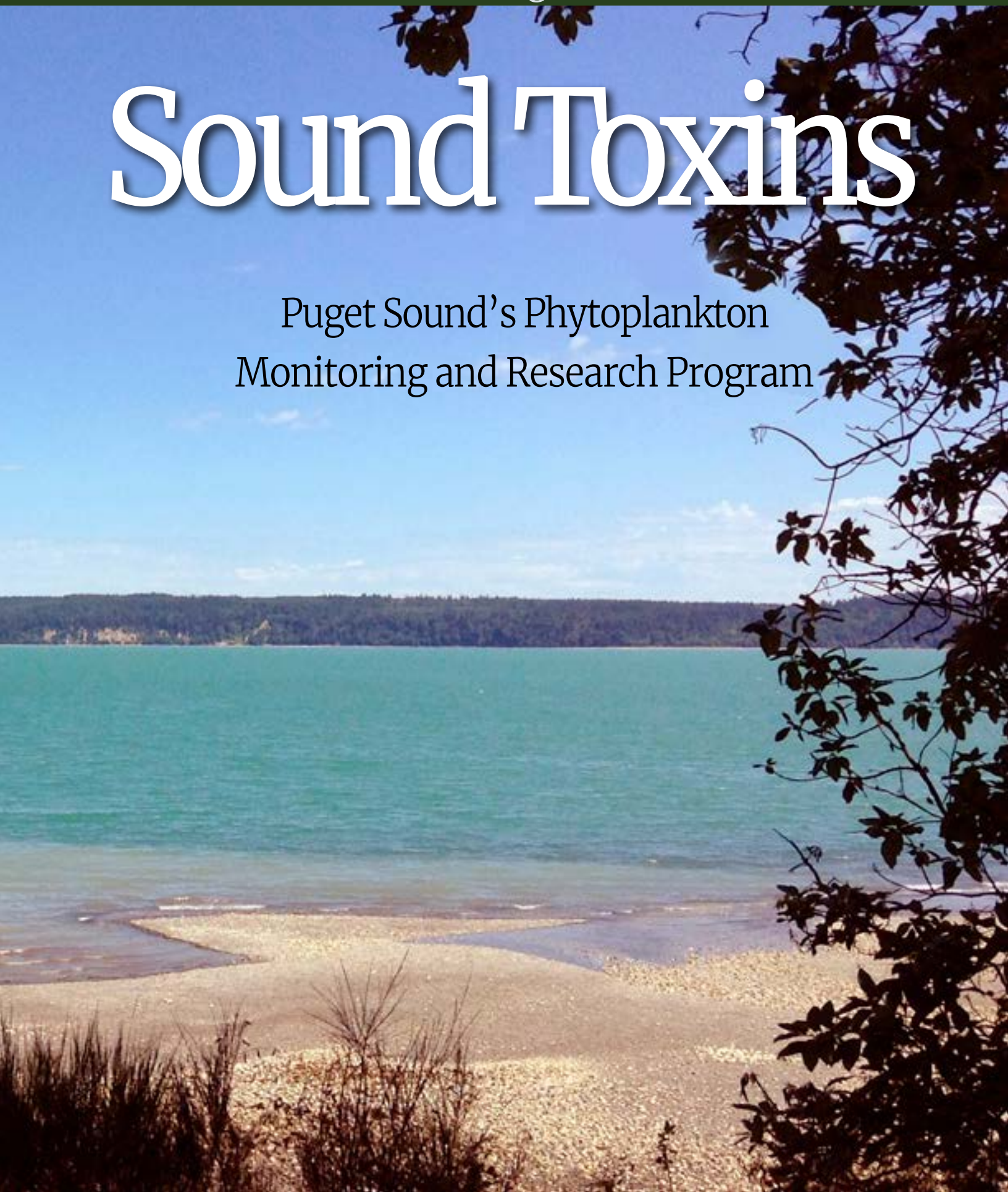
Sound Toxins

Puget Sound's Phytoplankton Monitoring and Research Program



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SoundToxins Overview

Welcome to SoundToxins!

