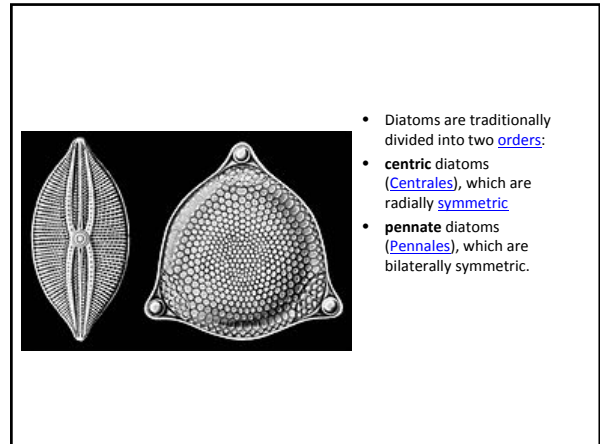
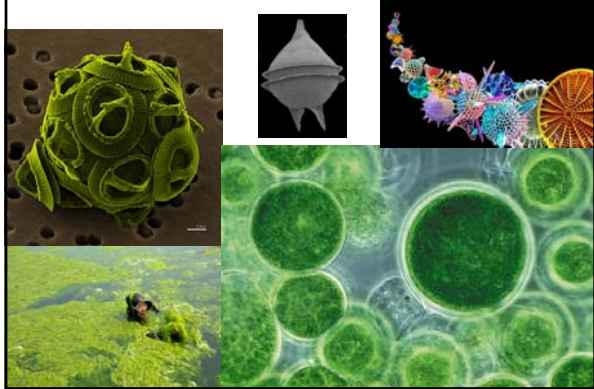


Classification of Algae



- Diatoms are traditionally divided into two [orders](#):
- [centric](#) diatoms ([Centrales](#)), which are radially [symmetric](#)
- [pennate](#) diatoms ([Pennales](#)), which are bilaterally symmetric.

The broad algae classification includes:

- Bacillariophyta – diatoms
- Charophyta – stoneworts
- Chlorophyta – green algae
- Chrysophyta – golden algae
- Cyanobacteria – blue-green
- Dinophyta – dinoflagellates
- Phaeophyta – brown algae
- Rhodophyta – red algae

stoneworts



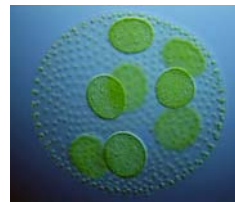
- freshwater [algae](#) in the division [Charophyta](#)
- macroscopic [thalli](#) growing up to 120 cm long, they are branched, [multicellular](#)
- can become encrusted in lime

Diatoms



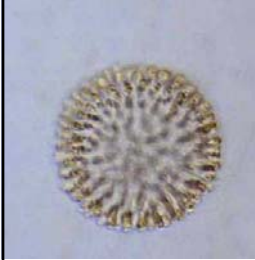
- enclosed within a unique cell wall made of [silica](#) called a [frustule](#)
- found in the [oceans](#), in [freshwater](#), in [soils](#)
- belong to a large group called the [heterokonts](#)
- Their yellowish-brown [chloroplasts](#) are typical of heterokonts, with four [membranes](#) and containing [pigments](#) such as the [carotenoid](#) [fucoxanthin](#)

Chlorophyta/ green algae

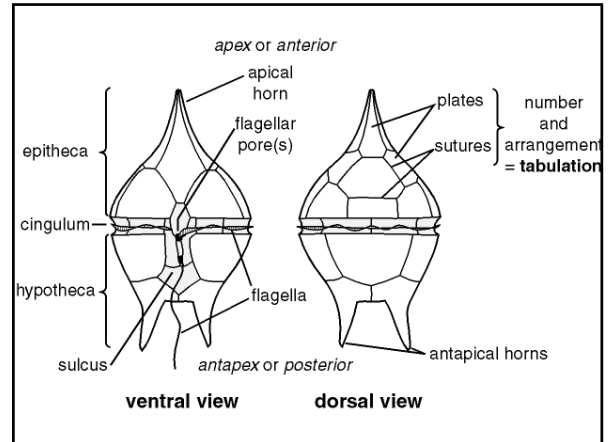


- include unicellular and colonial [flagellates](#)
- two [flagella](#) per cell, as well as various colonial, coccoid, and filamentous forms, and macroscopic [seaweeds](#)
- [chlorophylls](#) *a* and *b*, giving them a bright green color
- stacked [thylakoids](#)
- [mitochondria](#) with flat cristae

Chrysophyta – golden algae



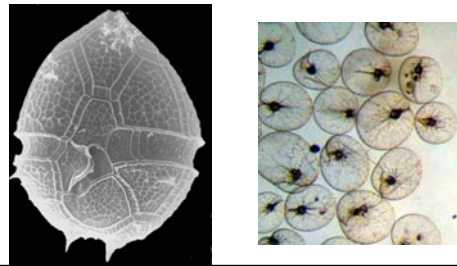
- mostly in freshwater
- unicellular [flagellates](#)
- no cell covering
- pigment [fucoxanthin](#)
- **facultatively heterotrophic**



