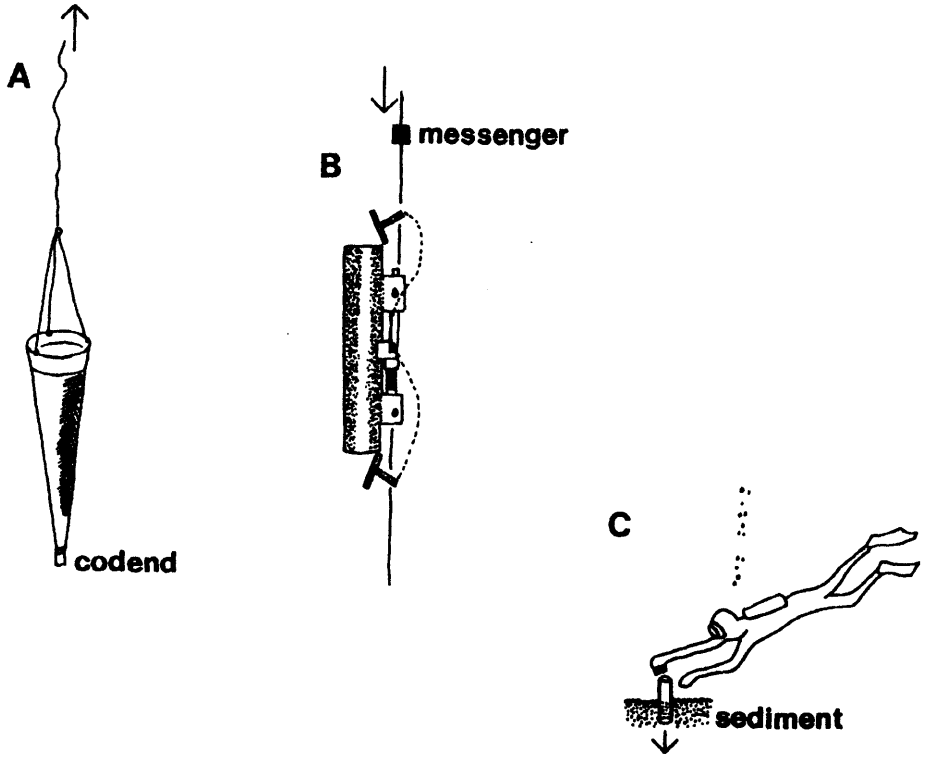
In general it is best to examine microalgae under the microscope as soon as possible after collection while they are still in the living state. If this is not possible, the sample needs to be *preserved* for later examination. A suitable preservative is Lugol's iodine fixative. It is prepared by dissolving 100 g potassium iodide in 1 litre of distilled water and then dissolving 50 g of crystalline iodine in this. Finally, 100 mls of glacial acetic acid are added. This fixative mixture is added to the sample until the water becomes a weak tea colour. Lugol has the disadvantage that it overstains many algal cells thus obscuring details important for identification. For some species 2% formaldehyde (buffered with hexamine) or 2% glutaraldehyde (buffered with phosphate buffer) are preferred. All these chemicals can readily be obtained through most scientific supply companies.

Quantitative samples can be collected with "water bottle" samplers. These commercially available samplers (e.g. Nansen, Niskin, Van Dorn) are lowered in the open position to the selected depth and a messenger is then lowered down the wire to trigger the closing mechanism in the bottle (Fig. 6b). Alternatively, a narrow-neck wine bottle, weighted at the bottom and with its cork in, could be lowered down a wire. At the selected depth the cork, which is attached to a separate line, is pulled out to allow the bottle to fill. SCUBA divers can also fill bottles at different depths. Water samples of 1 litre volume or more need to be preserved and can be concentrated by settling or centrifugation before further microscopic examination.

For plankton species (such as toxic dinoflagellates) which produce resting spores (cysts), the examination of *sediment samples* is also recommended. The choice of sampling site should be based on an inspection of local bathymetric maps (depth contours) and knowledge of local currents. Black undisturbed sediments from deep basins offer better opportunities for cyst surveys than coarse sandy sediments characteristic of strong current regimes. Perspex coring tubes (20

– 40 cm long) can be pushed into the sediment by SCUBA divers (Fig. 6c) and then withdrawn and capped. Bottom samplers such as dredges or grab buckets often lose the light fluffy material at the sediment surface and are less suitable.

Fig. 6 Sampling equipment used for the study of algal blooms; A. nylon plankton net (20 micron mesh) with codend container to retrieve the retained organisms; B. water bottle sampler, which is lowered in the open position; a messenger is then used to trigger the closing mechanism; C. SCUBA diver collecting a sediment sample by pushing a coring tube into the sediment and then capping it.



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SPECIES DESCRIPTIONS

2. DINOFLAGELLATES

The dinoflagellates are single-celled golden-brown algae, with a large nucleus with clearly visible chromosomes. They have two flagella, one protruding from the horizontal girdle groove and the other from the vertical sulcus groove, respectively. Some species are bounded by a membranous covering only (unarmoured dinoflagellates) while others have a cell covering of cellulose plates (armoured dinoflagellates). These plates are subdivided into several plate series, usually in parallel with the girdle groove. Apical plates touch the apical pore on top of the cell, precingular plates touch the girdle groove on the upper cell half (epitheca), postcingular plates touch the girdle groove on the lower cell half (hypotheca) and antapical plates are found at the bottom of the cell.

2.1 *Gymnodinium catenatum* Graham 1943
Figs. 7 A,C-H

Description

The green-brown cells form characteristic snake-like chains, most commonly 4, 8 or 16 cells but occasionally up to 64 cells long (Figs 7A,D). Each cell is 23-33 µm long and 27-36 µm wide. In *G. catenatum* the cells show a surface covering of roughly hexagonal amphiesmal vesicles (Fig. 7F). This species is unusual among the unarmoured dinoflagellates in that it withstands even harsh preservatives such as formaldehyde albeit with some deformation. The girdle groove is deep and shows a left-handed displacement over 1/5 - 1/3 of the cell length. The sulcus is deep and extends well into the upper and lower cell halves. The top of the cell has a horse-shoe - shaped apical groove (Fig. 7E).Under unfavourable conditions the chains break up to form single cells which can be confused with other *Gymnodinium* species (Fig. 7G).The chains can be confused with the non-toxic *Gymnodinium (Gyrodinium) impudicum,* which is narrower and forms mostly short (4-celled) chains (Fig.7B). Chains can also be confused with *Alexandrium catenella,* which forms short, straight chains (4 or 8 cells long) but its cells are covered with cellulose plates (Fig.11E).

As part of its life cycle, this species produces a brown, spherical, benthic resting cyst (42-52 µm diameter) with microreticulate surface markings that reflect the pattern of amphiesmal vesicles (Fig. 7H). Markings reflecting the girdle, sulcus and apical groove are also visible. These resting cysts can germinate after a maturation (dormancy) period as short as 2 weeks (even in the dark and at 4°C), thus seeding new plankton blooms in the water column. The similar microreticulate cyst-producing species *G. nolleri* and *G.microreticulatum* are apparently non-toxic.

Distribution

Within the Australian region, sediment cyst evidence suggests that this dinoflagellate species was introduced into Tasmanian waters around 1973 and started to cause blooms in the Huon River, d'Entrecasteaux Channel and Derwent River after 1980 and most seriously in 1993. The organism was first seen off Lorne in Victoria in 1993, at Port Lincoln, SA, in 1996, and off the NSW coast (Bega) in 2002. It has also been recorded from Argentina, Spain, Portugal, Italy, Phillipines, Palau, Singapore, Malaysia, Japan and Mexico. A major *G.catenatum* bloom also occurred in New Zealand in 2000.

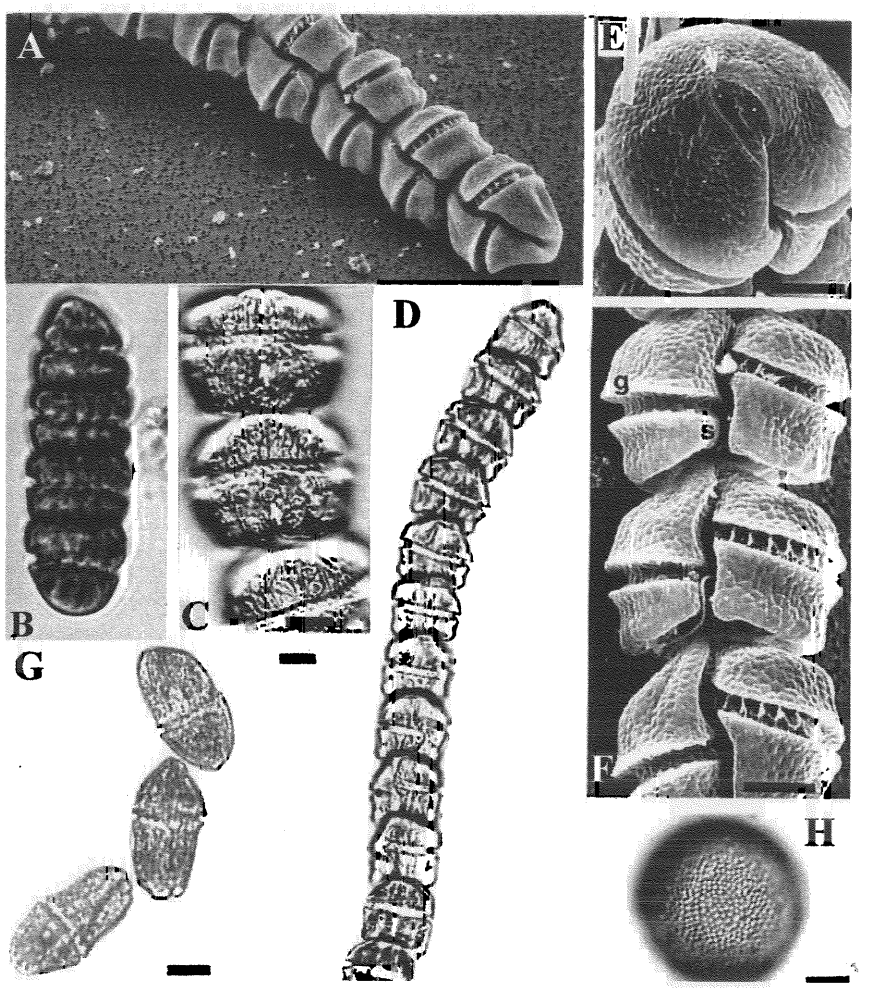

Fig. 7 Dinoflagellate *Gymnodinium catenatum* from Tasmanian waters and related species.

Figs. 7, A (SEM), D (LM). Characteristic snake-like chains; Fig.7B. Short 4-celled chain of the morphologically similar *Gymnodinium impudicum* from New South Wales; Fig. 7C (LM). Living cells showing branching chloroplasts with pyrenoids (arrows); Fig. 7E (SEM). Horse-shoe shaped apical groove on top of a cell (arrow);Fig. 7F (SEM). Cells in a chain, showing deep girdle (g) and sulcus (s) grooves and surface covering of hexagonal vesicles ; Fig. 7G (LM). Solitary, elongated cells from an old culture; Fig. 7H (LM). Brown, spherical resting cyst with microreticulate markings.

Micrographs Figs.7A,E,F. by C.Bolch; Scale bars: 10 µm.

Toxicology

Producer of paralytic shellfish poisons, which accumulate in shellfish such as oysters, mussels and scallops and thus can cause neurological and gastro- intestinal problems in human shellfish consumers. Although the toxin fractions produced by *G. catenatum* (mainly sulfamate saxitoxin derivatives) are less potent than the toxins of, for example, *Alexandrium minutum*, there is concern that preparation and cooking of seafoods can convert them to carbamate derivatives which are much more toxic. Only mild human poisonings have been recorded in Tasmania but human fatalities (3 children) have occurred in Mexico. In 1976, more than 100 people in Western Europe were made ill by Spanish mussels contaminated by *G. catenatum*. Dinoflagellate blooms in Tasmania were most extensive in 1986, 1991 and 1993 when they caused the closure of up to 15 shellfish farms. No effect on salmonid fish farms in the area has ever been observed, most likely because caged fish eat little natural food. Shellfish farm areas affected by this species are regularly surveyed for the dinoflagellate in plankton tows, and when they are present the shellfish meat is tested for toxins using mouse bioassay and/or HPLC techniques. Shellfish containing more than 80 µg toxin per 100 g meat are considered unsafe for human consumption.

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2.2 *Gymnodinium aureolum* (Hulburt) G.Hansen
Fig.8A

Basionym: *Gyrodinium aureolum* Hulburt 1957; *Non* North European *Gyrodinium aureolum*

Description

Small dinoflagellate, 18.9-38.7 µm long, 13.9-32,7 µm wide. Cingular displacement is 17-21 % of body length. A loop-shaped apical groove runs counterclockwise from the sulcal extension. The nucleus is generally located in the central part of the cell. The photosynthetic pigments of this species include peridinin and not fucoxanthin. This small dinoflagellate was first described by Hulburt 1957 from a Woods Hole pond, but without illustrating the apical groove. Subsequently, this species name has been widely but erroneously used for the European fish-killing species now designated as *Karenia mikimotoi.* Based on the presence of a loop-shaped apical groove, it was transferred to *Gymnodinium* by Hansen et al. 2000. Fragile unarmoured dinoflagellates may be difficult to identify; fixatives such as lugol and glutaraldehyde cause deformation of the cell shape, while living cells can deteriorate quickly under the microscope. Unfortunately, the diagnostic apical groove is a very delicate structure and it disappears almost immediately when the cells cease swimming.

Distribution

This species is known from Tasmania (*Gyrodinium* sp.1, Bolch & Hallegraeff 1990, Long Bay, Port Arthur; and East Coast Tasmania), Gippsland Lakes, Victoria, and from Adelaide, SA.

Toxicity

Not usually associated with fish kills.

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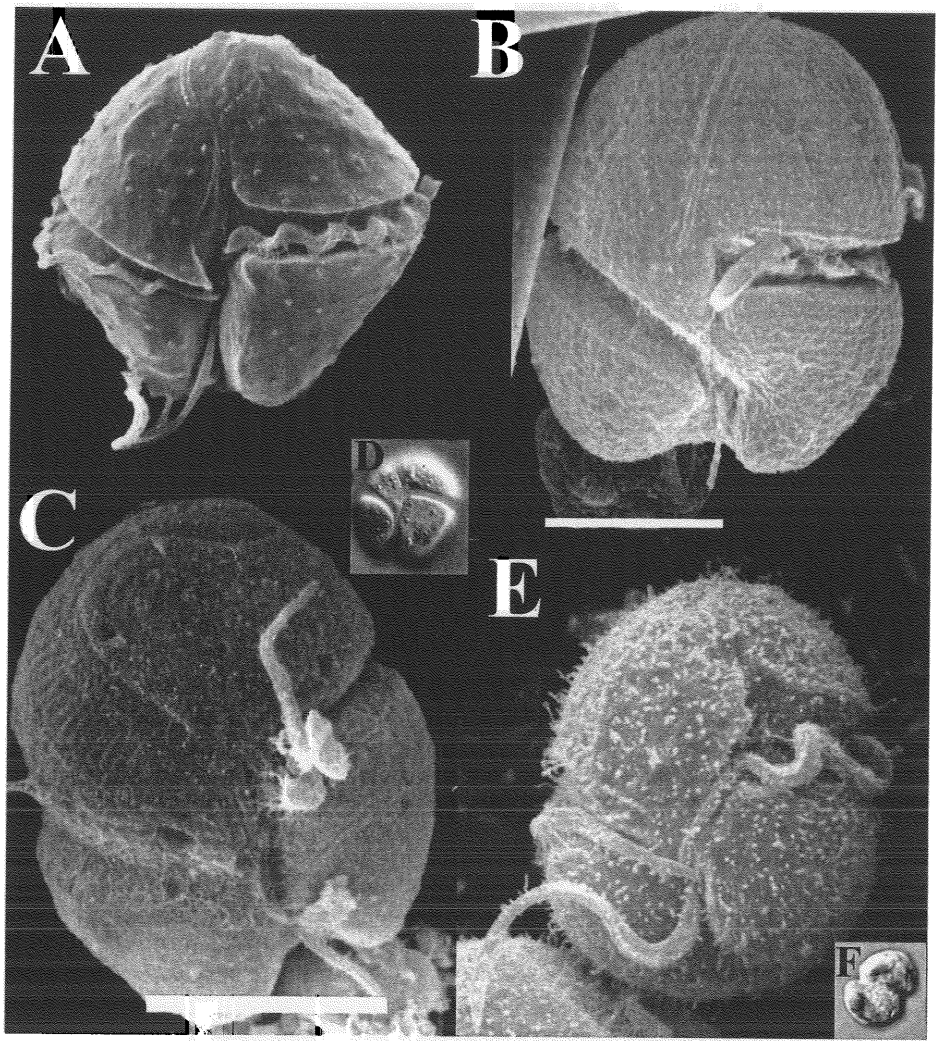

Fig. 8. Dinoflagellate *Karenia mikimotoi* and related species from Tasmanian waters.

Figs. 8A. *Gymnodinium aureolum* (non-toxic) with horse-shoe shaped apical groove; Fig.8B. Undescribed *Karenia* species from Tasmanian waters with straight apical groove; Fig.8C,D. *Gymnodinium pulchellum* with sigmoid apical groove; Fig.8E,F. *Karlodinium micrum* with straight apical groove and ventral pore.

Micrographs by C.Bolch. Scale bars: 10 µm.

2.3 *Gymnodinium pulchellum* Larsen 1994
Figs.8C,D,9B

Synonyms: *Gymnodinium* type '84K (Onoue *et al.*, 1985); *Gymnodinium* sp.1 (Takayama, 1985)

Description

The cells are broadly oval and only slightly flattened. The girdle is pre-median and describes a descending spiral which is displaced 1-1.5 girdle widths. The sulcus intrudes for a short distance onto the episome which also has a sigmoid apical groove. It has several irregular, chloroplasts with pyrenoids. The nucleus is located in the left part of the cell. Length 16-25 µm, width 11-16 µm. *Gymnodinium pulchellum* is distinguished from *K. mikimotoi* by the sigmoid apical groove. The precise generic affiliation of this species, in between *Karenia* and *Karlodinium*, is currently under revision.

Distribution

Gymnodinium pulchellum was described from the Melbourne area, Australia (Larsen, 1994). It has been reported also from Tasmanian waters and from Japan, Florida, New Zealand and Italy. Massive fish kills in Port Phillip Bay, Melbourne, in the early 1950s were associated with brown water discolorations caused by a similar *Gymnodinium* species.

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2.4 *Karenia brevis* (Davis) G.Hansen et Moestrup 2000
Fig.9A

Synonyms: *Gymnodinium breve* Davis, *Ptychodiscus brevis* (Davis) Steidinger

Description

The cells appear butterfly-shaped, almost square in outline, but with a prominent apical process; strongly flattened dorso-ventrally. The girdle is not or only slightly displaced and then describing a descending spiral; three vertical ridges are present near the distal end of the girdle. The sulcus intrudes onto the episome. An apical groove extends from near the sulcal extension on the episome across the apical process and onto the dorsal side of the cell. There are numerous chloroplasts with pyrenoids.

Distribution

K. brevis -like species have been reported in Australian waters from the Gippsland Lakes, Tasmania, Port Lincoln and Western Australia. . Some of them may represent different, closely related, species including *K. papilionacea, K. bidigitata* and *K. selliformis* to be described from New Zealand (Haywood & Steidinger, in prep).

Toxicology

The toxins are responsible for neurotoxic shellfish poisoning (NSP) which may be harmful to both fish and mammals, while aerosolization of the toxins may be responsible for asthma-like symptoms. NSP has been restricted so far to the southeastern part of the USA, particularly the Gulf of Mexico, and New Zealand. In Florida shellfish harvesting is banned at 10^4-10^5 cells/L, respiratory irritation occurs at 10^5-10^6 cells/L, and fish kills happen at $>10^6$ cells/L. Shellfish harvesting is reopened based on mouse bioassays for NSP.

References

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2.5 *Karenia brevisulcata* (Chang) G.Hansen & Moestrup 2000
Fig.9D

Basionym: *Gymnodinium brevisulcatum* Chang

Description

This is the smallest species in the *mikimotoi* group, 18-37 µm long; 18-22µm wide, slightly flattened, with a round nucleus, either located in the left hypocone, or stretching horizontally from the left to right hypocone. Girdle displacement is 11-27% of body length. This species has a straight, very short apical groove, located to the right of the sulcal axis, and extending 1/3 to 1/2 on the ventral side, and 1/3 down the dorsal side of the epicone A small triangular sulcus extension occurs into the epicone.

Toxicology

This species caused extensive fish kills in summer 1998 in Wellington harbour, New Zealand.

References

Chang, F.H. .(1999). *Gymnodinium brevisulcatum* sp.nov. (Gymnodiniales, Dinophyceae), a new species isolated from the 1998 summer toxic bloom in Wellington Harbour, New Zealand. Phycologia 38, 377-384.

2.6 *Karenia mikimotoi* (Miyake et Kominami ex Oda) G. Hansen & Moestrup 2000

Fig.9C.

Synonyms: *Gymnodinium mikimotoi* Miyake et Kominami ex Oda, *Gymnodinium nagasakiense* Takayama et Adachi, *Gymnodinium* type-'65 (Iizuka and Irie, 1969), *Gyrodinium aureolum* Hulburt, sensu Braarud and Heimdal (1970).

Description

The cell outline is variable, ovate to almost round, flattened dorso-ventrally. The girdle is wide and describes a descending spiral which is displaced about one fifth of the cell length. The sulcus continues for a short distance onto the episome, where an apical groove extends in a straight line from near the sulcal intrusion across the apex and a short distance down on the dorsal side of the cell. There are several more or less oval chloroplasts, each with a conspicuous pyrenoid. The nucleus is located in the left side of the hypocone. Length 24-40 µm, width 20-32 µm.

Distribution

This species is widely distributed and has formed blooms in Australasia, Denmark, Ireland, Japan, Korea, Norway and Scotland. The 1993 New Zealand outbreak of neurotoxic shellfish poisoning is now believed to have been largely caused by *K. mikimotoi*.

Toxicology

Karenia mikimotoi produces both hemolytic and ichthyotoxins, and has caused damage in fish farms in Northern Europe, Japan and New Zealand (Marlborough Sounds, 1987). This species has been implicated in causing massive kills of benthic invertebrates (lugworms, sea-urchins etc.) and of both wild and farmed fishes. In 1933, pearl oyster losses near Nagasaki in Japan amounted to $7 million. Young fish and fish contained in nets and pens are especially vulnerable. Characteristic histopathological symptoms are a severe necrosis and sloughing of epithelial tissues of the gills and digestive system. The precise mechanism of fish killing is still poorly understood; both purely physical mechanisms such as increased seawater viscosity due to secretion of algal mucilages and chemical mechanisms such as the production of haemolytic substances and reactive oxygen species have been proposed. Sensitive aquaculture areas need to be protected by intensive plankton monitoring programmes (eg. MARINET in Norway) and contingency plans need to be in place to allow cages being towed away from bloom-affected areas. Where dinoflagellate blooms are confined to surface waters, fish losses in cages can also be reduced by not feeding fish as this attracts them into the surface bloom area. No ill effects are known from human consumption of fish from bloom areas.

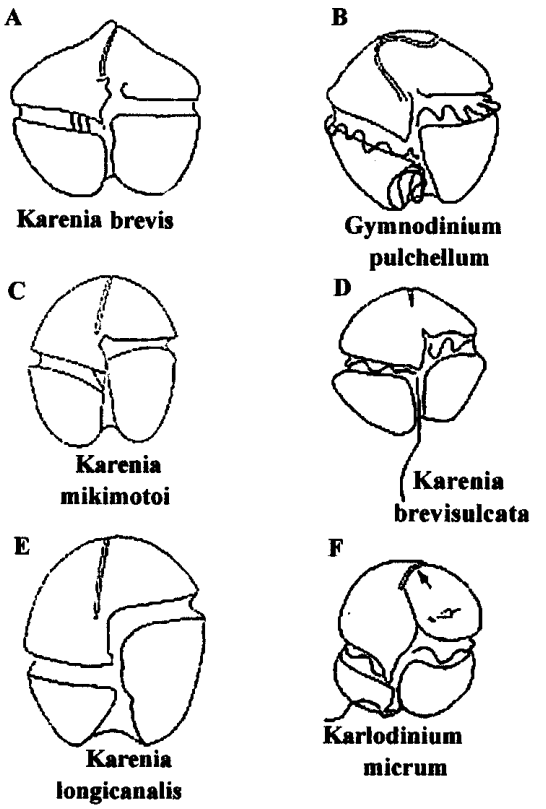

Fig. 9. Diagrammatic summary of gymnodinioid dinoflagellates. Fig.9A. *Karenia brevis*; Fig. 9B. *Gymnodinium pulchellum*; Fig. 9C. *Karenia mikimotoi*; Fig.9D. *Karenia brevisulcata*; Fig.9E.*Karenia longicanalis*; Fig.9F. *Karlodinium micrum.*

References

Partensky, F., Vaulot, D., Coute, A. & Sournia, A. (1988). Morphological and nuclear analysis of the bloom-forming dinoflagellates *Gyrodinium cf. aureolum* and *Gymnodinium nagasakiense*. J. Phycol. 24, 408-415.