Shellfish are critical to the culture, economy and ecology of Puget Sound. They shape habitats, provide food for humans and other species, and perform water-filtering functions important to all organisms that live in or near the Sound. Native American tribes have long incorporated shellfish into their daily lives through ceremony and art as well as food. Washington state is the national leader in farmed bivalve shellfish (USDA Aquaculture Census 2018). The majority of shellfish farming and recreational harvest opportunities are in rural communities. Recreation and tourism associated with shellfish harvesting on state-owned beaches is important to our food security, sense of place and economic well-being.

Ensuring safe and wholesome shellfish for consumption relies on water free of terrestrial and legacy pollutants and toxins from harmful algal blooms (HABs). A phytoplankton bloom can be harmful in several ways. Some blooms can produce anoxic (no oxygen) or hypoxic (low oxygen) conditions in the water column. This occurs when one or two species dominate a bloom and block the sunlight from other organisms in the lower water column. Other plankton begin to die and decompose. The process of decomposition consumes oxygen. Eventually, the massive bloom dies as well, removing any additional oxygen from the water column. This entire process can lead to a fish and/or shellfish die-off, resulting in negative economic impacts.

HAB events can lead to closures of shellfish beds, lost fisheries production, and reductions in tourism and associated service industries. Fisheries-related businesses close, insurance and unemployment rates rise, and public resources are redirected to advisories and monitoring programs.

Human health can also suffer during harmful algal blooms. Over 50 known species of phytoplankton produce toxins. As toxins move through the food web, they bioaccumulate in the tissues of large fish and marine mammals. Humans can contract illnesses from eating contaminated shellfish and fish, and medical treatment can be expensive.

Several types of algal toxin present in Puget Sound require vigilant monitoring to ensure safe shellfish consumption. The Washington State Department of Health (WDOH) monitors and regulates biotoxins including paralytic shellfish poison (PSP) from *Alexandrium catenella*, amnesic shellfish poison (ASP) produced by *Pseudo-nitzschia* spp., and diarrhetic shellfish poison (DSP) from *Dinophysis* spp.

In response to the threat HABs present to human health and shellfish harvests along Puget Sound, NOAA created the SoundToxins program in 2006, as part of its Oceans and Human Health Initiative. Washington Sea Grant (WSG) joined as a program partner in 2008 and assumed the tasks of monitor coordination, data management, data analysis, training and communications. NOAA funding expired in 2012 and WSG now manages the entire program and has succeeded in obtaining grants to support SoundToxins from sources such as the National Estuary Program's Toxics and Nutrients Ambient Monitoring Program through the Washington State Department of Ecology, the National Sea Grant Aquaculture Extension and Technology Transfer Program, NOAA Monitoring and Event Response for Harmful Algal Blooms (MERHAB), and the Northwest Association of Network Ocean Observing Systems (NANOOS).

SoundToxins is designed as a cost-effective community, tribal and industry-based monitoring program to provide enough warning of HAB events to enable early or selective harvesting of seafood, which ultimately minimizes health risks and reduces economic losses. The program employs twin strategies:

- Using phytoplankton data to provide early warning of HAB events. By rapidly determining the abundance of key HAB species, SoundToxins helps WDOH pinpoint shellfish-growing areas requiring regulatory testing and helps shellfish and finfish producers and natural resource managers identify mortality risks.

- Monitoring environmental parameters (e.g., water and air temperature, salinity, weather, tide, and wind conditions) to determine the key variables that promote the initiation of harmful events and encourage proliferation of HAB species. Identifying these variables may help provide early warning of events.

Participants throughout the region collect seawater samples routinely and analyze them for salinity, temperature and phytoplankton species. Monitors describe the phytoplankton species diversity and specifically identify and enumerate seven target varieties: *Pseudo-nitzschia* species (causing ASP), *Alexandrium* species (causing PSP), *Dinophysis* species (causing DSP), *Heterosigma akashiwo* (causing fish kills) as well as *Akashiwo sanguinea*, *Phaeocystis globosa*, and *Protoceratium reticulatum* (causing shellfish mortality). In addition, monitors at key locations also process samples for nutrients, chlorophyll and toxins.

Since 2006, the program has demonstrated the effectiveness of an early warning system. This became especially clear during the Sequim Bay DSP incident in August 2011, when SoundToxins monitors from the Jamestown S'Klallam Tribe raised an alert about elevated levels of *Dinophysis* spp.