Cyanobacteria

- Blue-green algae
- oceans, fresh water - even bare rock and soil
- thick, gelatinous [cell wall](#)
- No flagella, move by gliding or oscillation
- colour from the bluish pigment [phycocyanin](#)

- Can be naked (*Noctiluca*) or armoured (Proto-peridinium)
- thick cellulose **plates** with bounding **sutures**
- Bioluminescence (noctiluca)

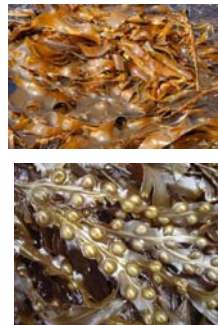


Dinophyta/dinoflagellates



- Unicellular
- 2 flagella, one trails behind cell (longitudinal flagellum) and other encircles the cell at right angles (transverse flagellum, in cingulum, ribbon-like)
- Xanthophyll pigment
- Produce toxins
- Marine and freshwater
- [mixotrophic](#), combining photosynthesis with ingestion of prey

Phaeophyta/Brown algae



- greenish-brown colored algae
- contain fucoxanthin, beta carotene and chlorophyll a and c
- Multicellular
- Mainly marine
- cell walls are composed of cellulose and alginic acid
- extraction of iodine, alginic acid and potash
- laminaria is cultivated on man-made algal ponds (using ropes) for the production of food supplements

Rhodophyta/Red algae



- Multicellular
- Marine, eukaryotic
- Phycobilins give red colour
- used as food, nori in Japan, Dulse in Ireland

- the physical presence of so many cells may suffocate fish by clogging or irritating the gills.
- when the densely concentrated algal cells die off, the decay process, assisted by bacteria, can deplete the water of oxygen, which in turn can lead to the death of oxygen-dependent marine creatures.
- some algal species produce deadly toxins which directly kill the animals that ingest the poisons.

Harmful algal blooms

- An **algal bloom** is a rapid increase or accumulation in the population in an aquatic system
- freshwater as well as marine
- only one or a small number of [phytoplankton](#) species are involved
- discoloration of the water may result from the high density of pigmented cells
- Algal blooms are often green, but they can be as yellow-brown or red, depending on the species of algae.
- Bright green blooms are a result of [cyanobacteria](#) such as [Microcystis](#)
- **harmful algal blooms** (HABs), algal bloom events involving toxic or otherwise harmful phytoplankton such as [dinoflagellates](#) of the [genus Alexandrium](#) and [Karenia](#), or [diatoms](#) of the [genus Pseudo-nitzschia](#).
- Such blooms often take on a red or brown hue and are known colloquially as **red tides**.



A spectacular “red tide” bloom (non-toxic) of *Noctiluca scintillans* in New Zealand



Dead fish from a *Karenia brevis* bloom in Texas. At high concentrations, toxins produced by this organism can cause massive fish kills.

EXAMPLES OF MARINE BIOTOXINS		
Human Health Impact	Toxicogenic Phytoplankton	Primary Toxin
Unnamed poisoning	<i>Pfiesteria piscicida</i> and related species	Unidentified toxins
Amnesic Shellfish Poisoning	Species of the diatom genus <i>Pseudo-nitzschia</i>	Domoic acid
Neurotoxic Shellfish Poisoning	<i>Gymnodinium breve</i>	Brevetoxin
Paralytic Shellfish Poisoning	<i>Alexandrium</i> spp., <i>Gymnodinium catenatum</i> , <i>Pyrodinium</i> spp.	Saxitoxins
Diarrhetic Shellfish Poisoning	Species of the dinoflagellate genus <i>Dinophysis</i> ; species of the dinoflagellate genus <i>Prorocentrum</i> have also been implicated	Okadaic acid
Ciguatera Fish Poisoning	Primarily <i>Gambierdiscus toxicus</i> , a benthic dinoflagellate, but also <i>Prorocentrum</i> , <i>Ostreopsis</i> spp., <i>Coolia monotis</i> , <i>Thecadinium</i> sp., <i>Amphidinium carterae</i>	Ciguatoxin/Maititoxin

HARMFUL ALGAL BLOOMS IN SOUTH AFRICA

- The Benguela system is characterised by upwelling circulation.
- Harmful Algal Blooms [HABs] are common
- harmful impacts are most often ascribed to dinoflagellate species
- associated with either their high biomass or the toxigenicity.
- blooms impact both commercial and recreational interests
- causing fish kills, contaminating seafood with toxins, or altering ecosystems in ways that are perceived by humans as harmful.