Roberts, R.J., Bullock, A.M, Turner, M., Jones, K. & Tett, P. (1983). Mortalities of *Salmo gairdneri* exposed to cultures of *Gyrodinium aureolum*. J. Mar. Biol. Ass. U.K. 63, 741-743.

Takayama, H. & Adachi, R. (1984). *Gymnodinium nagasakiense* sp. nov., a red-tide forming dinophyte in the adjacent waters of Japan. Bull. Plankton Soc. Japan 31, 7-14.

Tangen, K. (1977). Blooms of *Gyrodinium aureolum* (Dinophyceae) in North European waters, accompanied by mortality in marine organisms. Sarsia 63, 123-133.

2.7 *Karenia longicanalis* Yang, Hodgkiss et Hansen 2001
Fig. 9E

Description

Cells 17.5-35 µm long, 10-22.5 µm wide, slightly flattened. The nucleus is round, centrally located and surrounded by a nuclear capsule. The cingulum displacement is 22% of the cell body. The apical groove is straight, located to the right of the sulcal axis, and extending 2/3 down the dorsal side of the epicone.

Distribution

This species was described from a bloom in Hong Kong harbour in May 1998.

Toxicology

While *K. longicanalis* was not associated with fish kills in Hong Kong, a similar but not identical species (coded Gy2DE; Fig.8B) was associated with problems for finfish farms in Southern Tasmania (Murdunna) in 1989.

References

Yang, Z.B., Hodgkiss, I.J. and Hansen, G. (2001). *Karenia longicanalis* sp.nov. (Dinophyceae): a new bloom-forming species isolated from Hong Kong., May 1998. Bot.Mar. 44, 67-74.

2.8 *Karlodinium micrum* (Leadbeater & Dodge) J. Larsen 2000
Figs.8E,F, 9F

Synonyms: *Woloszynskia micra* Leadbeater & Dodge, *Gymnodinium micrum* (Leadbeater et Dodge) Loeblich III, non *Gymnodinium galatheanum* Braarud sensu Kite & Dodge, *Gyrodinium galatheanum* (Braarud) Taylor

Description

This very small oval to round gymnodinioid dinoflagellate, length 9-17 µm, width 8-14 µm, is characterized principally by an amphiesma with arrays of plug-like structures in a hexagonal configuration (only visible by electron microscopy). The apical groove is straight. The girdle is deeply incised, describing a descending spiral which is displaced almost one fifth of the cell length. The sulcus is strongly deflected in the inter-cingular region and extends onto the episome The cell contains usually 2 chloroplasts, one in the epicone and one in the hypocone which are visible by LM, and which have fucoxanthin or fucoxanthin derivatives as accessory pigments.The nucleus is located in the central part of the cell. The cells have chloroplasts with internal, lenticular pyrenoids.

Distribution

This species has been previously been reported from the North and South Atlantic. It is presumably wide-spread, but easily overlooked because of its small size. The first unambiguous documentation of this species in Australian waters derives from a fish-kill event in June-July 1991 in Lake Illawara, near Wollongong, NSW, involving dead eels, bream, flathead and mullet (S.Hardiman, pers.comm..). A recurrence of fish kills in Lake Illawara occurred in Dec. 2000 in association with a similar gymnodinioid bloom. Similar fish-kills in association with a bloom of a small (12-17 µm) gymnodinioid were reported from the Murray River, Western Australia, in June 1999. A reccurrence of such event occurred in the Swan River in March 2001.

Toxicology

Intraperitoneal mouse assays on a small culture were negative. The histopathological effects on juvenile cod (*Gadus morhua*) were documented by Nielsen (1993). This species is also toxic to mussels .

References

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Daugbjerg, N., G.Hansen, J.Larsen & O.Moestrup (2000). Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycologia 39:302-317.

Nielsen, M.V. (1993). Toxic effect of the marine dinoflagellate *Gymnodinium galatheanum* on juvenile cod *Gadus morhua*. Mar. Ecol. Progr. Ser. 95, 273-277.

2.9. *Gymnodinium uncatenum* (Hulburt 1957) Hallegraeff comb.nov. Fig.10A

Basionym: *Gyrodinium uncatenum* Hulburt 1957, Plate 4, Figs. 1-3

Description

Large gymnodinioid dinoflagellate, 40-54 µm long, 28-33 µm long, laterally flattened, with a deep excavation in the hypocone. The cells change shape and size as cultures age. Girdle displacement 1/3-1/2 of body length (less with moribund cells). Large spherical nucleus in anterior portion of epicone. Many chloroplasts are radially oriented. *Gymnodinium instriatum* (Freudenthal et Lee 1963) Coats 2002= *Gyrodinium instriatum* is very similar to *G. uncatenum*, from which it is claimed to "differ chiefly in being less dorsi-ventrally compressed, with a shallower antapical sulcus groove, chromatophores cortically displaced instead of centrally located, and occasional anterior displacement of the left ventral hypocone".

Distribution

First described from Great Pond, Uncatena Island, in the Woods Hole area of North America. Widespread non-toxic, red tide organism, known from Tasmanian waters and the Swan Estuary.

References

Freudenthal, H.D. and J.J. Lee (1963). *Glenodinium halli* n.sp. and *Gyrodinium instriatum* n.sp., Dinoflagellates from New York Waters. J. Protozool. 10, 182-189.

Hulburt, E.M. (1957). The taxonomy of unarmoured Dinophyceae of shallow embayments on Cape Cod, Massachusetts. Biol. Bull. 112, 196-219;

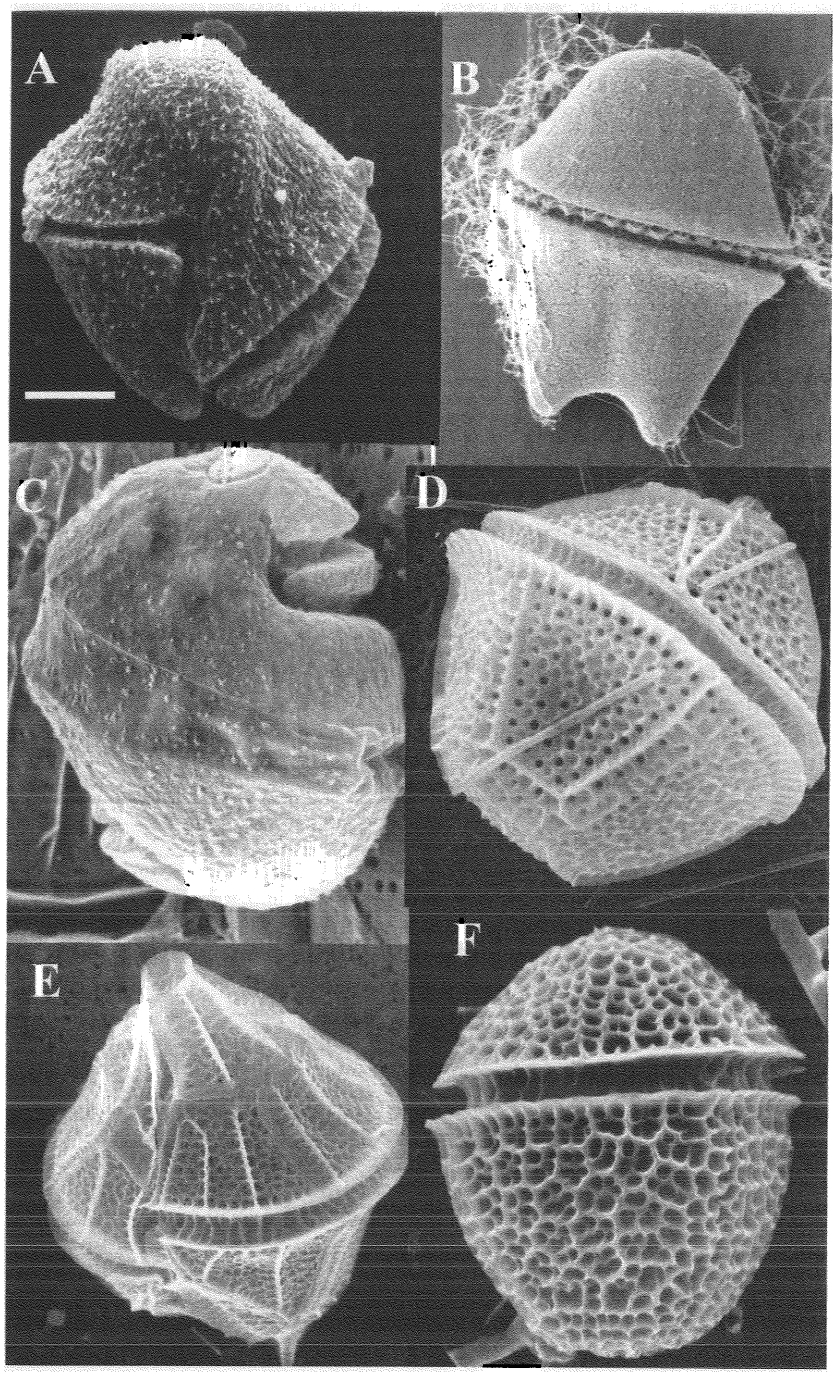

Fig.10 A. *Gymnodinium uncatenum.* Large cell with strongly displaced girdle.; Fig. 10B. *Akashiwo sanguinea*. Large flattened cell with two posterior lobes; Fig.10C. *Cochlodinium* sp. Unarmoured cell with characteristic spiral-shaped girdle; Fig.10D. *Lingulodinium polyedrum*. Large polygonal cell with areolate ornamentation; Fig.10E. *Gonyaulax polygramma* from Port Hacking (NSW). The cellulose plates are ornamented with characteristic longitudinal ridges and reticulations are visible between the ridges; Fig.10F. *Protoceratium reticulatum.* Large globose cell with reticulate ornamentation.

Micrographs Figs.10 A,C. by C.Bolch; Fig.10B by M.de Salas.

2.10. *Akashiwo sanguinea* (Hirasaka) G.Hansen & Moestrup 2000
Fig. 10B

Basionym: *Gymnodinium sanguineum* Hirasaka
Synonyms: *Gymnodinium splendens* Lebour,
Gymnodinium nelsonii Martin

Description

A large, 50 – 80 µm long, bloom-forming (akashiwo=red tide) unarmoured dinoflagellate (without plates on its surface). This species has a somewhat flattened shape, with two posterior lobes. The apical groove is a large clockwise spiral when seen from the front of the cell, and not visible by light microscopy.

Distribution

This species is common in Australian coastal waters. It has produced red water in New South Wales waters (e.g. Cooks River, Alexandra Canal, Sydney Harbour, Lane Cove River) and some New Zealand sounds.

Toxicology

Non-toxic. This species is an extremely important food source for certain zooplankton and the fish larvae of warm-water anchovy. However, there is evidence that some zooplankton avoid them and in British Columbia this species has been implicated in causing oyster losses, possibly through physical clogging of the gills.

References

Daugbjerg, N., G.Hansen, J.Larsen & O.Moestrup (2000). Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycologia 39:302-317.

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Kiefer, D. A. and Lasker, R. (1975). Two blooms of *Gymnodinium splendens*, an unamoured dinoflagellate. Fish. Bull. 73, 675 – 678.

2.11 *Cochlodinium* sp.
Fig. 10C

Description

Yellow-brown, unarmoured dinoflagellate, 20 – 30 µm long, with a spiral-shaped girdle which makes 1.5 turns or more. Some species (e.g. *C. catenatum*) can form short chains. These organisms are extremely fragile and readily disintegrate upon preservation or even when observing living cells under the microscope.

Distribution

Red tides in Moreton Bay, Brisbane, caused by a species similar to *Cochlodinium helix*, have been implicated in fish kills but no measurable mouse toxicity was detected from cell extracts (A. J. Moss, pers. comm.).

Toxicology

In Japan, *C. polykrikoides* Margalef (= *C. heterolobatum* Silva) and another unidentified *Cochlodinium* species have been shown to produce ichthyotoxic substances (toxic to fish). *Cochlodinium polykrikoides* has caused major damage to fish farming in Korea since 1989. In Costa Rica and Panama, blooms of *C. catenatum* have been implicated in causing coral mortality. This may have been caused by a combination of toxicity, oxygen depletion and smothering of corals by massive dinoflagellate mucus production.

References

Guzman, H. M., Cortes, J., Glynn, P. W. and Richmond, R. H. (1990). Coral mortality associated with dinoflagellate blooms in the eastern Pacific (Costa Rica and Panama). Mar. Biol. 60, 299 – 303.

Yuki, K. and Yoshimatsu, S. (1989). Two fish-killing species of *Cochlodinium* from Harima Nada, Seto Inland Sea, Japan. In: Okaichi, T., Anderson, D. M. and Nemoto, T. (eds), Red tides: Biology, Environmental Science and Toxicology, pp. 451 – 454. Elsevier, N.Y.

2.12 *Lingulodinium polyedrum* (Stein) Dodge 1989
Fig. 10 D

Synonym: *Gonyaulax poyledra* Stein

Description

Large, 42-54 µm diameter, polyedral shaped cell, with strongly reticulate ornamentation with pores in the depressions. Cell contents brown.

Distribution

Warm temperate to tropical waters.

Toxicology

Some strains produce Homoyessotoxin. Effect on humans is unknown.

References

Dodge, J.D. (1989). Some revisions of the family Gonyaulaceae (Dinophyceae) based on a scanning electron microscope study. Bot. Mar. 32, 275-298.

Tubaro, A., Sidari, L., Della Loggia, R., Yasumoto, T. (1998). Occurrence of yessotoxin-like toxins in phytoplankton and mussels from northern Adriatic Sea. In: Reguera, B., Blanco, J., Fernández, M. L., and Wyatt, T. (eds.), *Harmful Algae*, Xunta de Galicia and IOC/UNESCO, Santiago de Compostela, pp. 470-472.

2.13 *Gonyaulax polygramma* Stein 1883
Fig. 10E

Description

Large armoured dinoflagellate, 29 – 66 µm long, 26 – 54 µm wide. The cellulose plates are ornamented with longitudinal ridges and reticulations in between the ridges. The sulcus is slightly excavated and the girdle is displaced left-handedly about 1.5 times the girdle width. Short spines can be present on the bottom of the cell.

Distribution

A cosmopolitan species; it has produced red water in New South Wales estuaries.

Toxicology

Non-toxic, but dense blooms can generate anoxic conditions and cause fish kills (for example in South Africa and Hong Kong harbour).

References

Grindley, J. R. and Taylor, F.J.R. (1962). Red water and mass-mortality of fish near Cape Town. Nature 195: 1324.

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2.14 *Protoceratium reticulatum* (Claparède & Lachmann) Bütschli 1885
Fig. 10F

Synonyms.: *Peridinium reticulatum* Claparède & Lachmann, *Gonyaulax grindleyi* Reinecke.

Description

Oval, strongly pigmented cells with a *Gonyaulax*-like girdle displacement. The girdle is anterior to the midpoint of the cell (hypotheca is larger than epitheca). The surface is densely reticulated so that plate sutures are hard to see in an intact cell. The first apical plate is angular with parallel long sides and a prominent ventral pore on its left side. An apical pore complex is present. Length 28-53µm, transdiameter 25-45µm. The cyst is spherical with capitate spines.

Distribution

This is a widespread temperate and subtropical coastal species, very common in the summer although blooms are rare. Its cyst is the commonest in temperate coastal sediments in many regions.

Toxicology

Some (but not all strains) produce yessotoxin. Effect on humans is unknown.

References

Dodge, J.D. (1989). Some revisions of the family Gonyaulaceae (Dinophyceae) based on a scanning electron microscope study. Bot. Mar. 32, 275-298.

Satake, M., MacKenzie, L., Yasumoto, T. (1997a). Identification of *Protoceratium reticulatum* as the biogenetic origin of yessotoxin. Nat. Toxins 5: 164-167.

2.15 *Alexandrium minutum* Halim 1960
Figs. 11 A-C, 13A

Description

This small, inconspicuous, almost spherical dinoflagellate, 16 – 25 µm diameter, occurs as single cells or rarely in pairs. Conclusive identification requires careful study of the cellulose plates which surround the cell. The 6th precingular plate (the row of plates above the girdle groove) is narrow. A small pore plate (P_o) is found on top of the cell and this is either in direct contact or connected via a thin suture to the 1st apical plate (on the ventral side) (Fig. 11A). The latter plate has a characteristic small ventral pore. In Australian cells the cellulose plates are mostly smooth but in material from Italy and the Swan River, WA, the hypotheca (bottom half of the cell) can have a distinct reticulate ornamentation (Fig.11C).

As part of its life cycle, this dinoflagellate produces a colourless, mucoid cyst roughly hemispherical in shape, circular (24 – 29 µm diameter) when seen from above and bean-shaped (15 – 19 µm high) in lateral view (Fig. 12F).

Distribution

Within the Australian region, this species is known from the Swan River (since 1982) and Bunbury, WA, the Port River area near metropolitan Adelaide (since 1986), Hopkins Estuary and Portland, Victoria, and Shoalhaven (1993) and Newcastle, NSW. It has also been recorded from Egypt, France, Spain, Portugal, Italy, Turkey and New Zealand. A very similar species *A. angustitabulatum* Taylor has been linked with fish and shellfish kills in 1983 in North Island, New Zealand.

Toxicology

Producer of paralytic shellfish poisons (mainly gonyautoxin fractions) which can affect humans, other mammals, birds and possibly fish. This "weed species" tends to grow so densely that it can discolour the seawater red or reddish-brown ("red tide"). Wild mussels from the Port River area have been found to contain up to 2700 µg PSP/100 g (October 1987) but fortunately no commercial shellfish farms are located in the affected area. Finfish and crab samples from Port River were negative for PSP toxins. This species also produces a potent ichthyotoxic exudate toxin (Lush et al. 2001) and has been associated with fish kills (e.g. Alexandria aquarium in Egypt) and mortality of shrimp (Thailand, Taiwan).

References

Balech, E. (1989). Redescription of *Alexandrium minutum* Halim (Dinophyceae), type species of the genus *Alexandrium*. Phycologia 28, 206 – 211.

Bolch, C. J., Blackburn S. I., Cannon, J. A. and Hallegraeff, G. M. (1991). The resting cyst of the red tide dinoflagellate *Alexandrium minutum* Halim (Dinophyceae). Phycologia 30 (in press).

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Oshima, Y., Hirota, M., Yasumoto, T., Hallegraeff, G. M., Blackburn, S. I. and Steffensen, D. A. (1989). Production of paralytic shellfish toxins by the dinoflagellate *Alexandrium minutum* Halim from Australia. Bull. Japan Soc. Sci. Fish. 55, 925.

Fig.11. Dinoflagellate genus *Alexandrium.*

Fig. 11 A (SEM). Dinoflagellate *Alexandrium minutum* from Port River, Adelaide.

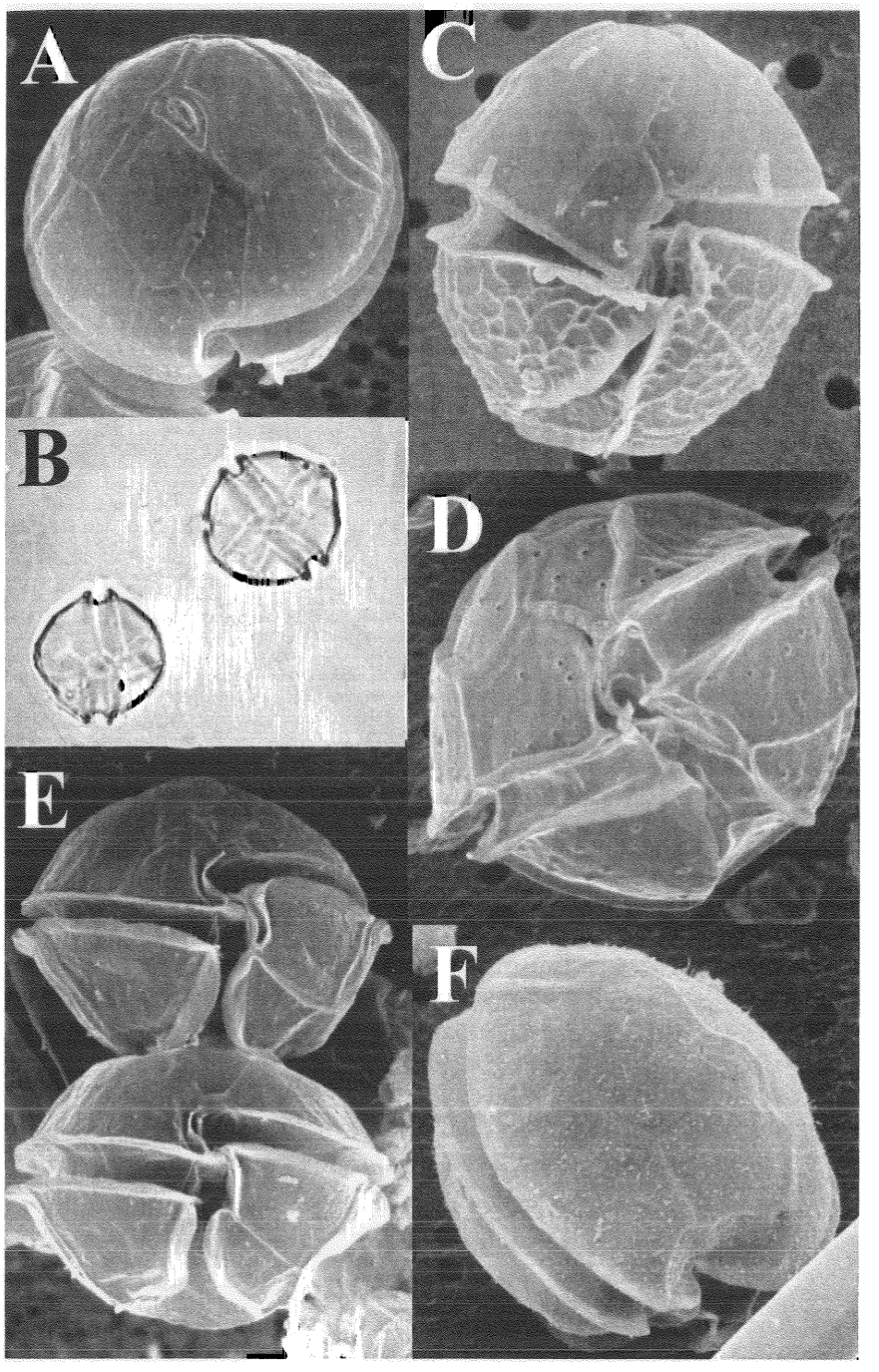
Details of plate tabulation showing direct contact between the first apical plate 1' (with ventral pore) and P_o; Fig. 11 B (LM). Small, nearly spherical cells showing details of plate tabulation; Fig.11C. Cell from Swan River, W.A., with areolate ornamentation on hypotheca;

Figs. 11D (SEM). Dinoflagellate *Alexandrium tamarense* from northern Tasmania. Single cells showing cell shape and details of plate tabulation including the shape of the first apical plate (1') (with ventral pore) and the triangular pore plate P_o (with anterior attachment pore).

Fig. 11E (SEM).Two-celled chain of dinoflagellate *Alexandrium catenella* from Port Phillip Bay, Melbourne.

Fig. 11 F (SEM). *Alexandrium margalefi* from Tasmanian waters.