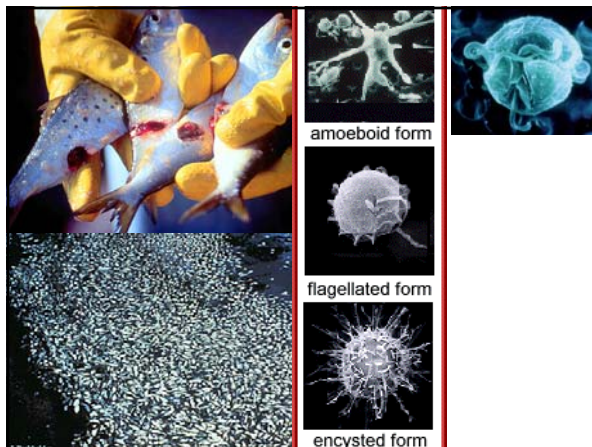
Pfiesteria

- micropredation
- *Pfiesteria* is a genus of **heterotrophic dinoflagellates** that has been associated with **harmful algal blooms** and fish kills.
- *Pfiesteria* acts as an "**ambush predator**" and utilizes a "**hit and run**" feeding strategy by releasing a toxin that paralyzes the respiratory systems of susceptible fish, such as **menhaden**, thus causing death by **suffocation**. It then consumes the tissue sloughed off its dead prey
- non-toxic forms of the *Pfiesteria* feed on the algae and bacteria
- *Pfiesteria* becomes toxic in the presence of fish, particularly schooling fish like menhaden, triggered by their excrement in the water
- conditions that support an *Pfiesteria* outbreak are 1) warm water temperatures of about 70 degrees F, 2) High levels of Phosphorous, Ammonium (NH4), and suspended solids, 3) moderate to low salinity levels, 4) increased rainfall or runoff, and 5) the presence of fish in particularly large numbers. Massive increases in the nontoxic flagellated stage have been observed where there are phosphorous enriched waters



Discolouration of an inner basin of Cape Town Harbour in November 2003 was caused by a bloom of the toxic dinoflagellate *Alexandrium minutum*.

This species is known to produce paralytic shellfish poisoning toxins and the bloom persisted in the harbour for several weeks. This was the first record of this species on the South African coast



- high biomass dinoflagellate blooms lead to low oxygen events and in some cases the production of hydrogen sulphide,
- red tides cause fluctuations in fish stocks in the Benguela.
- Monitoring has indicated that the distribution of these blooms is typically associated with the upwelling system, with few red tides reported east of Cape Agulhas [Pitcher and Calder, 2000].



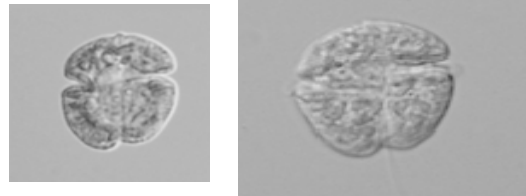
Bright green discoloration of the waters of Saldanha Bay in January 2003 was of concern to port authorities. Investigations determined that the bloom was dominated by an unknown species of *Tetraselmis*. Although these blooms show no signs of toxicity and pose no serious threat they are often considered aesthetically unpleasant and are in many cases perceived to be associated with eutrophied or polluted waters. For this reason blooms such as these have significant negative effects on the recreational uses of coastal areas.

- the Benguela is characterized by a variety of toxic microalgae
- The northern Benguela is characterised by the fish-killing dinoflagellate *Karlodinium micrum*
- Another fish-killing dinoflagellate is the newly described species *Karenia cristata*
- also responsible for large abalone mortalities in the 1980s.

- Anoxia in continental shelf waters may be ascribed to the advection of oxygen-deficient water from a remote source and from in situ formation.
- In the southern Benguela, in situ formation resulting from the degradation of organic-rich material derived from phytoplankton blooms is generally thought to be the controlling factor.
- The inshore decay of red tides after the exhaustion of nutrients has in many instances been responsible for marine mortalities as a consequence of oxygen depletion.

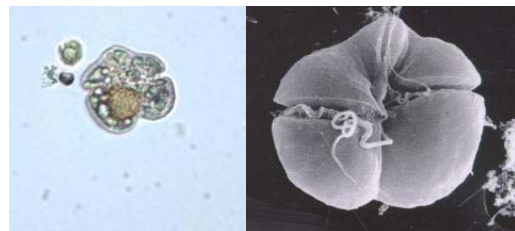
Karenia, a toxic unarmoured dinoflagellate

- *Karenia brevisulcata* produces an aerosol toxin responsible for respiratory and skin disorders.



In March 1994 large marine mortalities were experienced in St Helena Bay on the South African west coast as a consequence of hydrogen sulphide poisoning. This followed the decay of a red tide dominated by the dinoflagellates *Ceratium furca* and *Prorocentrum micans*. Oxygen concentrations were maintained at less than 0.5 ml.l⁻¹ in the bottom waters of the Bay and hydrogen sulphide, generated by anaerobic bacteria in the absence of dissolved oxygen, was recorded in excess of 50 µmol.l⁻¹. Although common in the northern Benguela, this was the first recorded case of hydrogen sulphide poisoning in the southern Benguela.

Karenia brevis



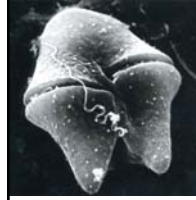
- Produces brevetoxin
- responsible for large die-offs of marine organisms and seabirds

Karenia mikimotoi (hemolysins)

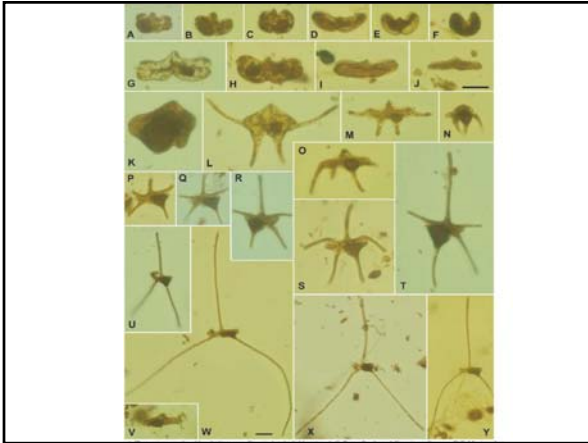


- mortalities of invertebrates
- inhibits growth of molluscs
- fish mortalities in the wild and in aquaculture
- pathology, asphyxiation
- osmotic shock

Akashiwo sanguinea (ichthyotoxic)

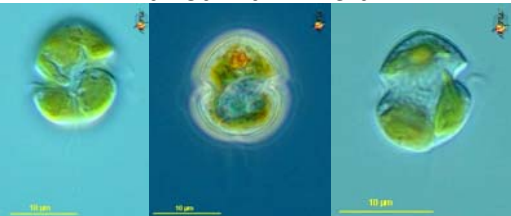


- can kill larval oysters and clams
- occasionally associated with fish kills
- water quality
- low dissolved oxygen
- produces surfactants associated with bird and fish kills

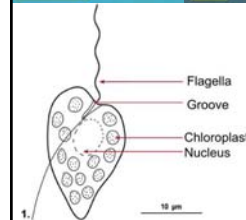
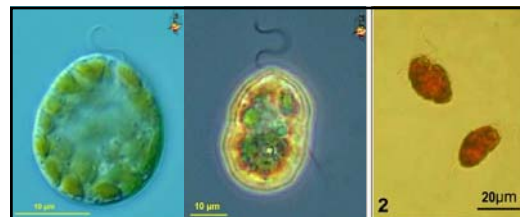


- phytoplankton belonging to other groups known to produce toxins are common in the Benguela.
- The raphidopyte *Heterosigma akashiwo* renowned for its ichthyotoxic properties has, for example, been observed to bloom in the northern and southern Benguela.
- Only recently have fish mortalities in the Benguela been associated with this species.

Karlodinium micrum



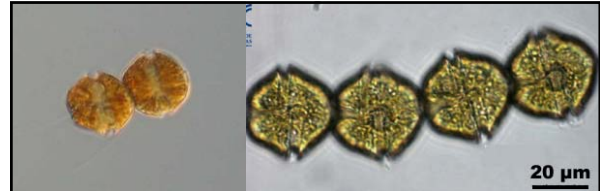
- Produces Karlotoxins, lethal to fish
- damaging to the gills
- serves to stun prey before ingestion



[Heterosigma akashiwo](#) have two flagella, one of which is used for swimming. Cells contain many yellow green chloroplasts. Mixotrophic, ingest bacteria

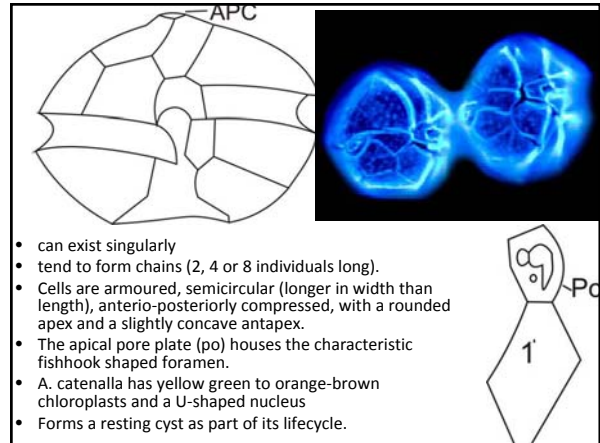


The first fish mortality in the Benguela attributed to *Heterosigma akashiwo* was observed in Saldanha Bay in March 2004. High concentrations of this raphidophyte caused a yellow brown discoloration of the water, and although the mortalities were not particularly extensive large shoals of disorientated fish were observed in shallow water [Photographs – © J. de Goede].



- *A. catenella* appears almost every year, typically during the latter part of the upwelling season.
- *A. catenella* in the Benguela can be found at exceedingly high cell concentrations of many million cells l⁻¹
- these blooms not only render shellfish toxic to consumers, but are also seemingly responsible for fish and shellfish mortalities [Pitcher and Calder, 2000].