Micrographs Figs.11 A, D, F. by C.Bolch



2.16 *Alexandrium catenella* (Whedon and Kofoid) Balech 1985
(= *Gonyaulax (Protogonyaulax) catenella*)
Figs. 11E,13B

Description

This small dinoflagellate occurs as single cells (similar to *A. fundyense*) or more often in characteristic short chains of 2, 4 or 8 cells long (Fig. 11E). The cells are often anteriorly-posteriorly compressed, 20 – 22 µm long, 25 – 32 µm wide. Conclusive identification requires careful study of the cellulose plates which surround the cell. The first apical plate is connected with the triangular apical pore plate (P_o) on top of the cell . Unlike *A. minutum*, no ventral pore is present.

As part of its life cycle, this dinoflagellate produces a colourless, mucoid cyst, roughly ellipsoidal (35 – 40 µm long) in shape with rounded ends (Fig. 12E).

Distribution

Within the Australian region, this species is known from Port Hacking (since 1954), Sydney Harbour (1993), Newcastle and Batemans Bay in NSW, Port Phillip Bay, Melbourne (since 1986), Port River, SA (since 1997) as well as Triabunna, Tasmania. It is also known from New Zealand. It is widespread in the Northern Hemisphere, from the west coast of North America between southern California and south-eastern Alaska, from Japan and most recently the Mediterranean (Spain). In the Southern Hemisphere, it has also been observed in South Africa and southern Chile.

Toxicology

Producer of paralytic shellfish poisons (C_1–C_4 toxins and gonyautoxins) which can affect humans, other mammals, birds and possibly fish. This species produced limited toxicity (up to 480 µg/100 g) in wild mussels from Port Phillip Bay in January/February 1988, and up to 300 µg/100 g in wild oysters from Sydney Harbour in late 1993.

References

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Hallegraeff,G.M., Bolch, C.J., Blackburn, S.I. & Oshima, Y. (1991).Species of the toxigenic dinoflagellate genus *Alexandrium* in south-eastern Australian waters. Bot. Mar.34, 575 - 587.

Hallegraeff, G.M., Marshall, J.A., Valentine, J., Hardiman, S. (1998). Short cyst dormancy period of an Australian isolate of the toxic dinoflagellate *Alexandrium catenella*. Mar. Freshw. Res. 49: 415-420

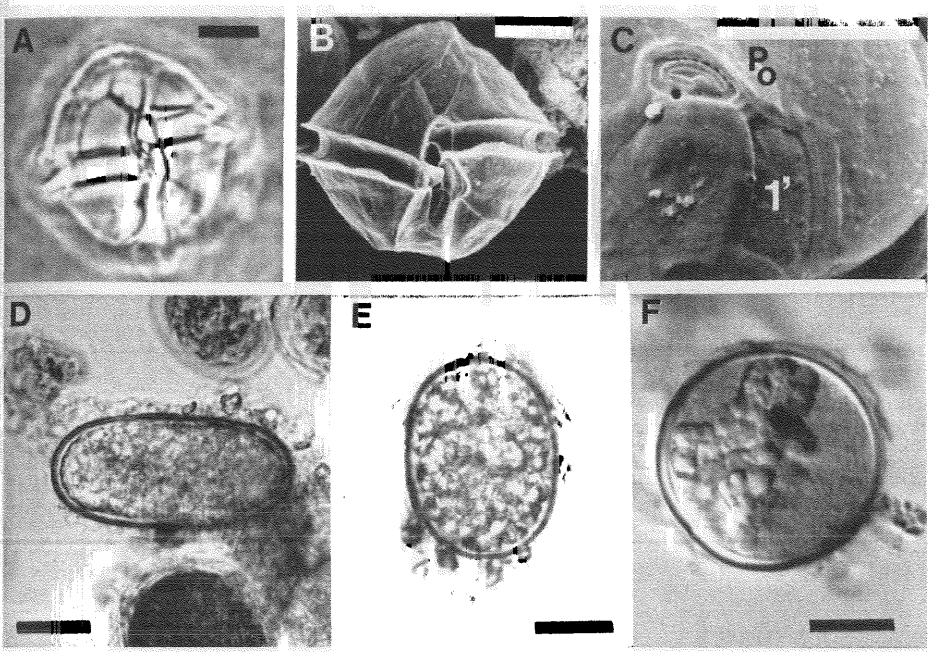

Fig. 12 A-C. Dinoflagellate *Alexandrium tamarense* from northern Tasmania. Single cells showing cell shape (A, B) and details of plate tabulation (C) including the shape of the first apical plate (1') (with ventral pore) and the triangular pore plate P_O (with anterior attachment pore).

Figs. 12 D-E. *Alexandrium* resting cysts.

Fig. 12 D *A. tamarense* from Port MacDonnell, South Australia; Fig. 12E *A. catenella* from Port Phillip Bay, Melbourne; Fig. 12F *A. minutum* from Port River, Adelaide.

Micrographs by C.Bolch. Scale bars: 10 µm.

2.17 *Alexandrium tamarense* (Lebour) Balech 1985
(= *Gonyaulax* (*Protogonyaulax*) *tamarensis* = *Gonyaulax excavata*)
Figs. 11D,12A-C

Description

This small dinoflagellate, 26 – 38 µm diameter, occurs as single cells or in pairs. Conclusive identification requires careful study of the cellulose plates which surround the cell. This species can be distinguished from other *Alexandrium* species by its cell shape, size and shape of the plates. Like *A. minutum,* the first apical plate carries a ventral pore.

As part of its life cycle, this dinoflagellate produces a colourless, mucoid cyst, roughly ellipsoidal (35 – 40 µm long) in shape with rounded ends (Fig. 12D). This cyst type cannot be distinguished from that of *A. catenella.*

Distribution

Within the Australian region, this species has been found in northern and southern Tasmania (Bell Bay, North West Bay), Port Phillip Bay (Melbourne) and off Port MacDonnell and Cape Jaffa (South Australia). It is common in Japan, North America and Europe.

Toxicology

Potential producer of paralytic shellfish poisons which can affect humans, other mammals, birds and fish. Both toxic and non-toxic forms occur; all Australian isolates tested thus far have proved non-toxic and belong to a unique ribotype not yet found anywhere else in the world. Normally these dinoflagellates transmit their poisons only through shellfish, but a bloom of this species in the Faroe Islands in 1984 was implicated in a fish kill of 27 metric tons of farmed rainbow trout and salmon. These fish suffered acute histopathological damage to their gills.

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2.18 *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen 1985
Fig. 13E

Synonym: *Goniodoma ostenfeldi* Paulsen, *Gonyaulax ostenfeldi* (Paulsen) Paulsen, *Protogonyaulax ostenfeldi* (Paulsen) Fraga & Sanchez, *Heteraulacus ostenfeldi* (Paulsen) Loeblich, *Gessnerium ostenfeldi* (Paulsen) Loeblich & Loeblich, *Triadinium ostenfeldi* (Paulsen) Dodge, *Gonyaulax globosa* (Braarud) Balech, *Protogonyaulax globosa* (Braarud) Taylor, *Gonyaulax trygvei* Parke.

Description

A distinctive, large (40-56 µm long, transdiameter 40-50 µm), non-chained, globose species in which both the epithecal and hypothecal profiles are smoothly rounded. The first apical plate is distinctive in shape, the right upper margin abruptly concave with a large ventral pore excavated from the margin at the point of inflection. It usually contacts the apical pore complex. The cingulum is only slightly excavated. The anterior sulcal plate is wide. It is very similar to *A. peruvianum* which is smaller and has a more smoothly curved right upper margin to its first apical plate.

Distribution

Known from Scandanavian, Icelandic, Spanish (Galicia), Washington State, British Columbian, Kamchatka, and Egyptian, New Zealand and Australian waters.

Toxicology

This species usually produces low concentrations of Paralytic Shellfish Poisons, but highly toxic strains are also known. Some (but not all) strains also produce spirolide shellfish toxins of uncertain human oral potency.

References

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Remarks

Several other *Alexandrium* species are present in Australian waters. *A. margalefi* Balech, 30 – 35 µm diameter, can be distinguished by its greatly displaced first apical plate (Fig. 11F). Cultures from southern Tasmanian waters have proved negative for PSP toxins. *A. pseudogonyaulax* (Biecheler) Horiguchi is a brackish lagoon species known from Western Australia (Fig.13F). *A. peruvianum* (Balech & Mendiola) Balech et Tangen and *A.concavum* (Gaarder) Balech (both non-toxic) are known from Port Phillip Bay.

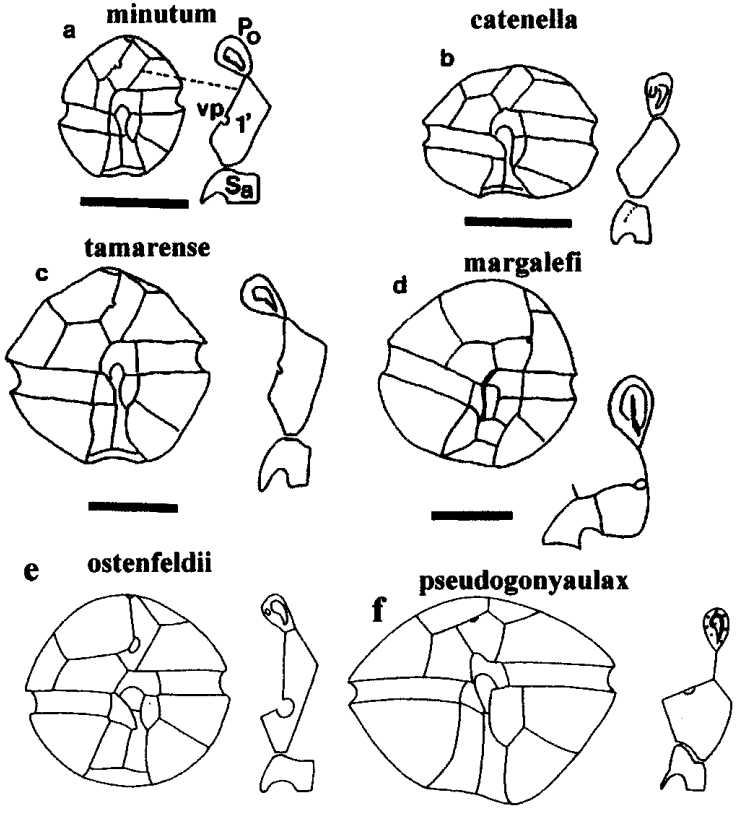

Fig. 13 Diagrammatic summary of ventral view of plate tabulation of *Alexandrium* species in Australian waters, including details of the apical pore plate P_o, the first apical plate 1' and the anterior sulcal plate S_a. The presence or absence of a ventral pore (vp) is also indicated. Fig. 13A *A. minutum* (toxic); Fig. 13B *A. catenella* (toxic); Fig. 13C *A. tamarense* (sometimes toxic); Fig. 13D *A. margalefi* (non-toxic); Fig.13E. *A. ostenfeldii* (sometimes toxic); Fig.13F. *A. pseudogonyaulax* (nontoxic).

Scale bars: 10 µm.

2.19 *Pyrodinium bahamense* Plate 1906 var. *compressum* (Bohm) Steidinger, Tester et Taylor

Fig. 14 A-E

Description

Tropical, green-brown dinoflagellate with strongly developed wall of cellulose plates, occurring as single cells (37 – 52 µm diameter) or in chains of 2, 4, 8, 16 or rarely 32 cells long (Fig. 14D). Cells in chains tend to be flattened (sometimes recognised as a separate variety *compressum*) (Fig. 14C). The girdle is displaced left-handedly by approximately one girdle-width and has strongly developed lists. Sulcal lists are also strongly developed and are supported by antapical spines. The first apical plate has a well-developed ventral pore and approaches (but does not usually touch) the triangular apical pore complex on top of the cell. Some confusion is possible with *Goniodoma (Triadinium) polyedricum* which, however, is more angular in shape, never occurs in chains and lacks prominent spines. Some species of *Gonyaulax*, such as *Gonyaulax (Lingulodinium) polyedra*, may also be confused with *Pyrodinium* but these occur singly, lack flanges and have a greater girdle displacement.

As part of its life cycle, this dinoflagellate produces a distinctive spinose resting cyst (50 – 70 µm diameter) which, upon germination, opens up equatorially to form two hemispheres (epicystal archeopyle) with characteristic sulcal notch (Fig. 14F).

Distribution

Within the tropical Indo-West Pacific region, this species is well known from mangrove areas of Brunei, Indonesia, Palau, Papua New Guinea, the Philippines, Sabah and the Solomon Islands. Its resting cyst has been discovered in recent sediments from Darwin and the Gulf of Carpentaria, but no plankton cells have ever been observed in these areas. In Latin-America this species is known from Guatemala, Venezuela, Mexico and the tropical Caribbean (notably Puerto Rico and Jamaica). Only 100,000 years ago *Pyrodinium* had a much wider distribution and fossil cysts (known as *Polysphaeridium zoharyi*) have been found in the Australian region as far south as Brisbane and Newcastle.

Toxicology

Producer of potent paralytic shellfish poisons (mainly saxitoxin, decarbamoyl saxitoxin and gonyautoxin 5), which can contaminate shellfish such as mussels, oysters, scallops and cockles, the gills and guts of small fish such as sardines and anchovies, and possibly even shrimp heads and crab fat. To date, this dinoflagellate has been responsible for more than 1,000 human illnesses and 60 fatalities. The first harmful blooms were recorded in 1972 in Papua New Guinea. Since then, toxic *Pyrodinium* blooms have apparently spread to Brunei and Sabah (1976), the central Philippines (1983) and the northern Philippines (1987). Until recently Latin-American populations of *Pyrodinium* were thought to be non-toxic, but in 1987 on the Pacific coast of Guatemala 187 people had to be hospitalised after consumption of toxic clams and 26 people died. Unfortunately, the tropical countries that are affected depend heavily on seafoods for protein and they have little prior experience in dealing with this problem.

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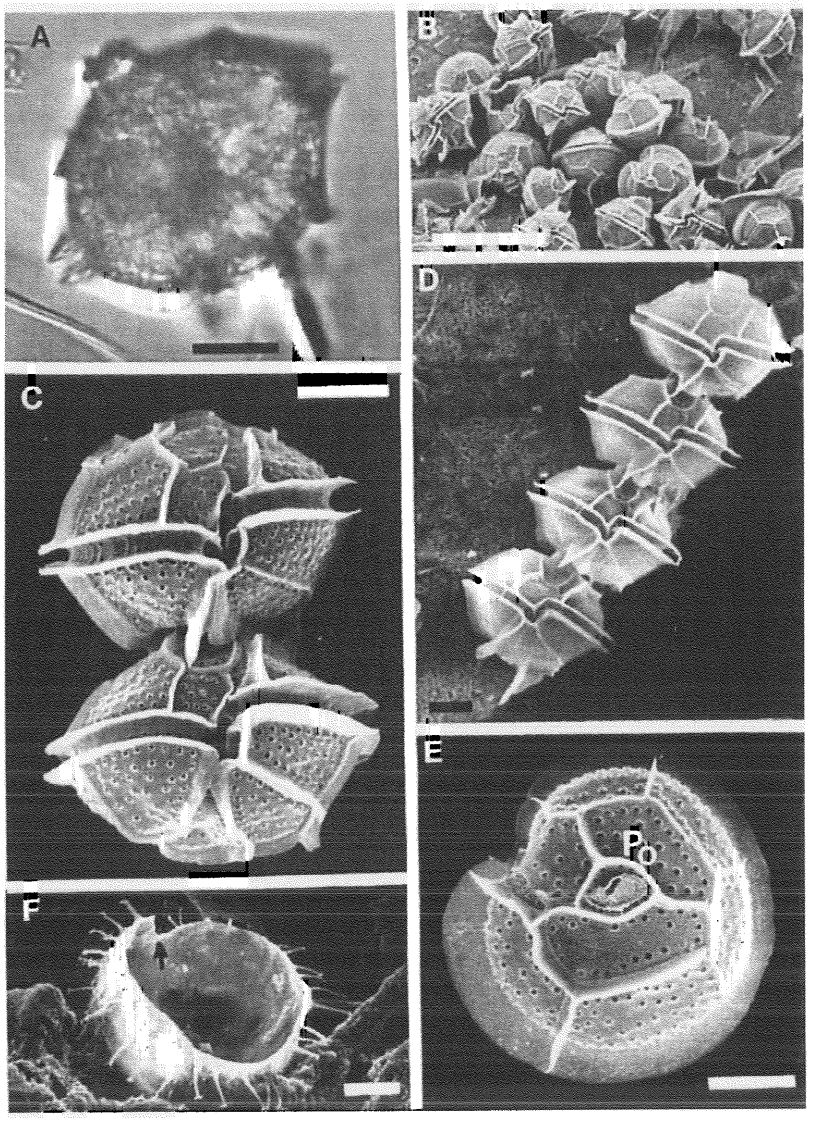

Fig. 14 Tropical dinoflagellate *Pyrodinium bahamense*.

Figs. 14A (LM), C, E (SEM). Cells from Papua New Guinea waters; Fig. 14B (SEM). Monospecific bloom from Oyster Bay (Jamaica); Fig. 14D (SEM). Four-celled chain from the Philippines; Fig. 14E (SEM). Top view of cell showing triangular pore complex (P_O); Fig. 14F (SEM). Spinose resting cyst from Port Moresby, showing characteristic sulcal notch (arrow).

Micrographs Fig. 14D by Y.Fukuyo; Scale bars: 10 µm, except Fig. 14B (100 µm).

2.20 *Gambierdiscus toxicus* Adachi and Fukuyo 1979
Figs. 15A-D

Description

Lens-shaped, benthic dinoflagellate, 24 – 60 µm long, 42 – 140 µm diameter. The thecal plates are thick and porulated and the apical pore platelet (on top of the cell) has a characteristic fishhook-shape pore (Fig. 15D). This species appears in epiphytic association with bushy red, brown and green seaweeds (up to 200,000 cells/100 g algae) and also occurs free in sediments and coral rubble. Living cells have dense yellow-brown cell contents (Fig. 15A) and may be difficult to recognise among coral rubble and seaweeds. Initially only one species, *Gambierdiscus toxicus*, which is flattened and benthic, was described by light microscopy. Scanning electron microscopy was subsequently used to describe *G. belizeanus* Faust with areolate thecal ornamentation and *G. yasumotoi* Holmes which is globular in shape like *Coolia*. Combined morphological and molecular classification has also allowed the discrimination of *G. australes* Faust & Chinain, *G. pacificus* Chinain & Faust and *G. polynesiensis* Chinain & Faust.

Distribution

In the Australian region this species is known from tropical coral reef areas of the Great Barrier Reef (between Brisbane and Cairns/Port Douglas). In Platypus Bay (Brisbane) the species also occurs in a noncoral environment as an epiphyte on the green alga *Cladophora*. It is especially prevalent in the South Pacific Islands and in the Caribbean. Evidence from French Polynesia suggests that reef disturbance by hurricanes, military and tourist developments may significantly increase the risk of ciguatera, probably by increasing benthic substrate for dinoflagellate growth.

Toxicology

This species produces the potent neurotoxins ciguatoxin analogues and maitotoxin, which accumulate through the food chain, from small fish grazing on the coral reefs into the organs of bigger fish that feed on them. Maitotoxin does not accumulate in the flesh of fish and has no proven role in ciguatera. Humans consuming contaminated fish suffer from gastro-intestinal and neurological illnesses (cold objects feel hot to touch) and in extreme cases can die from respiratory failure. These symptoms ("ciguatera") can persist for months and may recur up to several years later. More than 920 cases of ciguatera food poisoning (including one fatality) are known from Queensland in the period 1965-1992. In May 1987 a total of 63 people were poisoned after eating Spanish mackerel originating from Hervey Bay purchased from Sydney fish shops.

No adequate treatment is yet available but clinical trials using an intravenous infusion of mannitol are promising, provided it is administered within 48hrs after the onset of symptoms), and with the majority of patients reporting quick and complete relief from symptoms. The risk of ciguatera poisoning can be reduced by avoiding such species as red bass, chinaman fish, moray eel and paddle tail. Consumption of the viscera and gonads of reef fish should be avoided at all times and one should avoid eating large specimens of suspect fish. Ciguatera has caused severe public health problems and economic losses in the islands of the Caribbean and French Polynesia. The development of a rapid immnological stick-test (Cigua-check™ marketed by Oceanit) may in future allow for screening of toxic fish in the market place.

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Figs. 15 Benthic coral reef dinoflagellates.

Figs. 15 A-D. *Gambierdiscus toxicus* from Great Barrier Reef.

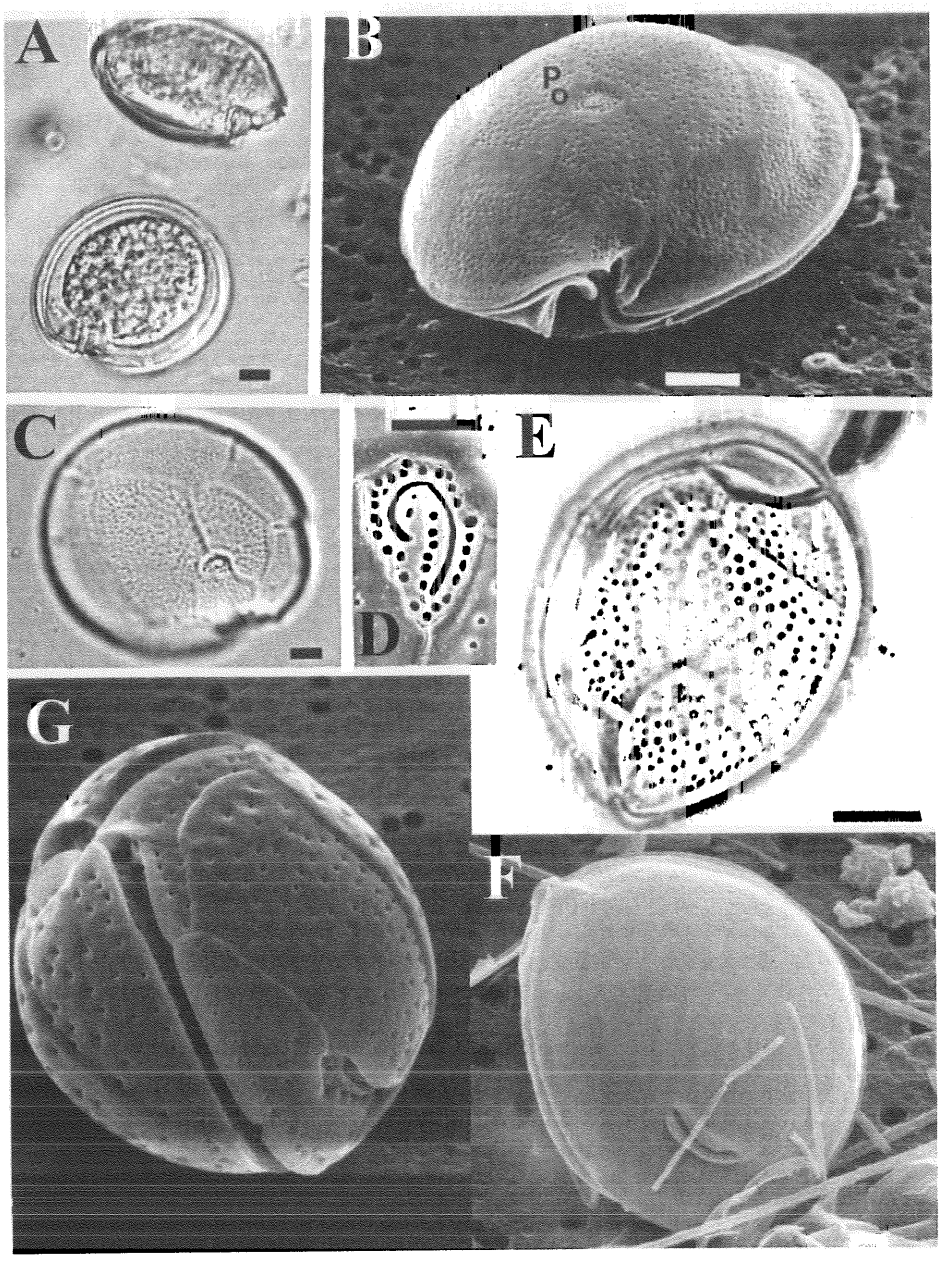
Fig. 15A (LM). Two living cells in side view (top) and top view (bottom), respectively; Fig. 15B (SEM). Ventral view of flattened cell showing apical pore plate on top (P_O); Fig. 15C (LM). Thecal plate pattern of upper cell half; Fig. 15D (SEM). Detail of fishhook-shaped apical pore plate.

Scale bars: 10 µm, except Fig. 15D (5 µm).

Figs. 15E-F. *Ostreopsis siamensis*.

Fig. 15E (SEM). Ovoidal flattened cell showing thecal plates with scattered trichocyst pores (Great Barrier Reef); Fig. 15F(SEM).Epitheca showing straight apical groove (Tasmania).

Fig.15G. *Coolia monotis* from New Caledonia.



2.21 *Ostreopsis siamensis* Schmidt 1901
Figs. 15 E,F

Description

A large, strongly flattened benthic genus with a tabulation fundamentally similar to *Gambierdiscus* but with a high degree of distortion due to dorso-ventral elongation. The apical pore complex is narrow and displaced dorso-laterally to the left. The cells are elliptical and pointed toward the sulcus and there is no obvious sulcal notch when seen in broad view (unlike *Gambierdiscus* with which it might be confused under low magnification). The species are all epibenthic, often attached to the substrate by a fine thread, the flagella beating while tethered in this manner. Currently at least 9 morphospecies are distinguished, *O. siamensis* Schmidt, *O. lenticularis* Fukuyo, *O. ovata* Fukuyo, *O. heptagona* Norris, *O. mascarenensis* Quod, *O. labens* Faust & Morton, *O. marinus* Faust, *O. belizeanus* Faust and *O. caribbeanus* Faust. These species differ in fine details of thecal plate size and shape, and thecal pore ornamentation. *O. siamensis* has flattened, tear-shaped cells, 60 – 100 µm dorso-ventral diameter, 40-50 µm transdiameter, with a ventral beak. The thecal plates have scattered trichocyst pores (Fig. 15E). The number and arrangement of the main thecal plates are identical for all known species of this genus, so cell shape and size are the primary taxonomic characters. This species swims very slowly and spins around the dorso-ventral axis.

Distribution

This dinoflagellate is found in ciguatera endemic coral reef areas in association with *Gambierdiscus toxicus* and *Prorocentrum lima*. Generally less substrate specific than *Gambierdiscus*. It tends to be abundant in reef flat situations around coral cays and inshore islands. First described from the Gulf of Thailand. Subsequently found at numerous Pacific Island locations, the tropical Caribbean and the Mediterranean. Similar forms are also known from temperate habitats in New Zealand and Australia, e.g. the East coast of Tasmania.

Toxicology

This species produces an unidentified water-soluble toxin fraction (coined ostreocin which is an analogue of palytoxin). Palytoxin can be sequestered in fish and crabs (considered to be responsible for clupeotoxicity) but bioaccumulation in shellfish has not yet been demonstrated.

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2.22 *Ostreopsis lenticularis* Fukuyo 1981

Description

Cells roundly lenticular, slightly pointed towards the sulcus. Plates covered with large pores as well as numerous fine pores. The girdle does not undulate and there is no girdle displacement. Dorso-ventral distance 60-100 µm, transdiameter 45-80 µm. Distinguished from *O. siamensis* chiefly by lack of undulation of the girdle and two types of pores.

Distribution

Probably circumtropical, benthic. Reported from the Great Barrier Reef. Presently known from French Polynesia (Gambier Islands), New Caledonia and several tropical Atlantic localities including Cayman Islands, Puerto Rico and the Virgin Islands.

Toxicology

The report of ostreopsis-toxin by this species appears to have been based on cultures of *O. siamensis.*

References

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2.23 *Coolia monotis* Meunier 1919
Fig.15G

Description

Globular cells, 21- 40 µm diameter, slightly compressed anterio-posteriorly. Cingulum descending but appearing straight in lateral view. The apex is displaced dorsally and to the left. In apical view, the epitheca is smaller than the hypotheca. Fan-shaped arrangement of postcingular plates, The hypothecal plate pattern is different than in *Ostreopsis*. *Coolia tropicale* Faust differs in having a large wedge-shaped first apical plate in the center of the hypotheca. *Coolia areolata* Faust has a strongly areolated surface ornamentation, and differs in shape and size.

Distribution

Worldwide distribution, planktonic, benthic and epiphytic, from temperate to tropical waters. Known from New Zealand, Queensland, NSW and Tasmanian coastal waters.

Toxicology

Coolia monotis produces yessotoxin analogues, shown to be toxic to larvae of *Artemia* and *Haliotis.*

References

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2.24 *Scrippsiella trochoidea* (Stein) Loeblich 1976
Fig. 16A

Description

Small pear-shaped dinoflagellate, 16 – 36 µm long, 20 – 23 µm broad, which can be confused with similarly sized *Alexandrium* species. The cellulose plate pattern (with six girdle plates) and tube-like apical pore (not fish-hook shaped as in *Alexandrium*) can be used to distinguish them.

Distribution

Common in Australian waters, sometimes in dense concentrations which can cause red-brown seawater discolourations (for example in West Lakes, South Australia, in 1983 and Hawkesbury Estuary, NSW, In 1991). This species (under the name *Glenodinium rubrum*) has also been implicated in causing fish kills in Sydney Harbour in the 1890s.

Toxicology

Non-toxic, but it can cause fish kills in sheltered bays through the generation of anoxic conditions.

References

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Fig. 16A. (SEM), Dinoflagellate *Scrippsiella trochoidea* from Port River (SA). Small pear shaped cells with tube-like apical pore.

Fig. 16B. Dinoflagellate *Noctiluca scintillans*. Large balloon-shaped cells with tentacle. Sample from British coastal waters (micrograph: I.A.N. Lucas).

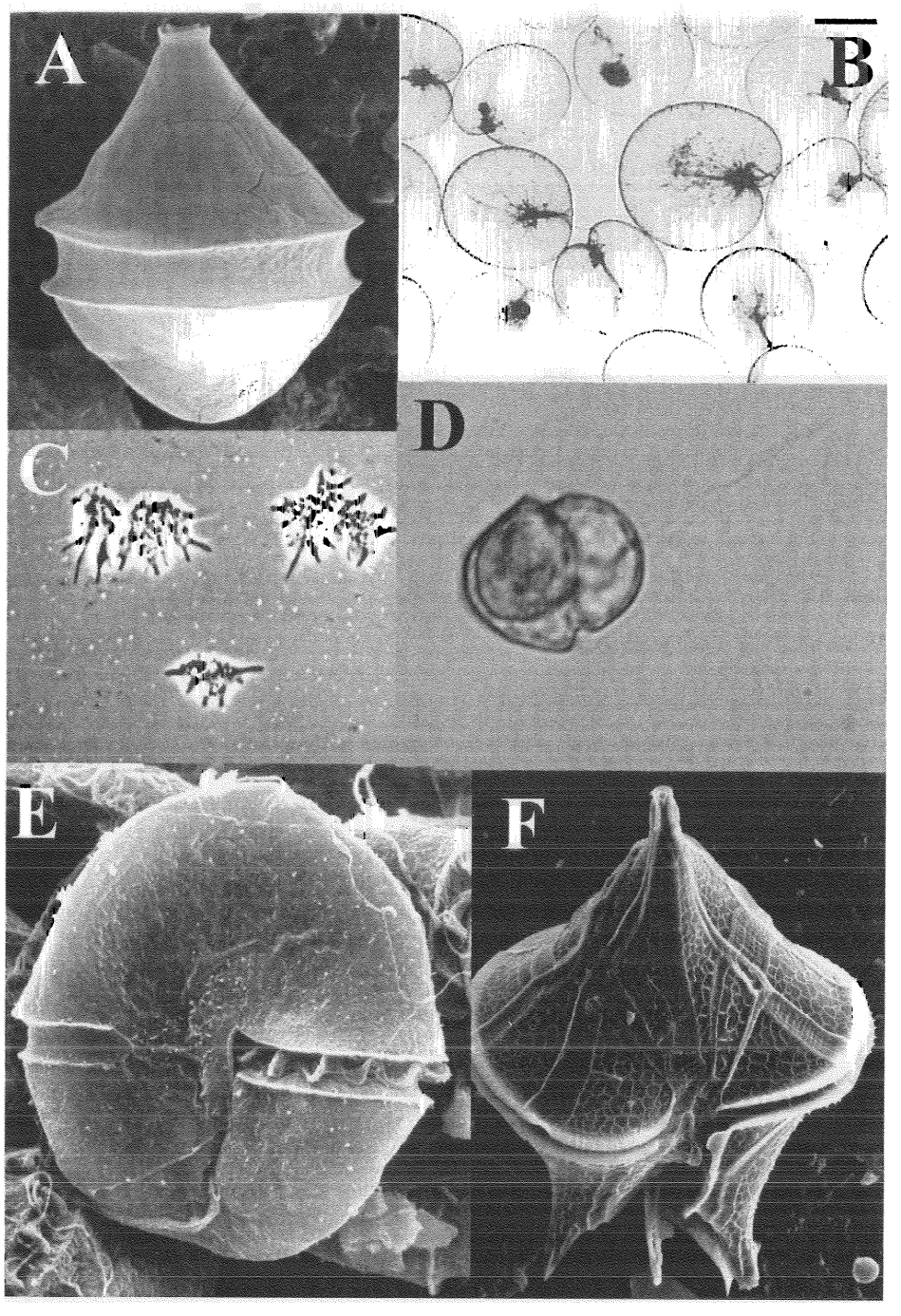
Scale bar: 100 µm.

Fig. 16C. Putative amoeboid stages of the dinoflagellate *Pfiesteria,* N.S.W.

Fig.16D. Zoospore of *Pfiesteria shumwayae* from Western Australia. Micrograph by S. Grigo and W. Hosja.

Fig. 16E. Leaf-like coastal lagoon dinoflagellate *Kryptoperidinium foliaceum.*

Fig. 16F. Dinoflagellate *Protoperidinium crassipes* from Tasmanian waters.



2.25 *Heterocapsa circularisquama* Horiguchi 1995

Description

Small photosynthetic dinoflagellate cells, similar to *Scrippsiella*, 20-29 µm long, 14-20 µm wide, with conical epitheca and hemispherical hypotheca and thecal plate arrangement Po, cp, 5', 3a, 7", 6c, 5s, 5"', 2"". The species is named for the diagnostic body scales with six radiating ridges on a circular basal plate, but which are visible only by TEM. All other known dinoflagellate body scales, such as from *Heterocapsa (Katodinium) rotundata* and *Heterocapsa triquetra,* are triangular in outline.

Distribution

This small dinoflagellate was first recognized in Japan in 1988, and since has spread to many other areas particularly of western Japan. It is unclear whether this species is indigenous to Japan or perhaps was introduced from tropical regions through transfer of shellfish stock or advection in prevailing northward currents. Not yet recorded from Australian waters, but being carefully monitored for.

Toxicology

This species has caused mass mortality of bivalves such as oysters, short-necked clams and pearl oysters in Japan. Australian trains of *H. rotundata* have also been demonstrated to be toxic to *Artemia* (LeRoi, unpublished).

References

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2.26 *Noctiluca scintillans* (Macartney) Kofoid 1920
Fig. 16B

Synonym: *Noctiluca miliaris*

Description

Very large, 200 – 2000 µm diameter, strongly buoyant, balloon-shaped cell with a striped tentacle. The cytoplasm is mostly colourless, except for the presence of minute carotenoid globules around the periphery of the cell. This species can cause spectacular bioluminescence phenomena.

Distribution

This dinoflagellate is mostly restricted to coastal waters and occurs especially in the vicinity of river mouths and following heavy rainfalls. This species can cause tomato-soup coloured water blooms in Australia, Japan and Hong Kong, whereas in Indonesia, Malaysia and Thailand the presence of green prasinophyte endosymbionts (*Pedinomonas noctilucae*) may cause green water blooms. From a rare bloom former in the 1980s (e.g. in Lake Macquarie, NSW, in 1982), starting in Sydney coastal waters in 1993 this organism has developed into one of the most common red-tide organisms in Australian waters, also affecting Port Phillip Bay (since 1993) and Tasmania (since 1994). It occasionally has forced closure of Sydney beaches, and has caused problems with feeding activity in salmonid pens in Tasmania (2002).

Toxicology

No toxic effects are known, but it has been demonstrated that the high ammonia content of the vacuole irritates fish, which generally avoid the bloom areas. This large, predatory dinoflagellate can feed, among others, on fish eggs floating in the water. *Noctiluca* has been known to bloom extensively off the east and west coasts of India, where it has been implicated in the decline of fisheries.

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2.27 *Pfiesteria piscicida* Steidinger et Burkholder 1996; *Pfiesteria shumwayae* Glasgow et Burkholder 2001 Figs.16C,D

Description

Small colourless dinoflagellates. The cells are heterotrophic and mixotrophic, capable of phagocytosis, and with klepto(=stolen) chloroplasts present .Very difficult to identify, even by electron microscopy. This species has a perplexingly complex life cycle which includes planktonic stages (5-18 µm size), filose and lobose amoeboid stages (5 -250 µm), and non-motile cyst stages (25-33 µm). Only a few of those stages can kill fish, and then only under special conditions. Bi-and triflagellate stages are typically planktonic and ephemeral and resemble small gymnodinioid dinoflagellates (such as *Karlodinium micrum*) but are actually small cryptic peridinioids with a plate formula of Po, cp, X, 4', 1a, 5" (*piscicida*) or 6" (*shumwayae*), 6c, 4s, 5"' and 2"''. Cyst stages, 25-33 µm, range from spherical testate forms with a honeycomb surface pattern to forms resembling scaled chrysophytes.

Currently, two species are recognised *P. piscicida* (described in 1996) and *P. shumwayae* (described in 2001). *P. shumwayae* can be distinguished morphologically by having 6" plates and a four-sided 1a plate, but in practice DNA probes are needed for species discrimination.

Distribution

Most likely a common, but normally benign inhabitant of estuarine systems over the world. First recognised in North Carolina in 1988 .Toxic strains of the two *Pfiesteria* species have overlapping distributions in the mid-Atlantic and southeastern United States and Scandinavia, with *P.piscicida* (rare) and *P. shumwayae* (common) also verified from New Zealand, Queensland, Victoria, Western Australia and Tasmania.

Toxicology

Pfiesteria normally exists in non-toxic forms, and only becomes "toxic" in the presence of fish, triggered by their secretions and excretions in the water. Cysts germinate in the presence of live fish, the dinoflagellates phagocytize the sloughed tissue of dead and moribund fish, and within hours encyst again after fish death (ambush-predator behavior). If live fish are not available the dinoflagellates switch to a microalgal diet and become non-toxic. Circumstantially linked to fish kills and fish lesions in poorly flushed, warm, brackish and nutrient-enriched Chesapeake Bay and North Carolina estuaries (USA) . Circumstantially linked to (temporary) human health problems such as skin irritation and cognitive impairment (Estuarine Associated Syndrome) through water or aerosol contact. There is no evidence for human illnesses from eating fish or shellfish exposed to *Pfiesteria*.

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2.28 *Kryptoperidinium foliaceum* (Stein) Lindemann 1924
Fig.16E

Synonym: *Peridinium foliaceum*

Description

Dorso-ventrally flattened, greenish-brown, large dinoflagellate with a convex dorsal and concave ventral surface, 14-50μm long, 11-45 μm wide, with a brick-red stigma located centrally on the hypocone. The slightly displaced, excavated girdle is without lists. A short slit-like straight apical groove is present. This dinoflagellate was originally allocated to the genus *Glenodinium* Ehrenberg, which is a poorly defined freshwater genus for species with a red stigma. Later workers resolved the presence of delicate plates in a peridinioid pattern. This species contains a chrysophyte symbiont and has fucoxanthin pigments.

Distribution

Brackish water, bloom-forming species, known from Cooks River, NSW (1993); Port Phillip Bay (1990) and South Australia (1996).

Toxicology

Non-toxic.

References

Dodge, J. D. (1982). Marine Dinoflagellates of the British Isles – Her Majesty's Stationery Office, London, 303 pp.

2.29 *Protoperidinium crassipes* (Kofoid) Balech 1974
Fig.16F

Basionym: *Peridinium crassipes* Kofoid 1907

Description

Large non-photosynthetic (phagotrophic) cell, 75μm long, with one apical (on top) and two antapical (on bottom) horns. The right antapical horn is wider and longer than the left horn. The cell is almost circular in cross section. The surface has a reticulate ornamentation. The first apical plate is 5 sided (meta) and the anterior intercalary plate is 4 sided (quadra). The cingulum is slightly displaced (descending) by one or two girdle widths.

Distribution

Cosmopolitan species. Can form blooms in warm water estuaries.

Toxicology

Putative producer of azaspiracid, which causes DSP-like symptoms in humans but a mixture of DSP-and neurotoxin-like effects in mice. Shellfish toxicity has only been associated with shellfish originating from Ireland to date.

References

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2.30 *Prorocentrum cordatum* (Ostenfeld) Dodge 1975
Figs.17A,B

Synonyms: *Exuviaella cordata* Ostenfeld, *Prorocentrum minimum* (Pavillard) Schiller, *Exuviaella marina* Pavillard, *Prorocentrum triangulatum* Martin, *E. mariae-lebouriae* Park et Ballantine, *P. cordiformis* Bursa, *P. mariae-lebouriae* (Park and Ballantine) Loeblich III

Description

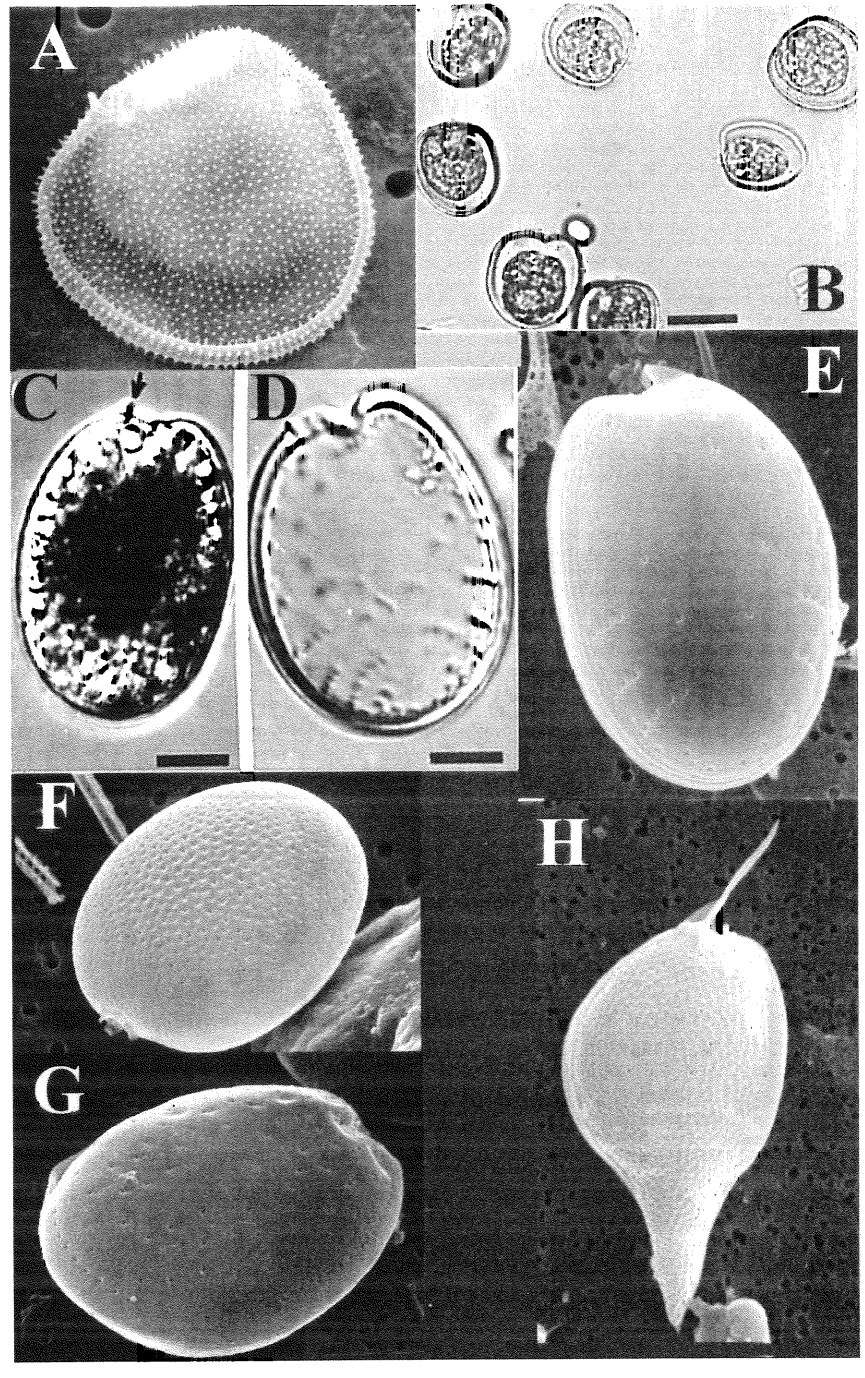
Very small ovate, triangular or heart-shaped cells, 14 – 22 µm long, 10 – 15 µm wide. The bottom (posterior) end is usually rounded and the top (anterior) end has a slight depression and minute apical spine (not always visible in the light microscope). The two large cell halves are covered with minute spinules. Trichocyst pores are found mainly around the margin of the plates.

Distribution

Planktonic species having a world-wide distribution, both in marine and brackish waters Common in the Australian plankton, sometimes in bloom proportions (e.g. Lake Macquarie and Hawkesbury Estuary (NSW), Hopkins Estuary (Vic), Peel–Harvey Estuary and Swan River, W.A.).

Toxicology

This species is often described as the causative organism of poisonings occurred by eating oysters and short necked clams. The toxic substance was named as venerupin, but the chemical structure and property has not been elucidated . So far six poisoning cases have been reported in Japan, and in total 542 people became sick with 185 fatal cases. As *P. cordatum* was observed abundantly in the digestive organs of toxic short necked clams, the species was thought to be responsible. Okaichi and Imatomi (1979) failed to detect venerupin in a culture of *P. cordatum*, although they could extract two substances having mouse toxicity. A possible outbreak of shellfish poisoning in Norway in 1979 was circumstantially linked to a *Prorocentrum cordatum* bloom. The human symptoms, which are distinct from those of diarrhetic (DSP) or paralytic shellfish poisoning (PSP), include gastrointestinal disorders, headache, feebleness and dizziness. The long latency period (36 – 48 hours) before the symptoms become manifest is similar to that of bacterial food poisoning. It is recommended to pay attention to shellfish toxicity, if a bloom of *P. cordatum* affects areas for shellfish aquaculture and collection. But it is not necessary to consider *P. cordatum* as a persistent toxic organism. A recent study has shown that senescent cultures can produce chemicals with i.p. toxicity to mice (Grzebyk et al. 1997). A bloom event in the Peel–Harvey estuary (August 1989) was negative for DSP toxins (Hosja., pers. comm.). Significant oyster mortalities near Lake Wonboyn, NSW, in 2002 were associated with blooms of *P.cordatum.*



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Figs. 17. Dinoflagellate genus *Prorocentrum.*

Fig. 17A-B. *Prorocentrum cordatum* from Peel-Harvey estuary.

Fig. 17A (SEM). Cell with small apical spine (arrow) and thecal ornamentation of minute spinules; Fig. 17B (LM). Ovate to triangular cells; Scale bars: 10 µm.

Fig. 17 C-E. *Prorocentrum rhathymum*

Fig. 17C (LM). Oval cell with small anterior spine (arrow); Fig. 17D (LM). Thecal half showing trichocyst pores; from Rottnest Island (W.A); Fig. 17E (SEM). Top view of cell showing apical pore platelets (arrow). Palau.Scale bars:
10 µm.

Fig.17F. *Prorocentrum compressum.* Great Barrier Reef; Fig.17 G. *Prorocentrum lima.* Rottnest Island; Fig.17H. *Prorocentrum micans.*

2.31 *Prorocentrum rhathymum* Loeblich, Sherley et Schmidt 1974
Figs.17C-E

Non *Prorocentrum mexicanum* Tafall

Description

Cell oval, length:30-40 µm, width: 22-25 µm, widest anterior or at the middle in valve view, lenticulate to ellipsoidal in lateral view. Anterior end slightly concave at the middle. Low list rising from periflagellar plate looks like a short spine. Valve surface smooth, having trichocyst pores lying in rows radially from the center perpendicular to the valve margin.