- One major category of public health impact from HABs occurs when toxic phytoplankton are filtered from the water by shellfish such as clams, mussels, oysters and scallops, which then accumulate the algal toxins to levels that are potentially lethal to humans and other consumers.
- Of particular concern in the Benguela are the shellfish poisoning syndromes known as Paralytic (PSP) and Diarrhetic (DSP) Shellfish Poisoning.
- In the southern Benguela, blooms of *Alexandrium catenella*, responsible for PSP are common
- accumulate [neurotoxins](#), called [Saxitoxin](#)
- Symptoms can appear ten to 30 minutes after ingestion, and include [nausea](#), [vomiting](#), [diarrhea](#), [abdominal pain](#), [tingling or burning](#) lips, gums, tongue, face, neck, arms, legs, and toes.^[5] [Shortness of breath](#), [dry mouth](#), a [choking feeling](#), [confused or slurred speech](#), and [loss of coordination](#) are also possible



- can exist singularly
- tend to form chains (2, 4 or 8 individuals long).
- Cells are armoured, semicircular (longer in width than length), antero-posteriorly compressed, with a rounded apex and a slightly concave antapex.
- The apical pore plate (po) houses the characteristic fishhook shaped foramen.
- *A. catenella* has yellow green to orange-brown chloroplasts and a U-shaped nucleus
- Forms a resting cyst as part of its lifecycle.

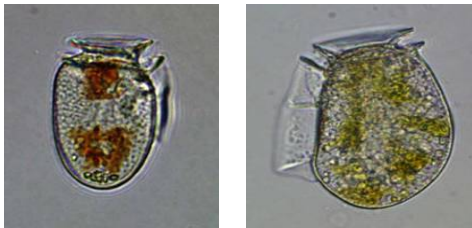
- PSP has been implicated as a possible cause of [sea otter mortality](#) and [morbidity](#) in Alaska, as one of its primary prey items, the butter [clam](#) (*Saxidomus giganteus*) bioaccumulates saxitoxin as a chemical defense mechanism.^[5] In addition, ingestion of saxitoxin-containing [mackerel](#) has been implicated in the death of [humpback whales](#)^[6]
- The full northern extent of *Alexandrium catenella* is not clearly defined, but it is known to extend into southern Namibia
- another toxic *Alexandrium* species has recently been isolated from the Western Agulhas Bank

- *Dormant cysts of A. catenella are able to settle to the seafloor and can survive there for several years. When favourable conditions return, cysts are able to germinate and reinoculate the water with vegetative cells that can again bloom.*

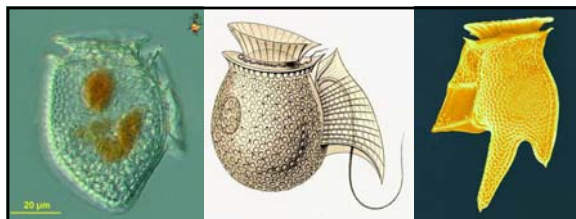
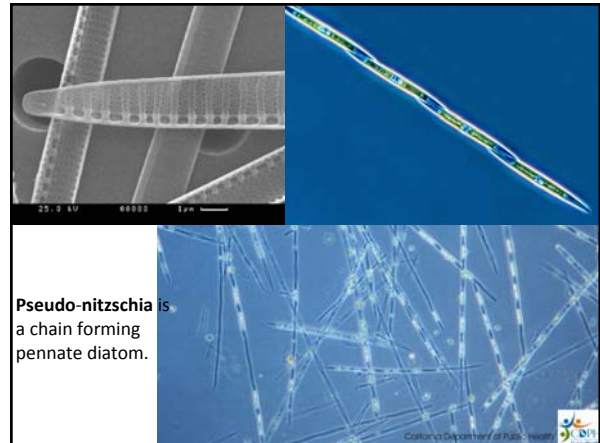
Diarrhetic (DSP) Shellfish Poisoning

- DSP symptoms occur half an hour after ingesting infected shellfish
- The causative poison is [okadaic acid](#), which inhibits intestinal cellular de-phosphorylation.
- This causes the cells to become very permeable to water and causes profuse diarrhea with a risk of dehydration.

- *Pseudo-nitzschia*, known to cause Amnesic Shellfish Poisoning
- Amnesic shellfish poisoning (ASP) is a human illness caused by consumption of the marine biotoxin [domoic acid](#).
- This toxin is produced naturally by marine [diatoms](#) belonging to the genus *Pseudo-nitzschia* and the species *Nitzschia navis-varinica*.
- the toxin can [bioaccumulate](#) in many [marine](#) organisms that consume [phytoplankton](#), such as [anchovies](#), and [sardines](#)
- domoic acid acts as a [neurotoxin](#), causing permanent [short-term memory loss](#), [brain damage](#), and death in severe cases
- no trace of the toxin domoic acid has been found in cultures of this species and no form of shellfish poisoning has been associated with these blooms in South Africa



- in the Benguela is DSP, usually attributable to *Dinophysis acuminata* or *Dinophysis fortii*.
- okadaic acid identified as the primary toxin



- Dinophysid dinoflagellate
- shape round to triangular,
- extended with projections or spines;
- two main hypothecal plates are often ornamented.

- In 1991, examination of the stomach contents of dead seabirds found along the beaches of Monterey Bay, California revealed high levels of domoic acid.
- The birds had been eating anchovies which had been consuming *Pseudo-nitzschia*.
- In 1998, sea lion deaths on the California coast also were associated with domoic acid, which entered the food chain via toxigenic diatoms, which were eaten by anchovies that in turn were eaten by the sea lions.