Distribution

This species is distributed very widely in tropical to temperate waters, forming benthic mats eg. on East coast Tasmania. Habitat is epibenthic on seaweeds and planktonic.

Toxicology

Hemolytic toxin and fast acting toxins were detected in culture of the species. A culture from Wilson Inlet (W.A.) was negative for DSP toxins (unpublished data).

References

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2.32 *Prorocentrum lima* (Ehrenberg) Dodge 1975
Fig.17G

Synonyms: *Exuviaella marina* Cienkowski (pro parte),
Prorocentrum marinum (Cienkowski) Dodge and Bibby

Description

Flattened oval cells, 32 – 50 µm long, 20 – 28 µm wide, broadest behind the middle and with a small anterior indentation. The two thecal halves are thick and have scattered trichocyst pores which are absent from the centre. A large number of small platelets surround the top (apex) of the cell where the two flagella arise .There is uncertainty about the distinction between this species and *Prorocentrum marinum*. *P. lima* is distinguishable by its smooth valve surface from other *Prorocentrum* species, especially from *P. hoffmannianum*, which has an areolate surface.

Distribution

A benthic species which is commonly found attached to seaweeds, in coral reef areas, epibenthic on seaweeds and benthic embedded shallowly in sand. After storms, it can also occur in the water column (Port Hacking (NSW), Rottnest Island, Wilson Inlet (W.A.), Port Phillip Bay, Gippsland Lakes (Vic)). Also known from Queensland (Heron Island) and east coast Tasmania.

Toxicology

Producer of okadaic acid and dinophysis–toxin–1, which can cause diarrhetic shellfish poisoning in humans.

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2.33 *Prorocentrum hoffmannianum* Faust 1990

Description

This species, 45-44 µm long, 40-45 µm wide, was described from mangrove habitats at Belize, Central America. This species is larger and broader than *P. lima* and also differs in that the valve surface is deeply areolated. Both valves are concave. The apical area is a broad triangle with a flared apical collar adjacent to the flagellar pore. The cell has a centrally located pyrenoid.

Distribution

Known from Fraser Island, Heron Island, Michaelmas Cay, Kelso Reef, Magnetic Island, Credlin Reef in Queensland.

Toxicology

Producer of okadaic acid.

References

Faust, M.A. ;Larsen, J.and Moestrup, O. (1999). ICES Identification Leaflets for Plankton. Leaflet no.184. Potentially Toxic Phytoplankton .3. Genus *Prorocentrum* (Dinophyceae). ICES, Copenhagen.

Remarks

Other potentially toxin producing *Prorocentrum* species in Australian waters include *P.compressum* (Bailey) Abe ex Dodge (Fig.17F), *P. elegans* Faust, *P . faustiae* Morton, *P.concavum* Fukuyo and *P.maculosum* Faust.

Planktonic *Prorocentrum* species such as *P. micans* Ehrenberg (Fig.17H), *P. gracile* Schutt and *P. triestinum* Schiller can form extensive blooms (e.g. in the Karamea Bight, New Zealand) but have never been found to be toxic.

2.34 *Dinophysis fortii* Pavillard 1923
Figs. 18A,E

Description

Large bag-shaped cells with well-developed sulcal lists and greatly reduced epitheca (upper cell half), 60 – 70 µm long, 33 – 40 µm wide. This species can be confused with *D. truncata* (Cleve) which, however, has a flat bottom (Fig. 18C).

Distribution

Common in Australian and New Zealand coastal waters but rarely abundant. Mixed blooms with *D. acuminata* occur in the Derwent River, Tasmania, in the period October – February.

Toxicology

Producer of okadaic acid, dinophysis- toxin-1 and pectenotoxin- 2 which can cause diarrhetic shellfish poisoning in humans. Low concentrations of these toxin fractions have been detected in wild Tasmanian mussels, but no incidents of human poisonings are known. Commercial Tasmanian shellfish thus far have proved negative for DSP toxins. This species has caused major problems for scallop fisheries in Japan (first recognised in 1976).

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2.35 *Dinophysis acuminata* Claparède and Lachmann 1859
Figs. 18A,D

Description

Strongly compressed, small cells with well-developed sulcal lists (flanges) and greatly reduced epitheca (upper cell half), 38 – 58 µm long, 30 – 38 µm wide, occasionally with small protrusions (knobs) on the hypotheca (lower cell half). This species is best identified by its small size and regular oval outline. The thecal plates are covered with circular areolae.

Distribution

Common in Australian and New Zealand coastal waters but rarely abundant. Seasonal blooms ($10^3 – 10^5$ cells l^{-1}) occur in the Derwent River, Tasmania, in the period October – February.

Toxicology

Producer of okadaic acid, dinophysis - toxin and ?pectenotoxins, which can cause diarrhetic shellfish poisoning (DSP) in humans. This species has caused major problems for the mussel industry in Holland, Spain, France and Ireland. Associated with a major DSP-like outbreak in New South Wales in 1997 after consumption of pipis, contaminated with PTX-seco acids and DTX-3. Shellfish farms in Boston Bay, S.A., have also been closed (e.g. in 2000-2001) because of concerns about PTX contamination.

References

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2.36 *Dinophysis acuta* Ehrenberg 1841

Description

The cells, 54 – 94 µm long, 43 – 60 µm wide, have a pointed bottom and are widest below the middle.

Distribution

A relatively rare species in Australian and New Zealand waters. This dinoflagellate has caused DSP problems in Chile.

Toxicology

Producer of okadaic acid, dinophysis - toxin-1 and ?pectenotoxins which can cause diarrhetic shellfish poisoning in humans.

References

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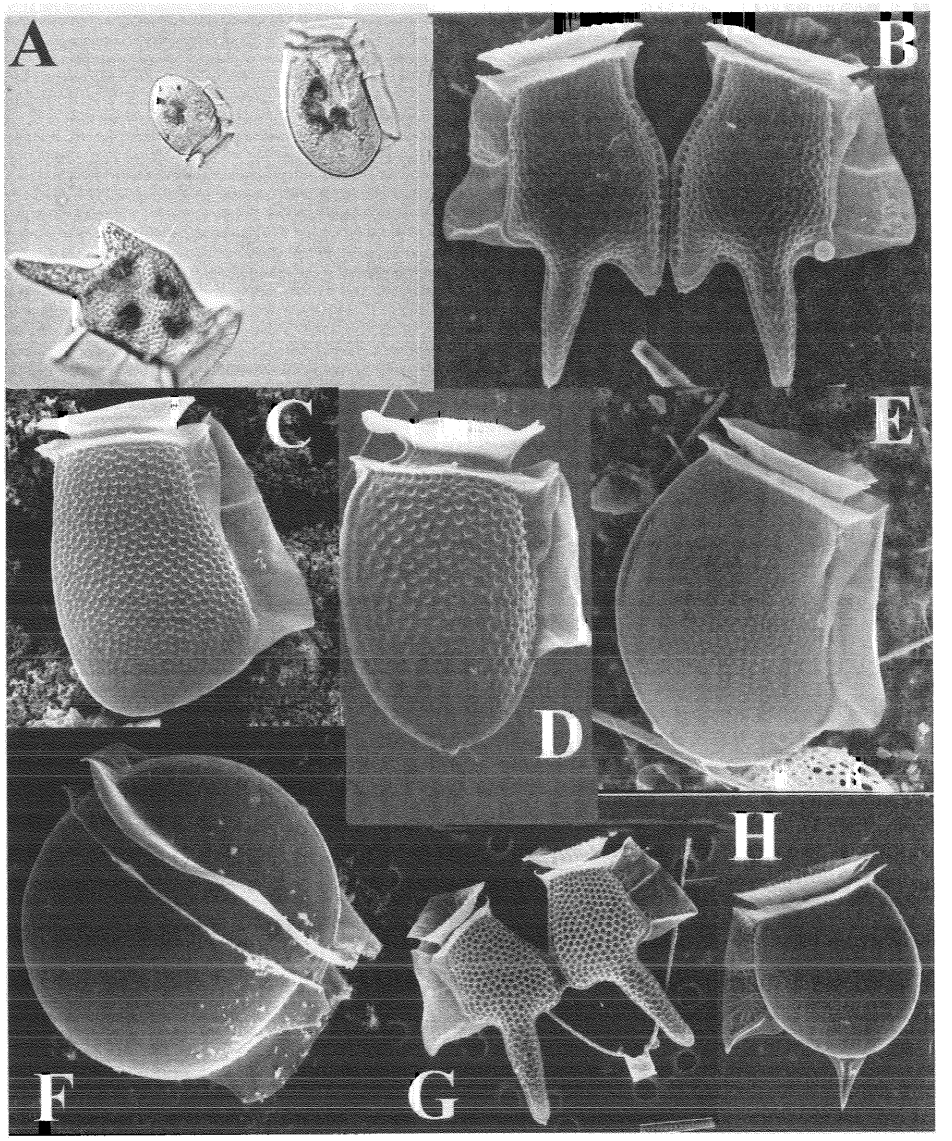
Fig. 18 Dinophysoid dinoflagellates.

Fig.18A (LM). Size comparison of *D. tripos, D.fortii* and *D.acuminata*; Fig.18B. *Dinophysis tripos* from Tasmanian waters; Fig.18C (SEM). *Dinophysis truncata* from the Huon River, Tasmania; Fig.18D (SEM), *Dinophysis acuminata* from Tasmanian waters; Fig. 18E(SEM). *Dinophysis fortii* from Tasmanian waters; Fig. 18F (SEM). *Phalacroma rotundata* from Storm Bay, Tasmania. Fig.18 G (SEM) *Dinophysis caudata* from North West Australia; Fig.18 H. *Dinophysis hastata* from Coral Sea.



2.37 *Phalacroma rotundatum* Kofoid and Michener 1911
Fig. 18F

Synonym: *Dinophysis rotundata*

Description

Round-to-oval cells, 36 – 56 µm long, with a well-developed upper cell half (epitheca). This species differs from the previous *Dinophysis* species in its absence of chloroplasts.

Distribution

Common in Australian oceanic waters but never abundant.

Toxicology

Japanese strains produce dinophysis-toxin-1, but North American strains of this species have proved to be non-toxic.

Remarks

Other *Dinophysis* species that are known to produce toxins include *D. caudata* Saville-Kent (Fig.18G), *D.sacculus* Stein, *D. tripos* Gourret (Fig.18B), *D. norvegica* Claparede et Lachmann (the latter not present in Australian waters) and *D. mitra* (Schutt) Abe, *D. hastata* Stein (Fig.18H) and *D. rapa* (Stein) Balech (all three found in tropical Australian waters).

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3. DIATOMS

Diatoms are single-celled golden-brown algae, with siliceous cell walls consisting of two overlapping symmetrical parts. Their pill-box shaped cell walls, called frustules, are ornamented with pores, ribs and spines radiating in spectacular geometry across the minute cell surfaces.

3.1 *Pseudo-nitzschia pungens* (Grunow ex P.T.Cleve) Hasle 1993
Fig.19G

Description

Approximately 20 species share the morphological features of the genus *Pseudo-nitzschia* including:

- Stepped chains formed by overlap of valve ends
- Cells are strongly elongate, spindle-shaped or rectangular in girdle view; narrowly lanceolate to spindle-shaped or linear with rounded or pointed ends in valve view
- Valves are shallow, flattened, weakly silicified
- Raphe is extremely eccentric, along one margin, not elevated above the general valve level

Identification to species level requires careful analysis of the presence/absence of a central nodulus (pseudonodulus in older literature); number of fibulae (keel punctae in older literature) compared to number of interstriae (formerly transapical costae) and the number of rows of poroids between the costae (the latter can often only be resolved by TEM)

Pseudo-nitzschia pungens has spindle-shaped cells, 80 – 140 µm long, 4.5 – 6 µm wide, with pointed ends and two chromatophores per cell. They form chains up to 38 cells long by overlapping of their ends. In this species, the central nodulus is absent, 9 – 15 costae per 10 µm can be distinguished, 2 rows of poroids occur between the costae and the poroids are spaced at 3 – 6 per 1 µm. Overlap of cell ends is approximately one-third of cell length, coarsely silicified, interstriae visible in water mounts, fibulae visible as continuation of interstriae (in acid-cleaned, mounted valves).

Distribution

A cosmopolitan species, which in the Australian region has been identified from the Arafura Sea, Port Hacking (Sydney), the Derwent and Huon estuaries (Tasmania), Bass Strait, and Wellington Harbour (New Zealand).

Toxicology

No toxin has been detected in any cultures of Australian *P. pungens* tested. Toxic clones have been reported from New Zealand, and the West Coast of the USA.

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Fig. 19 Diatom genus *Pseudo-nitzschia*

Figs. 19A (LM), B (SEM). Diatom bloom samples from Tasmanian waters showing overlapping of cells in chains;

Fig.19C. Diagrammatic summary of terminology of *Pseudo-nitzschia* cell structures

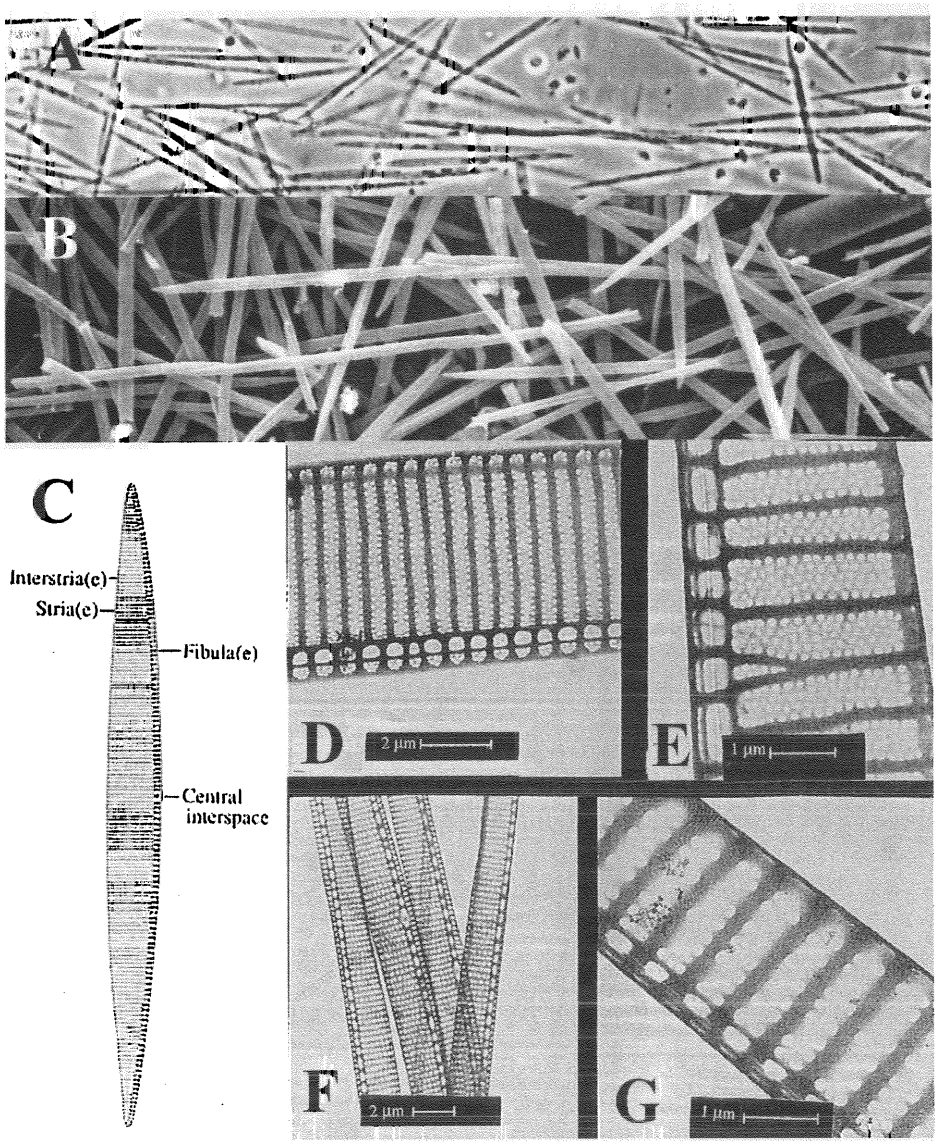
Fig.19D. (TEM). Detail of valve-structure of *Pseudo-nitzschia australis* from Tasmania;

Fig.19E. Detail of valve-structure of *Pseudo-nitzschia multiseries* from NSW

Fig.19F. Detail of valve-structure of *Pseudo-nitzschia pseudodelicatissima* from Tasmania;

Fig.19G. Detail of valve-structure of *Pseudo-nitzschia pungens* from Tasmania;

Micrographs Figs.19 D-G, by C.Lapworth



3.2 *Pseudo-nitzschia multiseries* (Hasle) Hasle
Fig.19E

Synonym: *Nitzschia pungens f. multiseries* Hasle 1965

Description

Spindle-shaped cells, 68– 140 µm long, 4– 5µm wide, with pointed ends and two chromatophores per cell. They form chains by overlapping of their ends. In this species, the central nodulus is absent, 10– 13 costae per 10 µm can be distinguished, 3– 4 rows of poroids occur between the costae and the poroids are spaced at 4– 6 per 1 µm. Overlap of cell ends is approximately one-third of cell length, coarsely silicified, interstriae visible in water mounts, fibulae visible as continuation of interstriae.

Distribution

Regularly present in Berowra Creek, NSW, and off-shore samples along the New South Wales coast.

Toxicology

Pseudo-nitzschia multiseries and *P. australis* are generally considered the most likely cause of Amnesic Shellfish Poisoning. In November/ December 1987 a diatom bloom of 15.10^6 cells/L *P.multiseries* (as *N. pungens)* in Cardigan Bay, Prince Edward Island, Canada, caused 3 deaths and 105 cases of acute human poisoning following the consumption of blue mussels containing 900 µg /g domoic acid. The symptoms included abdominal cramps, vomiting, disorientation and memory loss. Shellfish from bloom-affected areas need to be tested by HPLC or mouse bioassay, and seafoods containing more than 20 ppm (20 µg /g) domoic acid should be considered unfit for human consumption.

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3.3 *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle 1993
Fig.19F

Synonym: *Nitzschia delicatula* Hasle 1965

Description

Narrow spindle-shaped cells, 59 – 140 µm long, 1.5 – 2.5 µm wide, with pointed ends in valve and girdle views, and forming chains. A large central interspace is present. In this species 30 – 46 costae per 10 µm can be distinguished, 1 row of poroids occurs between the costae, and the poroids are spaced at 4 – 6 per 1 µm. Evidence of variation in morphology of the poroid hymen, toxin production and molecular probes suggests that more than one species may be included in this taxon.

Distribution

A cosmopolitan species. A common bloomformer in New South Wales (Berowra Creek), Victorian (Port Phillip Bay) and Tasmanian (Derwent River) waters.

Toxicology

Cultures of *Pseudo-nitzschia pseudodelicatissima* from the southwestern Bay of Fundy produced domoic acid, although at very low concentrations, highly toxic strains are known from the Gulf of Mexico, but the species was not found to be toxic in Danish and Californian waters. Natural bloom samples from NSW, Victoria and Tasmania never were found to be toxic. Of the five Australian cultures tested, only one produced trace levels of domoic acid, but the latter could have belonged to a morphologically similar (but distinct, yet undescribed) species.

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3.4 *Pseudo-nitzschia australis* Frenguelli 1939
Fig.19D

Synonym: *Nitzschia pseudoseriata* Hasle 1965

Description

Valve, 75-144 µm long, 6.5-8 µm wide, middle part (ca. one-third of cell length) with straight to slightly curved margins, valve ends slightly rostrate with rounded poles. Central nodulus is absent. The valves have 12-18 costae per 10 µm, and an intercostal membrane perforated by 2 rows of large poroids, 4-5 in 1 µm.

Distribution

Present in Tasmanian, Victorian and New South Wales waters.

Toxicology

Pseudo-nitzschia multiseries and *P. australis* are generally considered the most likely to cause Amnesic Shellfish Poisoning. A bloom event (7.10^5 cells/L) of *P. australis* off California in Sept.1991 was associated with mortality of 100 brown pelicans and cormorants, traced due to domoic acid in anchovies feeding on this diatom. Similarly, in Oct.1991 dozens of cases of human illnesses were reported along the Pacific coasts of Washington and Oregon, where razor clams, dungeness crabs, blue mussels and oysters were implicated (up to 154 µg/g DA).

Preliminary analysis using ELISA has confirmed domoic acid in Australian *P. australis* cultures tested.

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Remarks

Other common species of *Pseudo-nitzschia* in Australan waters include

P. delicatissima (P.T.Cleve) Heiden - Cell ends rounded in valve view, cut-off in girdle view, short overlap (one-ninth of cell length) ; weakly toxic in New Zealand; toxic strains also reported from Canada.

P. turgidula (Hustedt) Hasle- Short overlap of cells in chains (one-sixth of length), margins in girdle view somewhat convex, tapering toward truncated ends; cell ends in valve view rounded, and smaller specimens lightly rhomboid to lanceolate, larger specimens linear, except for a middle expansion ; weakly toxic in New Zealand.

P. multistriata (Takano)Takano- Overlap of cells in chains short; chains somewhat undulated, cells sigmoid in girdle view, poles slightly sigmoid in valve view, margins straight for the greater length of the valve, pointed poles ; nontoxic in New Zealand; toxic in Mediterranean.

P. fraudulenta (P.T.Cleve) Hasle)- Overlap of cell ends in chains fairly short, fusiform in valve view, valve structure not discernible in water mounts, fibulae and central larger interspace distinct on cleaned valves in permanent mounts; weakly toxic in New Zealand.

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3.5 *Thalassiosira mala* Takano 1965 and related species
Figs. 20A-D

Description

Minute disc-shaped cells, 4 – 9 µm diameter, embedded in cloud-like gelatinous masses of various shapes. These colonies can easily be mistaken for the prymnesiophyte *Phaeocystis globosa* (Fig. 23B). Conclusive identification of these delicate cells usually requires electron microscopic examination of the number and arrangement of the tube-like (strutted) processes, the location of the lip-shaped (labiate) process, the presence of spines and other (occluded) processes and girdle band morphology. In *T. mala,* there is one ring of marginal strutted processes (7 – 9 in 10 µm) and a single marginal labiate process is located in between two strutted processes. In addition a single central strutted process is found off centre and on the same side as the labiate process (Fig. 20C). In *T. partheneia* Schrader, one central strutted process, one ring of marginal strutted processes (4 – 7 in 10 µm) and one marginal labiate process are present. The last is found in between two strutted processes, just outside the marginal ring of strutted processes (Fig. 20D).

Distribution

T. mala and *T. partheneia* are common in Sydney coastal waters and the Gulf of Carpentaria. In August–September 1985 an extensive bloom of *T. partheneia* off the south east Australian coast (34–41°S) caused clogging of fishing nets. "Slimy waters", which since 1975 have caused great economic damage to tourism in the Adriatic (Mediterranean), are also caused by diatoms.

Toxicology

Not toxic, but the gelatinous masses may harm farmed oysters by clogging their gills (as has happened in Tokyo Bay, Japan).

Remarks

Several other species of *Thalassiosira*, such as *T. curviseriata, T. delicatula, T. diporocyclus, T. minuscula, T. stellaris, T. subtilis* and *T. weissflogii*, can also form gelatinous blooms in Australian waters.