Aquaculture

- Harmful algal blooms also can threaten the [aquaculture](#) industry.
- For example, unpredictable and destructive blooms of the small flagellate *Heterosigma* have threatened the commercial farmed salmon industry
- *Heterosigma* blooms also have destroyed some captive populations of threatened and endangered salmon being raised in **net pens** before their release to the wild.

- HABs can cause serious economic losses in aquaculture if they kill cultured organisms or cause consumers concern about food safety
- Toxic algal blooms caused \$38 million dollar loss to commercial abalone farmers

Pond Aquaculture

- Algal toxins can cause problems in the freshwater aquaculture of both vertebrates (fish) and invertebrates (shellfish).
- Such problems include:
 - • off-flavor
 - • indirect toxicity through changes in water quality
 - • direct toxicity

Algal bloom kills millions of abalone in E China

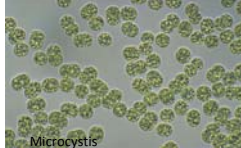


- Some algae are toxic only at very high densities, while others can be toxic at very low densities
- filter-feeding shellfish (clams, mussels, oysters, scallops) feed on toxic phytoplankton and accumulate harmful toxins that are passed up the food chain;
- fish, shellfish, birds and even mammals are killed by eating organisms that have consumed algal toxins;
- light cannot penetrate the water, thus changing the function and structure of the aquatic ecosystem;
- discoloration makes water aesthetically unpleasant;
- the decaying biomass of a bloom depletes dissolved oxygen (especially critical in aquaculture);
- blooms kill other algae important in the food web

Cyanobacteria

- produce substances that cause taste and odor problems in water supplies and aquacultural products
- Anabaena and Microcystis
- Cyanobacteria can colonize and rapidly grow to great masses in aquaculture ponds
- nutrient status, salinity or ionic strength, light conditions, turbulence and mixing, temperature and herbivory
- Cyanobacteria can affect the production of zooplankton and consequently the production of fish.
- They also produce allelochemicals that can inhibit competing algae and invertebrate grazers
- toxins (both neurotoxins and hepatotoxins) affect zooplankton

- Cyanobacteria can rapidly overtake an aquaculture pond and contribute to unstable conditions.
- Cyanobacteria blooms can decrease fish production and kill fish due to oxygen depletion.
- Cyanobacteria can also cause off-flavor and objectionable odour in fish.



Treatment

- Algicide
- physical mixing and aeration
- flushing to decrease hydraulic retention time
- decreasing or altering nutrient content and composition

Cyanotoxins

- dermatotoxins are produced primarily by benthic cyanobacteria, damage [skin](#), [mucous membranes](#), often leading to [necrosis](#)
- most cyanotoxins are either **neurotoxins** (anatoxin-a & saxitoxin) causing rapid paralysis of the peripheral skeletal and respiratory muscles or **hepatotoxins** (microcystins) symptoms of poisoning in fish include flared gills because of difficulty breathing and weakness or inability to swim

Prymnesiophytes

- Haptophyte
- form harmful blooms usually in brackish water
- responsible for fish kills
- produces ichthyotoxin, cytotoxin and hemolysin
- contain chlorophylls a and c as well as yellow-brown accessory pigments (Called golden algae)
- Bloom density does not correlate strongly with toxicity
- toxicity may be enhanced by temperature lower than 30 °C, pH greater than 7.0 and high phosphate concentration

Monitoring and diagnosing the problem

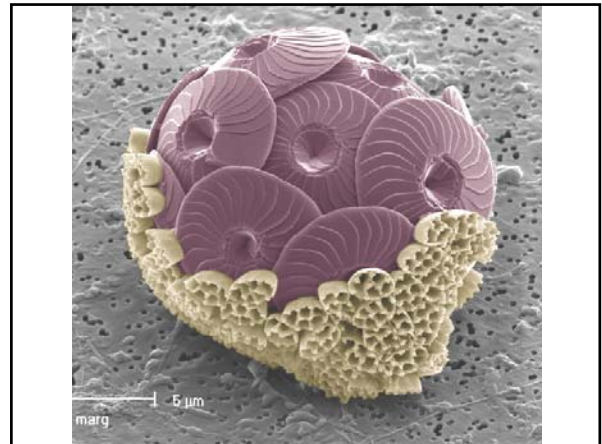
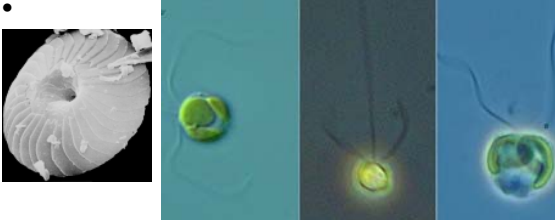
- While not all blooms of toxin-producing cyanobacteria result in toxin production, most do
- onset of toxicity will be rapid
- To confirm the problem, fresh samples (unpreserved) of the water containing the suspected cyanobacteria will need to be examined
- A sample of both sick and dead fish will also be needed, along with information on fish behavior and any other symptoms observed.
- Young fish are generally more sensitive than older fish.