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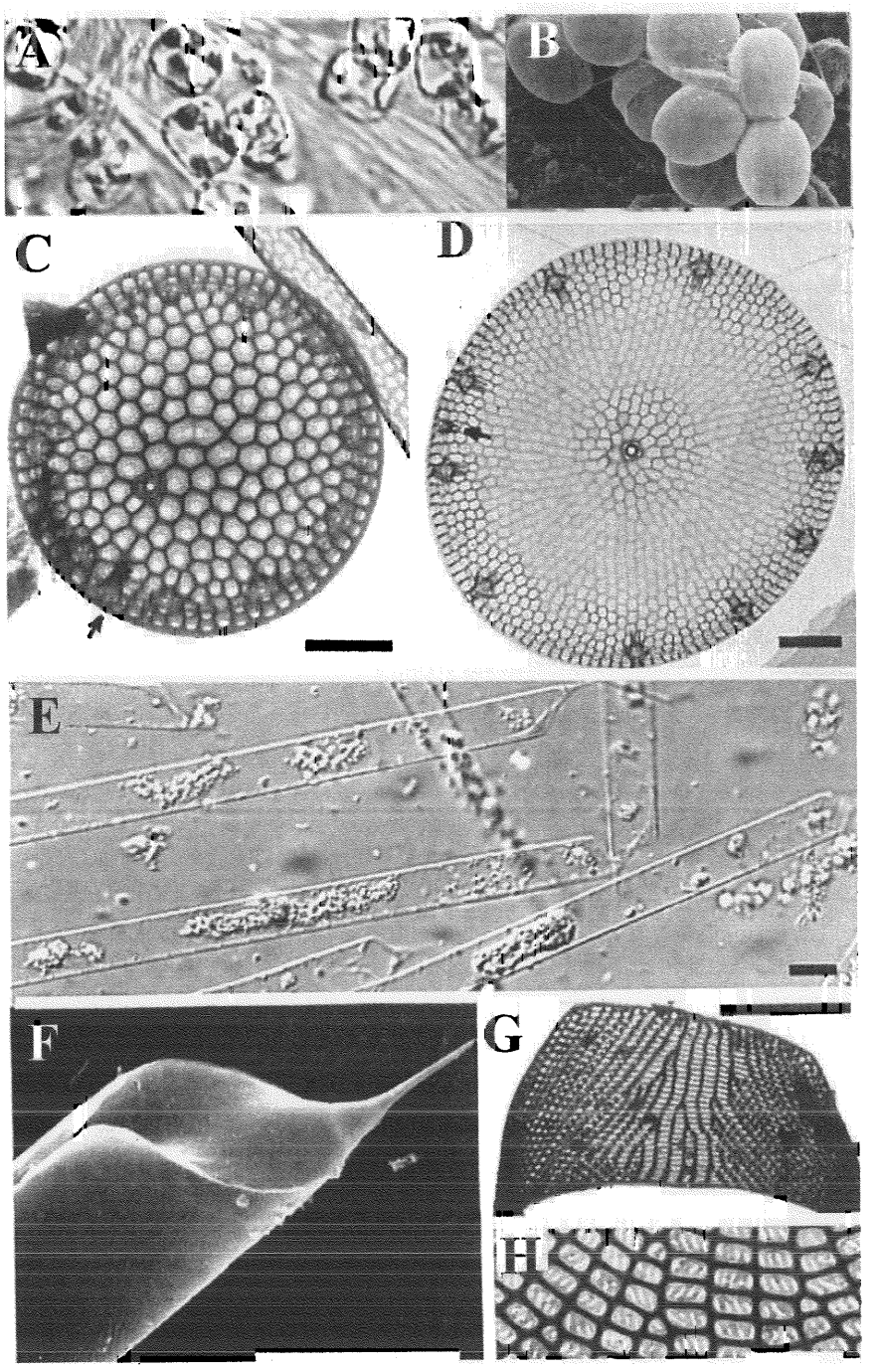

3.6 *Rhizosolenia cf. chunii* Karsten 1905
Figs. 20E-H

Description

Straight cylindrical cells (100 – 150 µm long, 20 – 30 µm diameter), often found in chains, bearing a stout pointed process at each terminal edge. The girdle bands form a characteristic zig-zag pattern. The striate pattern on these bands (copulae) (visible using the light microscope) converges on a central line (Fig. 20G). The membrane (velum) covering the individual punctae (areolae) (only visible using the transmission electron microscope) is perforated by diagonal slits (Fig. 20H). This diatom can be confused with *R. imbricata* (= *shrubsolei*) which, however, has a different velum structure.

Distribution

This species has only been recorded thus far from Port Phillip Bay (Melbourne) and Port Hacking (Sydney). *R. chunii* from the Antarctic is much shorter but has an identical ultrastructure.

Toxicology

A massive bloom of this diatom (10^6 cells l^{-1}) first occurred in Port Phillip Bay, Melbourne, in August – October 1987. Coincident with this bloom, mussels, scallops and oysters developed an unpleasant bitter taste, which rendered these seafoods unmarketable for up to 7 months. The shellfishes' digestive glands showed extensive inflammation and degeneration and high shellfish mortality occurred 3 to 8 months after the bloom. A less dense diatom bloom (10^4 cells l^{-1}) in 1989, 1991 and 1992 caused no bitter taste problems, but problems recurred in 1993 and 1994. A similar species has also caused gut pathology in scallops in Tasman and Golden Bay in New Zealand. Further research is needed to identify the chemical nature of the bitter taste, and experimental studies in which cultured diatoms are fed to shellfish are required to confirm that *Rhizosolenia* cf. *chunii* is the source of this "toxin".

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Fig. 20 Diatom *Thalassiosira mala* and related species.

Fig. 20A (LM). Gelatinous colonies of *T. partheneia* from NSW coastal waters; Fig.20B (SEM). Colonies of *T. diporocyclus* from NSW; Fig. 20C (TEM). *T. mala*. Detail of valve structure showing pattern of strutted and labiate processes (arrow); Fig. 20D (TEM). *T. partheneia*. Detail of valve structure showing pattern of labiate (arrow) and strutted processes.

Fig. 20 E-H Diatom *Rhizosolenia* cf. *chunii* from Port Phillip Bay.

Fig. 20E (LM). Cylindrical cells with pointed processes and zig-zag girdle bands; Fig. 20F (SEM). Detail of cell shape; Fig. 20G (TEM). Single girdle band with striate pattern converging on a central line; Fig. 20H (TEM). Detail of the areolar membrane perforated by diagonal slits.

3.7 *Chaetoceros convolutus* Castracane 1886
Figs. 21A-D

Description

Chainforming, bullet-shaped cells, 15 – 23 µm broad, with long hollow spines (setae) extending from their corners. The setae are studded with many smaller barbs along their length. There are more than fifty very similar species of *Chaetoceros* that possess spines (but not barbs) and form chains, but do not harm fish. In *C. convolutus* the chains are somewhat twisted and the setae do not increase in thickness from the base, while in *C. concavicornis* (Fig.21 B,D) the chains are straight and the setae increase in thickness from the base.

Distribution

A cold-water species, known from Bass Strait, New Zealand and Antarctic waters. It is a common bloom-forming diatom in Californian and British Columbian coastal waters.

Toxicology

Non-toxic, but the diatom's setae can break off and penetrate the gill membranes of fish. The small spines (spinules), which are directed toward the tip of the setae, function like barbs and thus prevent them from coming out. Deaths of cage-reared lingcod, sockeye, coho, chinook and pink salmon have been reported from British Columbia at diatom cell concentrations of about 5000 cells per litre. Death may be caused by capillary haemorrhage or by suffocation from an overproduction of mucus or even from secondary infection of the damaged tissue. Small fish are affected first. The application in feed of mucolytic agents such as L cystein ethyl ester has been suggested as a mitigating agent.

Remarks

Other *Chaetoceros* species in Australian waters which have been associated with fish farm mortalities are *Chaetoceros criophilum* (Tasmania, Nov.1991, Oct.1996) and *C.* cf. *danicus* (Tasmania, Oct. 1992).

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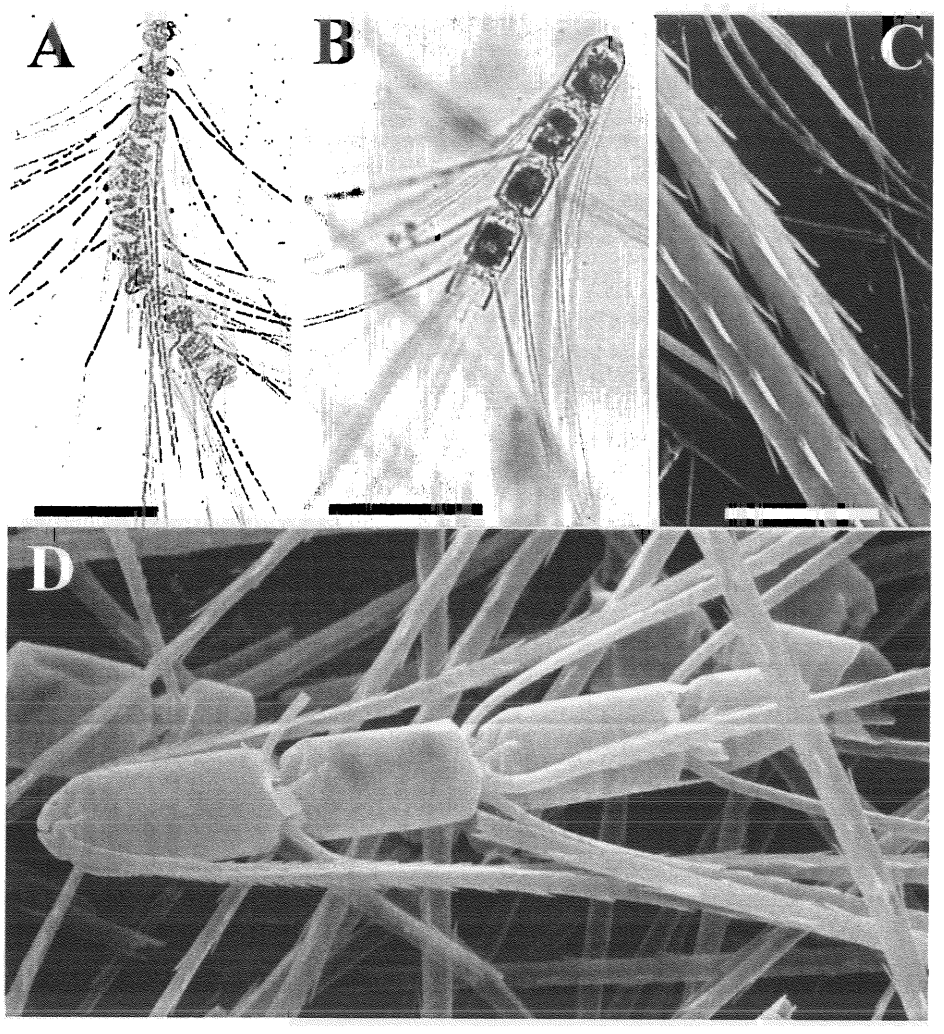

Fig. 21 Diatom *Chaetoceros convolutus* and related species.

Fig. 21A (LM. Chain of cells from New Zealand waters (Micrograph L. Mackenzie); Fig. 21B,D (SEM). *Chaetoceros concavicornis* from British Columbia; Figs.21C,D. (SEM). Detail of long spines (setae) with many smaller barbs along their length.

4. PRYMNESIOPHYTA= HAPTOPHYTA

(Golden-brown flagellates with haptonema)

These small golden-brown flagellates have two flagella which arise at the anterior end of the cell and a third threadlike appendage, the haptonema, is inserted between them. Flagellar appendages are absent. The cell surface is covered by minute unmineralised organic scales, only visible by electron microscopy.

4.1 *Prymnesium parvum* Carter 1937

Figs. 22 A-D

Synonym: *P. patelliferum* Green, Hibberd et Pienaar 1982

Description

The small golden-brown cells are more or less oval, 8 – 15 µm long, with two flagella and a very short haptonema. When swimming, the cells rotate around their longitudinal axis and the two flagella behave slightly differently (heterodynamic flagella). Identification to species level is only possible from investigation of the fine structure of the scales present on the cell surface. In *P. parvum* the two surfaces of the scales are distinct: the distal surface (away from the cell) has a concentric pattern and the proximate surface (nearest to the cell) has a radiating pattern of fibrils.

Distribution

In the Australasian region, *P. parvum* is only known thus far from New Zealand and the Vasse–Wonnerup estuary in Western Australia, which has been the site of recurrent fish kills (most commonly in January – March). This species is also known from Denmark, Germany, England, Spain, Bulgaria and Israel. It can grow at a wide range of temperatures (2° to 30°C) and salinities (especially in brackish waters). A closely related species, *P. calathiferum* Chang and Ryan, has been observed during fish and shellfish mortalities in 1983 in New Zealand, and *P. patelliferum* Green, Hibberd and Pienaar has been recorded from Wilsons Promontory, Victoria, as well as Tasmania. In studies of ploidy, *patelliferum* was found to be haploid, while forma *parvum* was diploid or haploid, indicating that the two forms belong to the same haplo-diploid life cycle. Furthermore, no genetic difference in the ITS region of the two taxa could be discerned.

Toxicology

There has been extreme confusion regarding the chemical nature of the toxins. This species produces cytolytic, haemolytic and neurologically active toxins which are excreted into the surrounding seawater. Igarashi *et al.* (1999) found two haemolytic and ichthyotoxic polyethers (glycosides), known as prymnesin-1 and -2. These toxins affect cell permeability, which leads to a disturbed ion balance. This has caused mass fish mortality (*Tilapia*) in brackish water culture ponds in Israel (since 1947), while mortality of salmon and rainbow trout in net-pens has been reported from Norway. Toxicity by this species is promoted by phosphorus deficiency.

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Remarks

A number of closely related species cause similar toxicity: *P. faveolatum* Fresnel, and *P. zebrinum* Billard.

4.2 *Chrysochromulina polylepis* Manton and Parke 1962
Figs. 22E-G

Description

Oval to pear-shaped golden-brown flagellates, 6 – 12 µm long, 5 – 9 µm wide, with two long flagella (2 to 3 times the cell length) and an additional stalk-like appendage (haptonema) that is 1 to 1½ times the cell length when fully extended (Fig. 22F). The cells swim rapidly, with frequent and sudden changes of direction. While swimming, the flagella and haptonema can be directed forwards or backwards but, when swimming rapidly, the haptonema is usually coiled. The cell surface is covered with minute organic scales, which can only be observed by electron microscopy (Fig.22G). The genus *Chrysochromulina* comprises about 50 very similar species. Important for species identification is the cell size, length of flagella and haptonema, and especially the morphology of the scales. Phagotrophic feeding is common among this genus although most species are photosynthetic.

Distribution

Most *Chrysochromulina* species have a world-wide distribution and some 15 species identified from east Australian waters are well-known in European coastal waters. *C. polylepis* has been reported from the North Sea and Skagerrak, but also from Port Phillip Bay and Tasmania.

Toxicology

Toxic to fish and invertebrates through the production of haemolytic and ichthyotoxic substances, especially under phosphate-deficient conditions. A massive bloom (60,000 km^2) of *C. polylepis* occurred in May–June 1988 in the Skagerrak, the Kattegat, the Belt and the Sound between Denmark, Norway and Sweden. Ecological effects of this bloom included mass mortality of invertebrates (gastropods, starfish, polychaetes, sponges), wild and cultured fish (some 600 tons of rainbow trout) and even red seaweeds. Fish deaths occurred due to damage to gill membranes which produced a lethal increase in the chloride concentration in the blood. This explains why fish cages moved into the less saline fjords were less affected. The toxins do not accumulate in fish flesh but have been found in mussels.

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4.3. *Chrysochromulina leadbeateri* Estep, Davis, Hargraves et Sieburth 1984
Fig.22H

Description

Cells more or less spherical, 3-8 µm in diameter. The two flagella are 13-16 and 16-20 µm long, the haptonema 20 µm long. All appendages are attached anteriorly but the haptonema is usually coiled. Cells contain two golden brown parietal chloroplasts, each with a pyrenoid. The cell surface is covered with two types of submicroscopic scale, arranged in two layers each with a circular outline, and measuring 0.35 µm in diameter. Scales of the underlayer are plate-like and consists of concentric and radiating ribs. It shows a central cross separating four perforations, surrounded by an elevated ring and a circle of 13-15 circular pores. Some 30 ribs radiate from the pores to the scale periphery, divided by a concentric rib into an outer ring of nearly square perforations and an inner ring of elongate ones. The other scale type is less ornamented but surrounded by an upright rim. It possesses a central cross separating four openings and surrounded by a distinct elevated ring, but most of the scale is more or less structureless. Along the base of the scale rim there is a circle of very small perforations, in the order of 30-35. In some scales a few scattered pores may be present immediately within this ring of perforations or around the central cross.

Distribution

C. leadbeateri has been reported from many parts of the world, including Australia. The species concept is uncertain due to the finding of slightly different scale morphotypes in different parts of the world.

Toxicology

C. leadbeateri was responsible for killing 600 tonnes of cultured fish in Northern Norway (Lofoten) in 1991 The toxic principle has not been isolated. Cultures of *C. leadbeateri*, proved non-toxic to the brine shrimp *Artemia salina* and three other methods also failed to demonstrate any toxicity.

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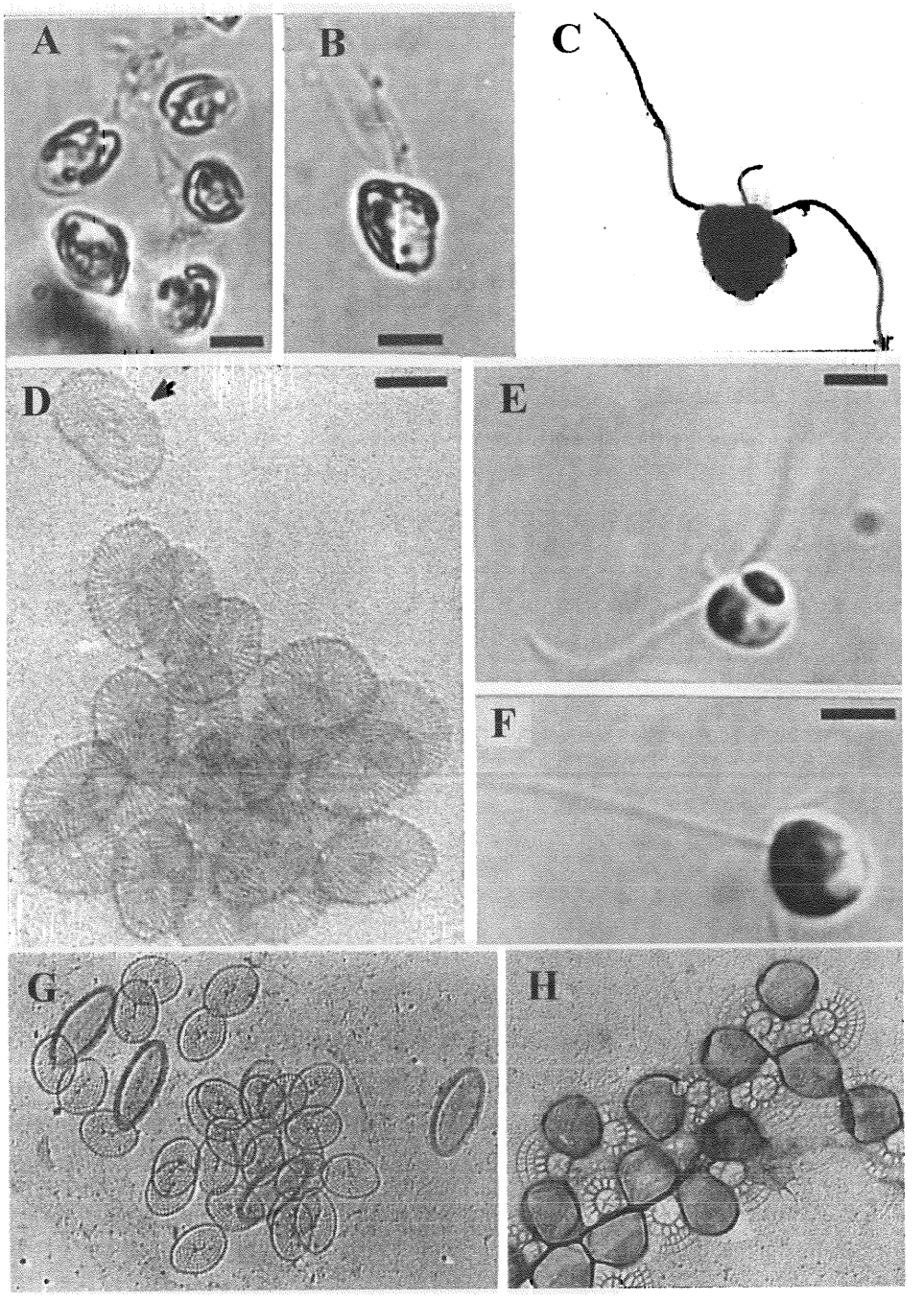


Fig.22.
(Page 101)

Golden-brown flagellates.

Fig. 22 A-D. Golden-brown flagellate *Prymnesium parvum* from the Vasse-Wonnerup estuary, W.A.

Figs. 22 A, B (LM). Lugol-preserved cells; Fig. 22C (TEM). Cell with two flagella and short haptonema (arrow); Fig. 22D (TEM). Detail of fine structure of the scales on the cell body. The distal surface of the scales has a concentric pattern (arrow) and the proximate surface has a radiating pattern of fibrils.

Scale bars: 10 µm (Figs. 22A–B); 0.2 µm (Fig. 22D)

Fig. 22 E,F (LM). Golden-brown flagellate *Chrysochromulina hirta* in different swimming positions. Scale bars: 10 µm.

Fig.22 G. Scale pattern of *Chrysochromulina polylepis* from Tasmanian waters (micrograph by J.M.LeRoi); Fig.22H. Scale pattern of *Chrysochromulina leadbeateri* from Tasmanian waters (courtesy J.M.LeRoi).

Fig.23.
(Page 103)

Golden-brown flagellates.

Fig. 23 A-E. Golden-brown flagellate *Phaeocystis globosa.*

Figs. 23A (TEM). Flagellate cell showing thread-like material (courtesy J.M.LeRoi); Fig.23B (LM). Colonial phase with cells embedded in a gelatinous matrix; Fig. 23C (TEM). Detail of cell surface with rimmed scales; Fig. 23D (SEM). Thread-like material arranged in a pentagonal figure; Fig.23E. Characteristic sphaerical colony.

Scale bars: 10 µm (Fig. 23B); 1 µm (Fig. 23C);0.5 µm (Fig. 23D)

Fig. 23F-G Golden-brown alga *Pelagococcus subviridis.*

Figs. 23F (LM). Minute cells from the East Australian Current; Fig. 23G (TEM). Thin section of a cell showing single chloroplast (chl) (micrograph: M. Vesk)

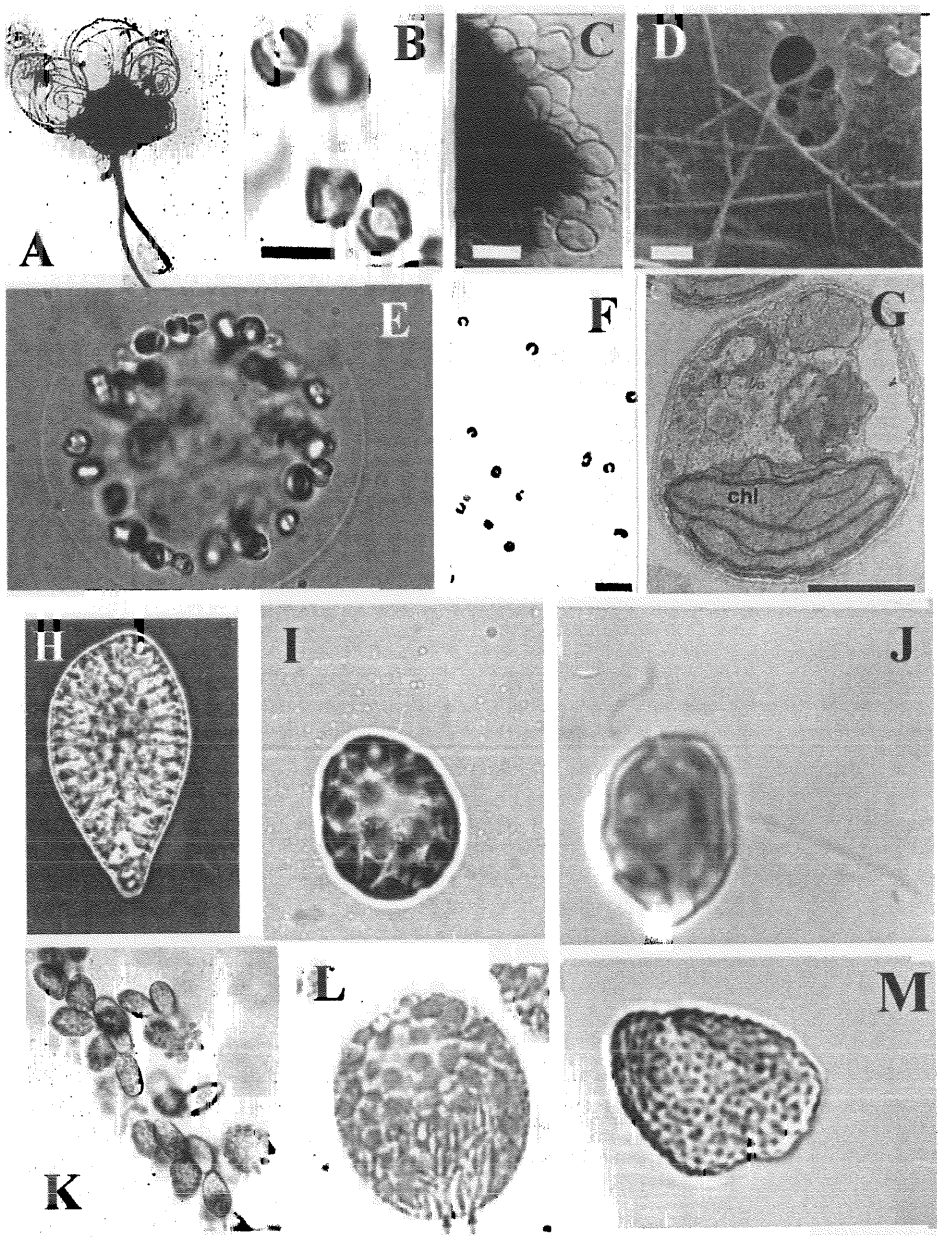
Scale bars: 10 µm (Fig.23F); 1 µm (Fig. 23G)

Fig.23 H. *Chattonella marina* from Port Lincoln

Fig. 23I-J. Raphidophyte flagellate *Heterosigma akashiwo*. (LM). Potato-shaped cells with one forward-directed flagellum and one trailing flagellum. Culture from Australian waters; Fig.23 K. Field sample from Cockburn Sound, WA.

Fig.23L. Raphidophyte *Fibrocapsa japonica,* showing mucocysts at posterior end.;

Fig.23M. *Haramonas* -like cell from Sydney Harbour.



4.4 *Phaeocystis globosa* Scherffel
Figs. 23A-E

Description

Phaeocystis usually forms colonies of highly variable morphology. Several species were described around the turn of the century but these were all merged into *Phaeocystis pouchetii.* This trend has now been reversed, and the genus comprises several species. At least two different stages occur in the life cycle, a colony-forming phase and one or more unicellular stages. In some species colonies may attain a length of 1 cm and thus become visible to the naked eye. The cells are embedded in mucilage, forming a monolayer along the periphery of the colony. Each cell contains 2 (1-4) parietal yellow-green chloroplasts but usually lack flagella and haptonema. The unicellular stages are biflagellate, 3-9 µm long, with a very short haptonema which is often difficult to see with the light microscope. The cell surface is covered with submicroscopic flat scales of two kinds. The cells also produce a thread-like material (believed to be trichocysts), the proximal parts of which are arranged in a pentagonal figure. In the closely related species, *Phaeocystis scrobiculata* Moestrup, known only as unicellular flagellate stage, the threads are arranged in a nine-ray figure. The life history of *Phaeocystis* is still unresolved.

Distribution

Phaeocystis globosa can form dense gelatinous blooms in the coastal waters of New South Wales, Tasmania and New Zealand. *P. pouchetii* (Hariot) Lagerheim is a cold-water species which occurs in the Arctic and in temperate waters of the Northern Hemisphere. Colonies are rather small, up to 2 mm. Small colonies are spherical, while colonies larger than 0.3 mm are lobed. Cells are generally in groups of four forming a square. *P. antarctica* Karsten is a cold-water species confined to the area around Antarctica. It grows to 9 mm, and colonies are spherical or derived from a spherical shape. Cells are uniformly distributed along the periphery of the colony. *P. globosa* Scherffel occurs in temperate waters of both hemispheres. It grows to 8-9 mm and the colonies are spherical or derived from a spherical shape. It also resembles *P. antarctica* in the uniform arrangement of the cells along the periphery.

Toxicology

Phaeocystis is a genus of foam-producing species distributed world-wide and causing problems for fishing. Not toxic to humans, but this species can form irritant substances (acrylic acid) and mucilage. The latter can interfere with fishing by clogging the gills of fish and bivalves and by fouling fishing nets (in 1981 in New Zealand). During blooms the gelatinous colonies can also be responsible for huge masses of foam on the beach. *Phaeocystis* blooms have been reported to deflect the migration path of herring in the North Sea. *Phaeocystis* blooms produce dimethylsulphide (DMS), which is believed to evaporate to the atmosphere and contribute to the acidity of rainwater. A directly toxic effect is believed to have occurred in Norway in 1992, causing death of farmed salmon valued at 1 million NKr .The toxic compound(s) has not been identified. *Phaeocystis* may also have a toxic effect on cod larvae .

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5. CHRYSOPHYTA, Class PELAGOPHYCEAE

5.1 *Aureococcus anophagefferens* Hargraves et Sieburth 1988
Aureoumbra lagunensis Stockwell, DeYoe, Hargraves et Johnson 1997
Fig.23F,G

Description

Minute yellow-brown coccoid algal cells of bacterial size (3 – 5 µm diameter), which can only be unequivocally identified by transmission electron microscope examination of thin sections. The cell contents include a single cup-shaped chloroplast with immersed pyrenoid and three-layered thylakoid lamellae. Analysis for the diagnostic pigments fucoxanthin and 19'-butanoyloxy–fucoxanthin or the application of species-specific immunological antibodies are alternative methods to identify these minute cells.

Distribution

Bloom events of *A. anophagefferens* (10^9 cells per litre) are known from Narragansett Bay, Long Island embayments (USA) and Norwegian coastal waters, but have never been reported from the Australian region. A similar species *Aureoumbra lagunensis* has had severe impacts on Texas bays and lagoons. The morphologically similar species *Pelagococcus subviridis* Norris has been identified from the East Australian Current.

Toxicology

Not known to produce toxins, but a dense bloom ("brown tide") in Narragansett Bay in summer 1985 badly affected the abundance, feeding and fecundity of crustaceans and bivalves such as scallops and mussels. Eelgrass beds were also decimated because of the reduction of light.

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6. CHRYSOPHYTA, Class RAPHIDOPHYCEAE (Chloromonads)

6.1 *Heterosigma akashiwo* (Hada) Hada 1967
Figs. 23 I-K, 24A

Basionym: *Entomosigma akashiwo* Hada 1967
Synonyms:? *Heterosigma inlandica* Hada 1968, "*Olisthodiscus luteus* " Plymouth cultures 12A and 239, *Olisthodiscus carterae* Hulburt 1965, *Heterosigma carterae* (Hulburt) Taylor 1992.

Description

Biflagellate potato-shaped cells, 11 – 25 µm long, 8 – 13 µm wide, 8 – 11 µm thick. The forward-directed flagellum has two rows of fine hairs (only visible by electron microscopy) while the trailing flagellum is smooth and lies close to the surface (Fig. 23J). The cells rotate during swimming. They contain numerous yellow-brown disc-shaped chloroplasts (Fig. 23I) and many mucus-producing vesicles. The naked cells (without scales) are extremely fragile and identification usually requires live cells. This organism has often been misidentified as *Olisthodiscus luteus*. As part of its life cycle, this species produces benthic resting stages consisting of agglutinated masses of non-motile brown cells of variable size and shape.

Distribution

In the Australasian region this species is known from Port Stephens and Berowra Creek (NSW), West Lakes (South Australia), Cockburn Sound (W.A.), Queensland waters and Stuart Island (New Zealand).

Toxicology

Toxic to fish. This species has been circumstantially linked to deaths of caged fish in Japan, Canada (1985, 1986), Chile (1988), New Zealand (1989) and possibly Singapore. In January 1989 a bloom event in Big Glory Bay, Stuart Island (New Zealand) killed 12 million NZ dollars worth of cage-reared chinook salmon. A bloom event in June 2000 at Sea World, Surfers Paradise, was also associated with fish kills. The killing mechanism of *Heterosigma* blooms is poorly understood. Both physical clogging of gills by mucus excretion, breve-like neurotoxins as well as gill damage by haemolytic substances and reactive oxygen species may be involved.

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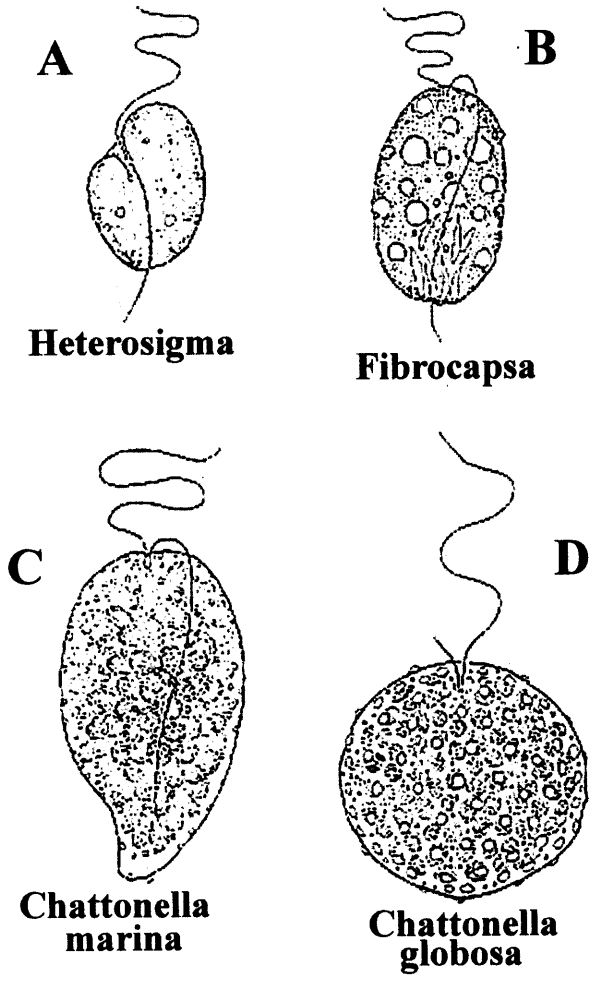

Fig.24. Diagrammatic drawings of raphidophyte flagellates.

Fig.24 A. *Heterosigma akashiwo*; Fig. 24B. *Fibrocapsa japonica*; Fig. 24C. *Chattonella marina*; Fig. 24D. *Chattonella globosa.*

6.2 *Fibrocapsa japonica* Toriumi et Takano 1973
Fig.23L,24K

Synonym: *Chattonella japonica* (Toriumi et Takano) Loeblich III et Fine 1977, "*Exuviella* sp." (isolate FCRG 51)

Description

Slightly flattened cell, ovate to obovate in ventral view, 20-30 x 15-17 µm size. The anterior flagellum is as as long as the cell, and the posterior flagellum is 1.2 times the cell length. Both emerge from an anterior gullet. Many discoid, yellow-brown to golden-brown chloroplasts are densely packed in the cell, giving the appearance of a single reticulate chloroplast. A pyrenoid is present in each chloroplast. Rod-shaped mucocysts in the posterior end of the cell eject long threads (up to 300 µm). The nucleus is located in the centre of the cell. Eyespots and contractile vacuoles are absent. Spherical cyst stages can be found adhering to diatom frustules.

Distribution

Coastal waters of Japan, Australia, New Zealand, California, northern Europe and Brazil.

Toxicology

Toxic to fish. Producer of neurotoxins similar (but not identical) to brevetoxins, provisionally named fibrocapsin.

References

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6.3 *Chattonella marina* (Subrahmanyan) Y.Hara et Chihara 1982 Fig.23H,24C

Basionym: *Hornellia marina* Subrahmanyan 1954

Description

The cell, 30-70 µm long x 20-30 µm wide, is asymmetrical in lateral view, slightly flattened, oblong to obovoid in shape, with a posterior tail. The two subequal flagella are approximately equal to the length of the cell and emerge from the bottom of an anterior depression. Many, green to yellowish-brown, ellipsoid chloroplasts, are arranged radially. A naked pyrenoid is located on the inner pole of the chloroplast.The tear-shaped nucleus is situated in the centre of the cell. Contractile vacuoles, eyespots and mucocysts are absent. Asexual reproduction is by binary division. Cyst formation occurs after meiosis in vegetative cells. The hemispherical cysts have a simple pore on top.

Distribution

Brackish coastal areas rich in organic material from India, Australia, New Zealand, China, Korea, Japan, Brazil, USA and the Netherlands. In the Australian region, known from Port Lincoln (South Australia), Western Australia and Tasmania (Derwent River).

Remarks

This species is often regarded as synonymous with *Chattonella subsalsa* Biecheler 1936 but Hara and Chihara 1982 discuss reasons to keep them separate, i.e. no thylakoids penetrating the pyrenoids, and oboe-shaped mucocysts present in *C. subsalsa* but not *C. marina*. The form described as *C. minima* Hara et Chihara may be an ecotype of *C. marina* and is still only known from the Japanese type locality. The separation of *C.antiqua* and *C.marina* also requires reexamination, because it is based solely on cell size and is not supported by chemotaxonomy of pigments and lipids nor molecular sequencing.

Toxicology

An aquaculture mortality ($45 M loss) of cultured bluefin tuna in Boston Bay, South Australia, in April-May 1996 was circumstantially associated with a bloom (up to 66,000 cells/L) of *Chattonella marina*. This species has caused significant problems for yellowtail aquaculture in the Seto Inland Sea in Japan (US $0.5 Billion damage in 1972). However, the precise fish killing mechanism remains poorly understood. Research is focusing on the production of polyunsaturated fatty acids, reactive oxygen species (superoxide, hydrogen peroxide, hydroxyl radicals), or neurotoxins (similar to brevetoxins).

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6.4. *Chattonella globosa* Y.Hara et Chihara 1994
Fig.24D

known as "flagellate X" (Scotland, Ireland)

Description

Nearly globose cell, 40-55 µm diameter.Two unequal flagella emerge from a shallow depression at the cell anterior. Numerous pale-brown to golden-brown, small elliptical chloroplasts without pyrenoid are located throughout the cytoplasm. A spherical nucleus is located in the centre of the cell. Several large mucocysts with nail-shaped inclusions are distributed along the cell periphery. No contractile vacuoles nor eyespot are present. Asexual reproduction takes place by binary fission while swimming. Cyst formation and sexual reproduction are unknown.

Distribution

Eutrophic coastal areas of Japan, southeast Asia, Australia and Canada. This species is often confused with rounded cells of *C. antiqua*, but can be distinguished by characteristics of chloroplasts, flagellation and mucocysts. This species is known to cause respiratory damage to fish, similar to that caused by other *Chattonella* species. Observed In Port Lincoln and Sydney Harbour.

References

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6.5. *Haramonas dimorpha* Horiguchi 1996 and related species
Fig.23 M

Description

Club-shaped cells, 24-39 µm long, 10-15 µm wide. Two heterodynamic flagella emerge from the cell anterior. Numerous(10-20) disc-shaped, yellow-brown chloroplasts arranged peripherally in an overlapping manner. A diagnostic tubular invagination occurs at the posterior end of the cell. Mucocysts are distributed along the cell periphery but trichocysts are absent. The organism occurs in two distinct life cycle stages ('*dimorpha*'), a club-shaped motile form and a spherical non-motile benthic form.

Distribution

Only known thusfar from the type-locality in tropical mangrove waters of N.E. Australia and a coral lagoon in Saipan.

Toxicology

A similar (but not identical) organism was associated with fish kills in Sydney Harbour in 1996.

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7. CHRYSOPHYTA, class DICTYOCHOPHYCEAE (Silicoflagellates)

7.1 *Dictyocha speculum* Ehrenberg 1837

Figs. 25 A,C

Synonym: *Distephanus speculum*

Description

Silicoflagellates are yellow, unicellular flagellates, 10 – 20 µm diameter, which are commonly surrounded by a characteristic basket-shaped, siliceous skeleton but they can also occur in a naked stage. The cytoplasm is very fragile and contains 30 to 50 yellow chloroplasts around the periphery (Fig. 25A). Cells of the skeleton-less form of *Dictyocha* may be distinguished from *Heterosigma* by the presence of a single anterior flagellum and the absence of a trailing flagellum.

Distribution

A cosmopolitan species. Bloom events are known from Danish, French and Mediterranean coastal waters but have never been observed in the Australian region. A bloom event of the related species *Dictyocha octonoria* Ehr. (Fig.25B) off Newcastle in 1993 was associated with extensive fish mortality.

Toxicology

Perhaps toxic to fish. Bloom events in Denmark ("naked stage") in 1983 and in France in 1987 ("siliceous skeletons") have been responsible for fish kills. No toxic effects have been reported from the Australian region.

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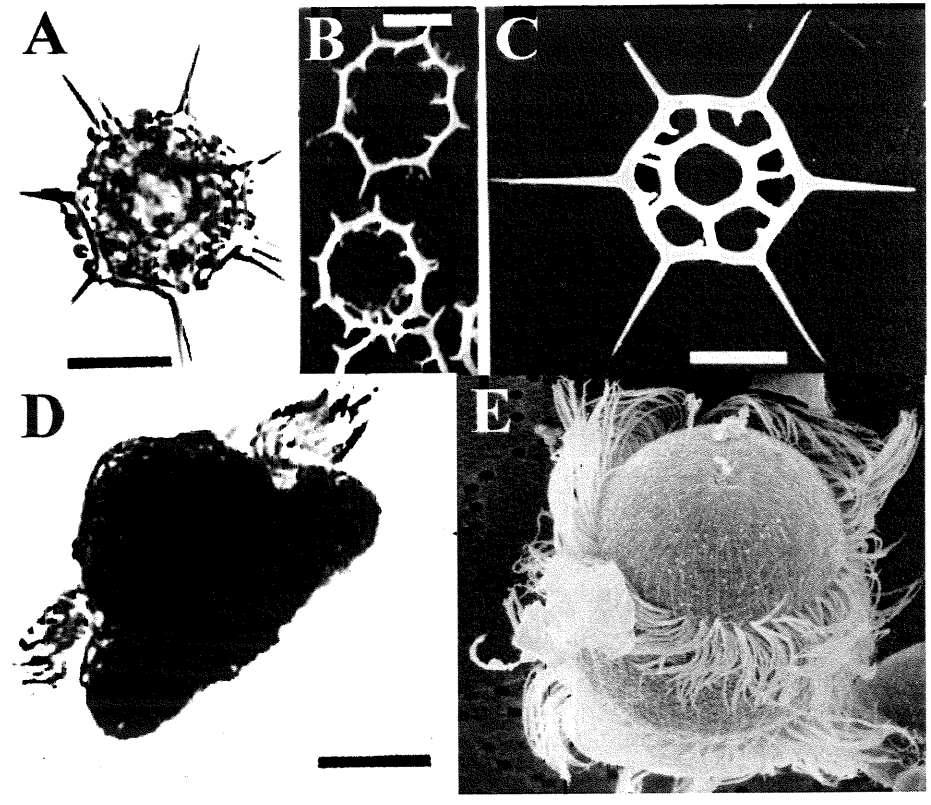

Fig. 25. Silicoflagellate *Dictyocha* and photosynthetic ciliate *Mesodinium.*

Fig. 25 A-C. Silicoflagellate *Dictyocha.*

Fig. 25A. *Dictyocha speculum.*(LM). Living cell with fragile cytoplasm and numerous chloroplasts contained in a basket-shaped skeleton; Figs. 25B. *Dictyocha octonaria*; Fig.25C. *Dictyocha speculum* (SEM). Siliceous skeletons.

Fig. 25 D-E. Photosynthetic ciliate *Mesodinium rubrum.* Two-lobed cell with a fringe of cilia around its middle.

Fig. 25D. (LM). Lugol-preserved cell from New Zealand waters (micrograph: L. Mackenzie); Fig.25E. SEM. Tasmanian waters.

8. MISCELLANEOUS

8.1 *Mesodinium rubrum* Lohmann 1908
Figs. 25 D,E

Synonyms: *Myrionecta rubra, Cyclotrichium meunieri*

Description

This ciliate, about 30 µm diameter, can usually be distinguished by its two-lobed shape and the presence of many flagella which are arranged in a fringe around its middle. When alive it is easily recognised under the microscope by its jumping swimming pattern. This unusual species is a small animal that carries cryptomonad algal symbionts. Preservatives such as formalin destroy these delicate cells beyond recognition. Water discolourations by this species may look red, brick red, wine red, maroon or muddy.

Distribution

This cosmopolitan species has caused purple water in Wellington Harbour, New Zealand and in Parramatta River and Lane Cove River, New South Wales. *Mesodinium* red tides were already observed by Charles Darwin in 1832 off the coast of Chile.

Toxicology

No harmful effects to shellfish, finfish or humans have ever been observed, although red discoloration of shellfish has caused occasional consumer complaints.

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9. CYANOBACTERIA (Blue-Green Algae)

The Cyanobacteria (also called Cyanophyceae) are primitive single-celled or filamentous bacteria, characterised by the absence of a nucleus and other membrane-bound organelles, and their often blue-green colour. Problem species are prevalent in freshwaters (*Anabaena, Cylindrospermopsis, Microcystis,*), but *Nodularia* occurs in brackish water, and *Lyngbya* and *Trichodesmium* cause problems in true marine environments. Cyanobacterial toxins can kill domestic and wild animals that drink from the shores of eutrophic ponds, lakes and reservoirs, contaminate human drinking water with teratogens and tumour promotors, but they can also accumulate in the digestive system of freshwater and brackish water shellfish. Microcystins of unknown origin have been related to salmon net pen liver disease in British Columbia and Puget Sound. Adequate regulatory levels for cyanobacterial toxins in seafood have yet to be established, while safe concentrations for peptide toxins in drinking water as adopted by WHO are < 1 µg /L. Testing facilities (HPLC) for peptide and alkaloid cyanobacterial toxins are available at the Australian Centre for Water Quality Research, Salisbury, SA, and the National Research Center for Environmental Toxicology and CSIRO Division of Land and Water, both in Brisbane.

9.1. *Anabaena circinalis* Rabenhorst ex Bornet & Flauhault 1888
Figs 26A,C

Description

Both the genera *Anabaena* and *Anabaenopsis* are filamentous cyanobacteria with more or less spherical cells. The trichomes resemble string of beads. The trichomes can be embedded in mucilaginous matrix and are sometimes forming large colonies. They have different shaped heterocytes (nitrogen-fixing cells) and akinetes (spores or resting cells). The position of the heterocytes on the filaments is an important diagnostic feature. *Anabaena* has single heterocytes intercalary (mid-trichome) positioned on the filament. *Anabaenopsis*, on the other hand, has terminal and paired, intercalary heterocytes. *Anabaena circinalis* has widely coiled trichomes, regularly or irregularly twisted, spirals 70-120 µm wide, free floating or in larger colonies. The cells are spherical to short barrel-shaped with many aerotopes, 7 to 11 µm in diameter and with heterocytes 7-13 µm wide. The akinetes are 25-30 µm long and 12-13 µm wide.

Distribution

Anabaena is a freshwater genus that is sometimes present in brackish and marine waters. In Nov.-Dec 1991 a bloom of *A. circinalis* covered 1000 km of the Darling-Barwon river system and killed an estimated 10,000 live stock and required emergency water supplies for several towns.

Toxicology

Within this genus there are nine species associated with toxin production. These toxins are the potent neuromuscular blocking alkaloid anatoxin - a, several of the paralytic shellfish poisons (PSP), the anticholinesterase organic phosphate anatoxin - a(s) and the hepatotoxic cyclic peptide

microcystins. The species associated with one or more of these toxins include: *Anabaena circinalis*, *A. flos-aquae,* A. *macrospora*, *A. spiroides*, and *A. variabilis*. In addition, in the related genus *Anabaenopsis,* the species *A. milleri* has also been shown to be toxic.

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Fig. 26. Filamentous cyanobacteria.