Signs of intoxication

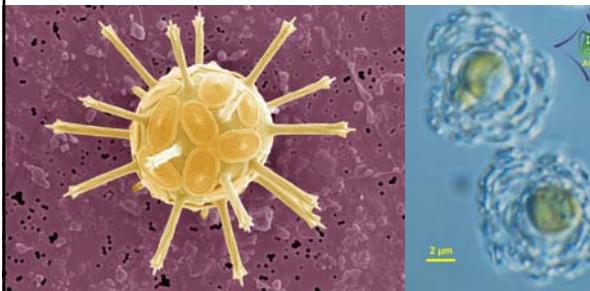
- Dense growths of *Prymnesium parvum* may color the water yellow to copper-brown or rust.
- water may foam if aerated or agitated
- fish behave erratically, accumulate in the shallows and try to leap from the water to escape the toxins
- fish may have evident blood in gills, fins and scales and may be covered with mucus
- If fish are removed to uncontaminated water in the early stages of intoxication, their gills can recover within hours but growth will be affected if they survive
- Aquatic insects, birds and mammals are not affected by *P. parvum* toxin

Managing prymnesiophytes

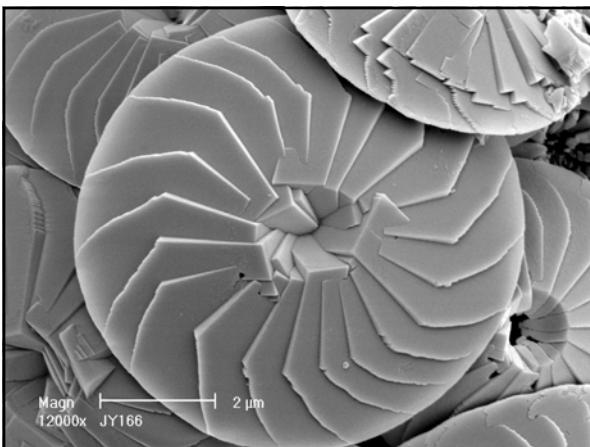
- identification requires examining unpreserved water samples
- Includes haptophytes and coccolithophores
-



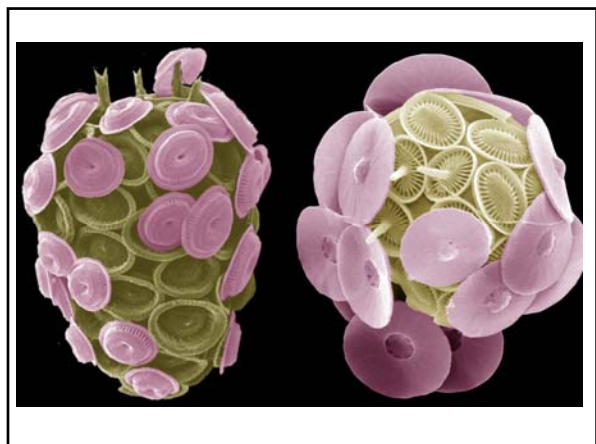
Calcifying : coccolithophores



Coccolithophore blooms



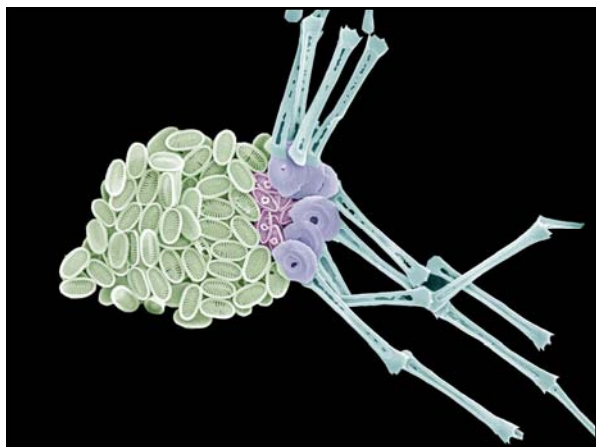
- Some parts of the ocean floor are covered by layers of coccoliths many meters thick
- Coccolithophores can act as important carbon sinks. By taking CO₂ out of the atmosphere, they may have a direct cooling effect on global temperatures
- Coccolithophore blooms also produce large amounts of dimethylsulfide (DMS), a molecule that is thought to enhance cloud formation



Phaeocystis foam



- Produced during bloom decline in coastal areas
- Clogs fish nets and fish gills
- Stinks!



Pymnesiales

- 75+ species
- 2 families : Pymnesiaceae / Chrysochromulinaceae
- Mostly marine, some brackish
- Some toxic species, can cause massive fish kills

Non-calcifying : Phaeocystales Pymnesiales

-



Treatment

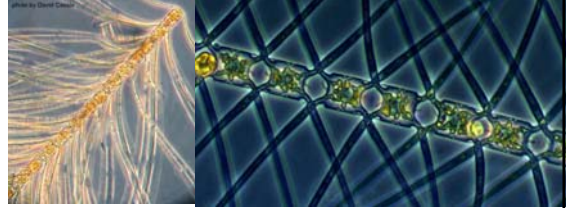
- applying ammonium sulfate and copper sulfate
- If not carefully used, the copper sulfate may kill desirable algae
- decrease salinity
- Algicides

Euglenoids

- Euglena species produce an ichthyotoxin in freshwater aquaculture
- hybrid striped bass, channel catfish, tilapia, sheepshead minnows
- Diagnosis by microscopic examination of a water sample



- For example, in 1987, phytoplankton blooms of the non-toxic diatom *Chaetoceros convolutus* were linked to the mortality of 250,000 Atlantic salmon valued at over \$500,000. The diatoms lodged in the gill tissues, causing excessive mucus production, suffocation, and death



- progression of symptoms from begins with the fish going off feed for no apparent reason.
- Within 24 hours of cessation of feeding, the fish swim at or near the surface in an agitated or disorientated state
- dorsal fin extending out of the water
- swimming on their sides and even upside down.
- If steps are not taken immediately after observing this state, the fish will be dead in 24 hours
- Gill tissue may be observed to redden

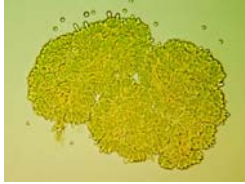
- A single algal species may change shape, composition and color in a single day based on culture variables such as available light energy, nutrients, temperature and acidity, pH
- the mere presence of toxin-producing algae does not necessarily mean that enough toxin will be produced to harm fish in culture.

Treatment

- try to minimize mixing of the water because mixing will disburse the bloom throughout the pond
- Aerate only if needed to save the fish from acute oxygen depletion
- potassium permanganate applied at a rate of 2.5 times the permanganate demand of the pond apparently detoxified it
- Algicide

Taxonomic Group	Chloro phyll	Carotenoids	Storage products
Bacillariophyta	a, c	β -carotene, \pm -carotene rarely fucoxanthin	Chrysolaminarin oils
Chloro phycophyta (green algae)	a, b	β -carotene, \pm -carotene rarely carotene and lycopene, lutein	Starch, oils
Chrysophycophyta (golden algae)	a, c	β -carotene, fucoxanthin	Chrysolaminarin oils
Cyanobacteria (blue green algae)	a, c	β -carotene, phycobilins	
Phaeo phycophyta (brown algae)	a, c	β -carotene, \pm fucoxanthin, violaxanthin	Laminarin, soluble carbohydrates, oils
Dinophyta (dinoflagellates)	a, c	β -carotene, peridinin, neoperidinin, dinoxanthin, neodinoxanthin.	Starch, oils
Rhodo phycophyta (red algae)	a, rarely d	β -carotene, zeaxanthin, \pm β carotene	Floridean starch, oils

Bioprospecting

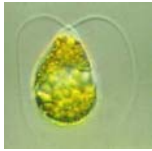


- *Botryococcus* species, with oil droplets being released.
- ability to produce high amounts of hydrocarbons
- 30-40 percent of their dry weight

Algal ponds



Dunaliella



- Halophilic algae
- Balances osmotic pressure by producing glycerol
- Produces B-carotene



- Algae have the potential to produce considerably greater amounts of biomass and lipids per hectare than terrestrial biomass, and can be cultivated on marginal lands, so do not compete with food or other crops. Algae can be cultivated photosynthetically using sunlight for energy and CO₂ as a carbon source.

Photobioreactor



Algae may be used to produce biofuels in several ways:

- Conversion to bioethanol (e.g. [AlegenoI](#))
- Extraction of oils (e.g. [SGI](#), [Solix Biofuels](#), [Sapphire Energy](#), [Algasol](#)).
- Production of oils from feedstock via dark fermentation (e.g. [Solazyme](#))
- Oil plus ethanol (e.g. [Green Star](#))
- Conversion of whole algae to biocrude via pyrolysis (e.g. [BioFuel Systems SL](#))
- Jet fuel (e.g. [Sapphire Energy](#))
- Algal biorefinery - biofuels and other products ([Petro Algae](#), [HR Biopetroleum](#))
- Following extraction, algal oils will need to be further refined (e.g. by hydrocracking and hydrogenation) to produce gasoline or jet fuels.

What kind of media do you need???

- **Sea Water Enriched Media**
- **Fully Defined Media**
- **Diluted Media?**
- **Other – total inorganic/organic**

Isolation

- Collect samples gently in a towed plankton net
- 10 minutes with 200um or 600um net
- View as soon as is practical
- Don't freeze or preserve before viewing
- Can use single-cell isolation

- Newly isolated strains are difficult to maintain
- To speed up optimization of media place alga into several different media and see which one grows best
- Light, do not over expose, they need day night cycle



ERD-SCHREIBER MEDIUM

- 1000ml filtered seawater
- 50ml soil extract
- 0.2g NaNO₃
- 0.02g Na₂HPO₄·12H₂O

- Autoclave