Fig. 26A-C. Spiral-shaped colonies of freshwater *Anabaena circinalis*;

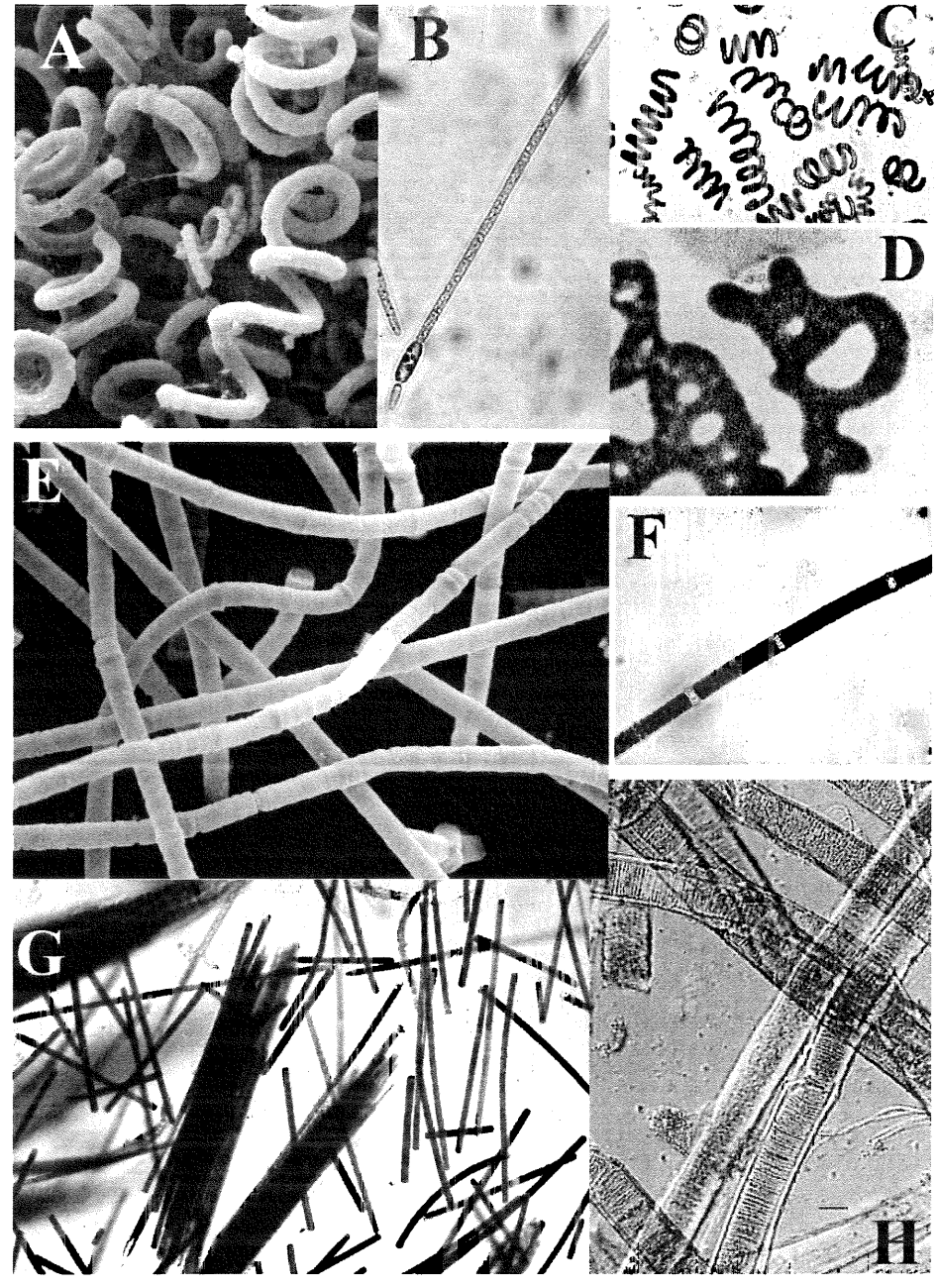
Fig. 26B. Colony of freshwater *Cylindrospermopsis raciborskii* (image courtesy P.Baker).

Fig. 26D. Irregular sheet-like colonies of freshwater *Microcystis aeruginosa* (image courtesy P.Baker);

Fig. 26E-F. Filamentous colonies of the brackish water *Nodularia spumigena*. Filaments of disc-shaped cells, including a slightly bigger heterocyte.

Fig. 26G. Raft-like aggregations of *Trichodesmium erythraeum* from tropical Australian waters.

Fig. 26H. Filaments of *Lyngbya majuscula* from Moreton Bay, Qld (image courtesy G.Cronberg).



9.2. *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya et Subba Raju 1972
Fig.26B

Description
Solitary, straight or slightly curved filaments with a length up to 200 µm. The cells are cylindrical, slightly constricted at the cross-walls, 2.5-4 µm wide and 2.5-16 µm long. The terminal heterocytes are long, conical, 5-6 x 2-2.5 µm. The akinetes are elongated-oval situated adjacent to the heterocyte or terminal vegetative cell, 2.8-3.2 µm wide and 4.5-16 µm long.

Distribution
This species occurs in eutrophic ponds, lakes, dams and rivers, especially in tropical Australia.

Toxicology
Producer of the hepatotoxic alkaloid toxin cylindrospermopsin. In 1979 140 children and 10 adults of the aboriginal population of Palm Island off Queensland, Australia, received hospital treatment after a *Cylindrospermopsis raciborskii* bloom in the drinking water reservoir. Also responsible for numerous cattle deaths in Queensland.

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9.3. *Microcystis aeruginosa* Kützing 1845
Fig.26D

Description
Microcystis is a freshwater genus, which occasionally occurs in brackish water. The colonies can be spherical, ellipsoidal, or irregularly lobed, up to 8 µm in diameter. Within the colony cells are spherical, 3-6 µm in diameter, surrounded by 5-8 µm wide mucilage with many aerotopes (gas vesicles), which results in a granular appearance. The slime edge of the colony is diffuse and narrow.

Microcystis flos-aquae has mostly spherical, compact colonies. The mucilaginous envelopes hardly exceed the aggregated cells in the colony. The cells are 3.5-4.8 µm wide and contain many aerotopes. This species occurs in the plankton of mesotrophic to slightly eutrophic temperate water bodies.

Microcystis wesenbergii has spherical to elongate, often lobed and clathrate colonies, sometimes composed of sub-colonies with distinct, refractive mucilage edge up to 6 µm long. The cells are evenly spread in the colony, 14-7 µm in diameter with many aerotopes. Cosmopolitan distribution.

Distribution
Widespread In Australia, e.g. causing nuisance blooms in the Swan River, Perth, and Craigbourne dam, Tasmania. In three successive summers 1998-2000 the Torrens Lake (in the centre of the City of Adelaide) had heavy blooms of *Microcystis*, believed to be the cause of waterfowl deaths.

Toxicology
Producer of the polypeptide microcystin which causes liver damage, and can be a serious problem in terms of drinking water contamination. (safe oral level for peptide toxins is 1 µg/L). In 1996 55 human fatalities occurred in Brazil when microcystins from *Microcystis panniformis* contaminated water used for kidney dialysis.

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9.4 *Nodularia spumigena* Mertens 1822
Figs. 26 E, F

Description

The blue-green filamentous colonies of *N. spumigena* have filaments (trichomes) enclosed with more or less firm sheaths. The unbranched filaments have disc-shaped vegetative cells as well as disc-shaped, slightly bigger heterocytes thought to be responsible for nitrogen fixation. Gas vacuoles, which cause this species to float to the surface, appear as dark grains in the cells. Filaments are straight or often twisted, and cells are constricted at cross-walls, from 8 – 14 µm in diameter and only about 3 - 5 µm long. The sheath is thin and colourless. Heterocytes are compressed and elliptical in shape, 14 µm in diameter and 7 µm long. The thick-walled spore stages (akinetes) are transversely oval, 6-11 x 10-12 µm and rarely spherical 8-10 µm in diameter, with brownish cell wall, in series, rarely solitary or in pairs. Akinetes allow this species to over-winter in sediments.

Distribution

This brackish water species has produced dense blooms in the Gippsland Lakes system of eastern Victoria, Orielton Lagoon In Tasmania, in the Peel-Harvey, Serpentine River and Vasse-Wonnerup estuaries of Western Australia, and in the Darling and Murray Rivers of South Australia. Blooms of this species were reported from Lake Alexandrina, South Australia, as early as 1878, but their increasing frequency and distribution appear to be caused by phosphorus from soil and agricultural fertilisers being washed into the river systems. In early 1990 domestic water supplies to metropolitan Adelaide had to be partly diverted due to contamination of water reservoirs by *Nodularia*. Also known from the Baltic Sea and brackish water lakes and estuaries of New Zealand, United States and Uruguay. It can live either in the plankton or attached to macroalgae or sediments. Coastal engineering approaches which modify the brackish habitat of *Nodularia* have been successful in eradicating this bloom organisms from e.g. Orielton Lagoon (Tas.) and the Peel-Harvey Estuary (WA).

Toxicology

Producer of the peptide hepatotoxin, nodularin, which can kill domestic and wild animals that drink from the shores of eutrophic ponds, lakes and reservoirs. Animals affected include horses, cows, sheep, dogs, pigs, fowls, turkeys, mice and probably birds and fish. These toxins are known to contaminate drinking water and also can accumulate in mussels, prawns and fish (safe oral level for peptide toxins is 1 µg/L). Fish and crabs avoid estuaries affected by *Nodularia* blooms and commercial fish catches can be reduced considerably. With humans stomach complaints, headaches, eczema and inflammation of the eyes have been reported. Ingestion of toxins by laboratory mice have resulted in degeneration of liver cells, tumour promotion and death from hepatic haemorrhage and the failure of pulmonary circulation.

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9.5. *Trichodesmium erythraeum* Ehrenberg 1830
Fig.26G

Synonym:*Oscillatoria erythraea*

Description

Tropical filamentous alga, 7 – 12 µm wide, filaments 60 – 750 µm long. *T. erythraeum* has straight trichomes oriented parallel in bundles; the cell width is 7-11 µm (rarely 21 µm) and cell length 5.4-11 µm. Most of the cells are shorter than wide and red-coloured . A calyptra is present at the end of the trichomes.

In contrast, colonies of *T. thiebautii* consist of many trichomes which are bundled together parallel or twisted in a rope-like fashion. A large fraction of the population may have trichomes in a radiate or spherical form. Colonies are usually buoyant and are about 1 x 3 µm in size. They usually appear golden brown in colour, but colour can vary from grey to brown to red. Cells diameter ranges from 7 to 16 µm, and cells are usually as long as they are wide, or can be up to twice as long as wide.

At the start of the bloom the filaments usually appear throughout the water column, but during late-bloom stages the strong gas vacuoles cause a massive rise of the alga to the surface layers. These algae can then appear as yellow-grey (early bloom) or reddish-brown (late bloom) coloured windrows spreading over up to 40,000 square kilometres.Differentiated cells within the centre of the colony are capable of fixing atmospheric nitrogen, which allows the alga to thrive under nutrient-impoverished oceanic conditions, where they readily outcompete other phytoplankton. Wave action can break up the bundles and inactivate the central nitrogenase enzyme, which is why calm seas are a prerequisite for *Trichodesmium* blooms.

Distribution

This tropical species produces seasonal (February – April) water blooms in the Java, Banda, Arafura and Coral seas. The East Australian Current and Leeuwin Current transport these algal masses as far south as Sydney (Wollongong, Ulladulla) and Perth. *Trichodesmium* red tides ("sea sawdust") were observed as early as 1770 during Captain Cook's voyage through the Coral Sea. There is no evidence of a relationship with pollution.

Toxicology

T.erythraeum is not normally considered toxic, and has tested negative for PSP toxins in Indonesia and Australia but microcystin-like compounds have been detected in some strains. The alga can be a nuisance to swimmers on Australian beaches, but harmful effects on fish are seldom observed except in sheltered bays where the decaying bloom may generate anoxic conditions (e.g. in India). An unusual death of corals caused by the decomposition of masses of *Trichodesmium* driven ashore by the wind has also been reported (New Caledonia).

Trichodesmium thiebautii from the Caribbean has been shown to possess a neurotoxin, which is similar to PSP. This planktonic tropical marine species has been shown to be toxic to some, but

not all, marine invertebrates, which graze on it. Furthermore, there are reports of breathing difficulties from people who have been near "red tide" blooms of *Trichodesmium*. ("*Trichodesmium* fever").

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9.6. *Lyngbya majuscula* (Dilwyn) Harvey
Fig.26H

Description

The non-heterocystous trichomes of *L. majuscula* ("Mermaids Hair") are straight and long (up to 80cm) and enclosed in a distinct unbranched sheath. Cells are about 10-12 µm in diameter and 1-1.5 µm long. The sheath is 4 µm thick. Cells multiply through fragmentation or hormogonia (trichome fragmentation into small motile parts). This species forms dense consolidated mats.

Distribution

This species has been reported from the Pacific and Atlantic Oceans, and has been a regular nuisance in Moreton Bay outside Brisbane. It is distributed from tropical to temperate locations.

Toxicology

Lyngbya majuscula produces lyngbyatoxin A and debromoaplysiatoxin. Direct external contact causes "Swimmers Itch", which results in irritation of human skin which may blister and peel off in severe cases. Generally *L. majuscula* grows attached to rocks or on sediments, but it can tear loose and drift for long distances. The toxins can also be concentrated by some marine invertebrates (e.g. sea hares), which graze on the cyanobacterium.

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10. BACTERIA

10.1 *Escherichia coli* and other pathogenic bacteria and viruses

Description

Seafood products that have not been subjected to severe heat treatment, such as raw oysters, can contain large numbers of bacteria, including pathogenic species. Identification of these minute organisms (mostly 0.2 – 2 µm size) is complex and involves cultivation on solid or in liquid media, biochemical staining (e.g. Gram staining), morphological examination by electron microscopy and increasingly molecular approaches such as DNA microarray techniques and fluorescence in-situ hybridisation (FISH). The presence of *Escherichia coli* in estuarine waters is almost always attributable to faecal contamination from human or animal wastes. Possible pathogenic bacteria tend to occur in much lower numbers and thus may be more difficult to detect. Although as a rule *E. coli* itself is not a pathogen, total faecal coliform counts in water samples from shellfish growing areas are used to indicate the possible presence of pathogenic bacteria such as *Salmonella* and *Vibrio* as well as pathogenic viruses. Water quality standards set by the US Food and Drug Administration and adopted in Australia require that faecal coliforms in the water should not exceed a median level of 14/100 ml and not more than 10% of the samples should exceed 43–49/100 ml. Similarly, the US Food and Drug Administration require that shellfish meat shall not contain more than 230 *E. coli* per 100 gram. Tasmanian shellfish farm waters, for example, are regularly monitored for faecal coliforms (every 2 months in "approved leases"; every month in "conditional leases") and monitoring is intensified after heavy rainfall. Occasionally the shellfish meats are also tested. If bacterial counts exceed the quarantine levels, shellfish farm areas are temporarily closed. Natural depuration by the shellfish will usually allow shellfish farm leases to be re-opened within a week. In New South Wales all shellfish are routinely treated for 36 hrs in dedicated depuration plants to allow them to purge their digestive system under UV radiation conditions.

Toxicology

Bacterial contamination of seafood can cause severe gastrointestinal disturbances in humans (abdominal pain and diarrhoea, with or without vomiting) due to microbial toxins formed in the food (short incubation time), toxins produced in the human intestine or due to true bacterial infection (long incubation time). In 1924 a widespread typhoid fever outbreak in New York, Chicago and Washington (USA) was traced to sewage-polluted oysters. In 1978, 1989 and 1990 hundreds of people suffered from viral poisoning (Norwalk virus, Parvo virus) after eating sewage-contaminated Sydney rock oysters. In 1997 in New South Wales an outbreak of 150 cases of hepatitis A was also linked to oyster consumption.

Remarks

More precise alternative methodologies are now being developed to test for *Salmonella* bacteria directly (e.g. by immunological test kits) and for faecal contamination directly (e.g. by measuring the biochemical marker coprostanol).

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12. Glossary

Alga (plural: algae)
Any of various primitive, chiefly aquatic, one-celled or multicellular plants that lack true stems, roots and leaves, and which usually contain the green pigment chlorophyll. Included among the algae are large kelps and other seaweeds (*macro-algae*) and the microscopic diatoms and dinoflagellates (*micro-algae*).

Amnesic shellfish poisoning
Human illness caused by eating shellfish contaminated with domoic acid (e.g. from the diatom *Pseudo-nitzschia multiseries*). In extreme cases this can lead to short-term memory loss (amnesia) and seizures.

Antapex
Bottom end of the dinoflagellate cell.

Apex
Top end of the dinoflagellate cell.

Apical pore
Distinctive pore-like structure, present on top of some dinoflagellate cells. A plate bordering the pore is termed the apical pore plate (designated P_o).

Armoured
Dinoflagellate cells with a thick cellulose wall (*theca*) composed of rigid plates.

Bacteria
Primitive single-celled micro-organisms, mostly 0.2 to 2 µm in size, characterised by the absence of a nucleus and other membrane-bound organelles, and having a wide range of biochemical (often pathogenic) properties. See *faecal coliforms.*

Blue-green algae (cyanobacteria)
Primitive single-celled algae, characterised by the absence of a nucleus and other membrane-bound organelles, and their (often) blue-green colour.

Ciguatera
Human illness caused by eating certain tropical coral reef fish that are contaminated with dinoflagellate toxins (e.g. from *Gambierdiscus toxicus*).

Colony
A group of the same kind of micro-organisms, which are connected and coexist in close association.

Cyst
A thick-walled stage in the life-history of certain algae, especially dinoflagellates; also called resting spore. The cyst wall is often made up of resistant sporopollenin and may be ornamented with spines. Cysts are often produced at the onset of adverse conditions, and when conditions are suitable, they may germinate to seed new plankton blooms.

Diarrhetic shellfish poisoning (DSP)
Human illness caused by eating shellfish contaminated with certain dinoflagellate toxins (e.g. from *Dinophysis acuminata*).

Diatoms
Single-celled golden-brown coloured algae, with siliceous cell walls consisting of two overlapping symmetrical parts.

Dinoflagellates
Single-celled golden-brown algae, characteristically having a large nucleus with clearly visible chromosomes, and with one flagellum protruding from the girdle groove and another from the sulcus groove. Some species have a cell covering of cellulose plates (armoured dinoflagellates), while others are bounded by a membranous covering only (unarmoured dinoflagellates).

Electron microscope
A microscope that uses electrons rather than light to produce magnified images, especially of objects whose dimensions are smaller than the wavelengths of visible light; see *scanning electron microscope* and *transmission electron microscope*.

Faecal coliforms
A group of bacteria resembling the colon (gut) bacterium *Escherichia coli* and which are capable of producing gas from lactose in a suitable liquid culture medium within 24 hours at 44.5°C.

Flagellum (plural: flagella)
Whip-like extensions of the cells of certain micro-organisms, called flagellates. Mainly used for locomotion.

Girdle groove
The horizontal groove that encircles the dinoflagellate cell and contains the transverse flagellum.

Green flagellates
Single-celled motile plankton algae, characterised by the presence of the olive-green pigment chlorophyll *b*.

Haemolysis
The breakdown of red blood cells in fish gills through the action of microalgal toxins (e.g. from *Karenia mikimotoi*).

Hepatotoxins
Toxins that cause degeneration of liver cells (e.g. produced by *Nodularia spumigena*).

Ichthyotoxins
Toxins that selectively kill fish by inhibiting their respiration.

Labiate process
Hollow tube-like projection or opening through the diatom cell wall, which is supported internally by a flattened tube or longitudinal slit, often surrounded by two lips.

Light microscope (LM)
An optical instrument that uses a combination of glass lenses to produce magnified images of objects too small to be seen by the unaided human eye. Magnification up to 1,000 x.

Micron
One thousandth of a millimetre (µm).

Naked
Plankton cells bounded by a membranous covering only. These organisms are very easily damaged beyond recognition.

Neurotoxins
Toxins that disrupt the normal functioning of the human nervous system.

Paralytic shellfish poisoning (PSP)
Human illness caused by eating shellfish which are contaminated with certain dinoflagellate toxins (e.g. from *Gymnodinium catenatum* or *Pyrodinium bahamense*). In extreme cases this can lead to death through respiratory paralysis.

Phytoplankton
Plant plankton; see *plankton*.

Plankton
Plant and animal organisms, mostly microscopic, that float or drift passively with the currents, both in fresh and salt water.

Plankton bloom
Prolific growth of plankton algae; see *red tide*.

Red tide
Dense concentrations of plankton organisms (mostly dinoflagellates) that can colour the sea red or brown.

Scanning electron microscope (SEM)
A type of electron microscope in which a beam of electrons scans the outer surface of objects previously coated with a thin layer of gold or platinum. Magnification up to 30,000 x.

Silicoflagellates
Single-celled golden-brown coloured flagellate algae with characteristic external siliceous skeleton.

Strutted process
Hollow tube-like projection of the diatom cell wall, supported at the base by struts.

Sulcus
The vertical groove on the dinoflagellate cell, extending onto epitheca and hypotheca, which guides the longitudinal flagellum.

Theca
The complete cell covering of the dinoflagellate cell. Thecal plates are subdivided into several plate series, usually parallel to the girdle groove. Apical plates usually touch the apical pore, precingular plates touch the girdle groove on the upper cell half (epitheca), postcingular plates touch the girdle groove on the lower cell half (hypotheca), and antapical plates are found at the bottom of the cell (antapex). Cingular plates are the plates in the girdle groove and sulcal plates are located in the sulcus groove.

Transmission electron microscope (TEM)
A type of electron microscope in which electrons pass through sections of thin biological specimens. Magnification up to 100,000 x.

Virus
A submicroscopic pathogen consisting essentially of a core of a single nucleic acid surrounded by a protein coat, and having the ability to replicate only inside a living host cell.

13. Relevant Web Sites

Australian Research Network for Algal Toxins (ARNAT). This is a volunteer network of researchers within Australia linked by their common interest in all aspects of both marine and freshwater toxic algae including cyanobacteria and their toxins

www.aims.gov.au/arnat

Cyanobacteria (initiated by Ben Long at Monash University, now transferred to Purdue University)

http://www-cyanosite.bio.purdue.edu/cyanotox/cyanotox.html

Intergovernmental Oceanographic Commission (IOC) of UNESCO, Harmful Algal Bloom program

http://ioc.unesco.org/hab/

International Society for the Study of Harmful Algae (ISSHA)

http://www.cbr.nrc.ca/issha/

Fossil Dinoflagellates, maintained by Rob Fensome, Andrew MacRae & Graham Williams, at Geological Survey of Canada

http://agc.bio.ns.ca/dinoflaj/

Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP)

http://ccmp.bigelow.org/data/Catalog.txt

Woods Hole Institution, Harmful Algal Bloom website (supported by NSF, NOAA)

http://habserv1.whoi.edu/hab/

14. Acknowledgements

I am greatly indebted to my students and colleagues Dr Chris Bolch, Dr Susan Blackburn, Dr Tony Rees, Dr Graeme Lush, Dr Martina Doblin, Malcolm McCausland, Miquel de Salas, Imojen Pearce, Hugh Forehead, Jonathan Smith, Susan Lambert, Judi Marshall, Ian Jameson, Caroline Lapworth and Marion Reynolds for working with me on Australian harmful microalgae.

Numerous persons provided me with algal bloom samples from Australian waters, especially: Mr D. Cannon (Metropolitan Water Sewerage and Drainage Board, NSW); Ms J. Cannon (University of Adelaide); the late Ms B. McGrath (NSW State Pollution Control Commission); the late Dr N. Gillespie and Dr M. Holmes (ex Southern Fisheries Research Centre, Qld); Mr V. Hosja (Waterways Commission, Perth); the late Dr J. Langdon (Fisheries Department of Western Australia), Dr R. Wetherbee (University of Melbourne), Ms Penny Ajani and Mr Sean Hardiman (NSW EPA) and Mr Peter Christy (PIRSA SA).

The Institute of Medical and Veterinary Science (Adelaide) carried out mouse bioassays, and Professor T. Yasumoto, Dr Y. Oshima (Tohoku University, Japan), Dr Andrew Negri (AIMS, Townsville), Dr Dan Baden (University of Miami), Dr Mike Quilliam (NRC Canada) elucidated the toxin chemistry of Australian samples.

Professor Balech (Argentine) confirmed identification of the *Alexandrium* species and Dr J. Larsen (University of Copenhagen) assisted with identification of the unarmoured *Gymnodinium* species.

I greatfully acknowledge funding from the Fisheries Research and Development Cooperation, Australian Biological Resources Study, Australian Research Council and support from the Fishing Training Board of Tasmania.