The WDOH maintains its own monitoring network of sentinel mussel cages throughout Puget Sound, but until 2011, only tested these mussels for PSP toxins and domoic acid. The addition of testing for DSP after the 2011 illnesses and other emerging toxins stresses the already stretched state monitoring resources. SoundToxins monitoring targets high-risk sites that have an immediate need for testing because of shellfish resources and past toxin history. SoundToxins phytoplankton data, entered into a database in near real time, allows WDOH to allocate expensive shellfish testing for DSP toxins only when *Dinophysis* species are present. WDOH has hailed the importance of SoundToxins as an early warning system. In fact, WDOH acquired a modest amount of funding in early 2012 to create a new, easy to use database for participants and to support data management. Jerry Borchert, marine biotoxin shellfish specialist for WDOH stated, "There is an urgent need for us to use SoundToxins data in real time to pinpoint those shellfish growing areas where monitoring for DSP toxins is essential. This will help us to focus our resources in high risk areas."

By monitoring with SoundToxins, you are helping to ensure a safe, sustainable harvest of shellfish and fish throughout Washington.

A Brief Introduction to Marine Phytoplankton

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Definition

The term "plankton" comes from the Greek planktos, meaning "to drift or wander"; thus these organisms float passively or have only limited ability to swim. Phytoplankton are plant plankton, mostly unicellular algae that are usually photosynthetic. They are sometimes called "floating pastures" because they provide food for so many marine organisms.

Features

Phytoplankton underlie all animal production in the ocean, supporting the food webs that supply the world's fisheries. They provide half of the world's oxygen and are key players in the global biogeochemical cycling of carbon, nitrogen and sulfur.

Phytoplankton range in size from about 2 microns (µm) to 2 millimeters (mm) but commonly fall between 10 and 50 µm. They inhabit a wide range of environments, from puddles, lakes, and streams to ice, snow and oceans. They live in a wide range of temperature, salinity, light and nutrient conditions and across a wide range of temporal and spatial scales. Their distribution is limited by light and nutrients.

The cells have a variety of shapes ranging from round to elongate. They may have spines, horns, setae or lists and may live as solitary cells or in colonies, often attached together in long or short chains. Some cells have flagella, whip-like structures that enable them to swim, albeit slowly. They have the usual organelles (nuclei, Golgi bodies, and mitochondria) and may also have chloroplasts, eye spots, ejectile structures (trichocysts), mucocysts, and skeletons.

Reproduction is usually asexual but in some cases sexual. It may produce resting cells, spores (e.g., from diatoms), or cysts (e.g., dinoflagellates).

The earliest studies of phytoplankton were taxonomic. They used light microscopy to observe such features as size, shape, color and swimming mode. Electron microscopes are now used: scanning electron microscopes (SEM) to view surface features and transmission electron microscopes to view ultrastructure and external scales.

Kinds of Organisms

1. Diatoms

Diatoms are the best known and the most abundant group of phytoplankton in terms of both number of species (5,000 to 100,000 or more depending on the authority) and biomass. They occur in all the oceans from the poles to the tropics, but are most common in polar and temperate seas. They range in size from 2 to 200 µm, but may be as long as 4 mm.

Diatoms have traditionally been divided into two taxonomic groups based on the type of symmetry they display. These definitions are changing, but for **SoundToxins**' purposes we will stick with the old groups. The centric diatoms (the Centrales order) have radial symmetry with surface patterns arranged along a central point. The pennate diatoms (Pennales order) have longitudinal symmetry with surface patterns arranged along an axis (Fig. 1).

A diatom's cell wall (frustule) is a box-like silica shell with an organic covering. It has two halves, each consisting of a flat plate, valve, and marginal girdle bands. The larger half is called the epitheca; the smaller half, the hypotheca, fits into the epitheca. The rounded area between the valve and girdle is called the mantle (Fig. 1).

Diatom shape varies, usually round or boat-shaped, but may be triangular, square, or elliptical. The silica walls are usually highly patterned with pores, ribs, minute spines, processes (natural projections or appendages), marginal ridges, and elevations, which indicate genera and species.

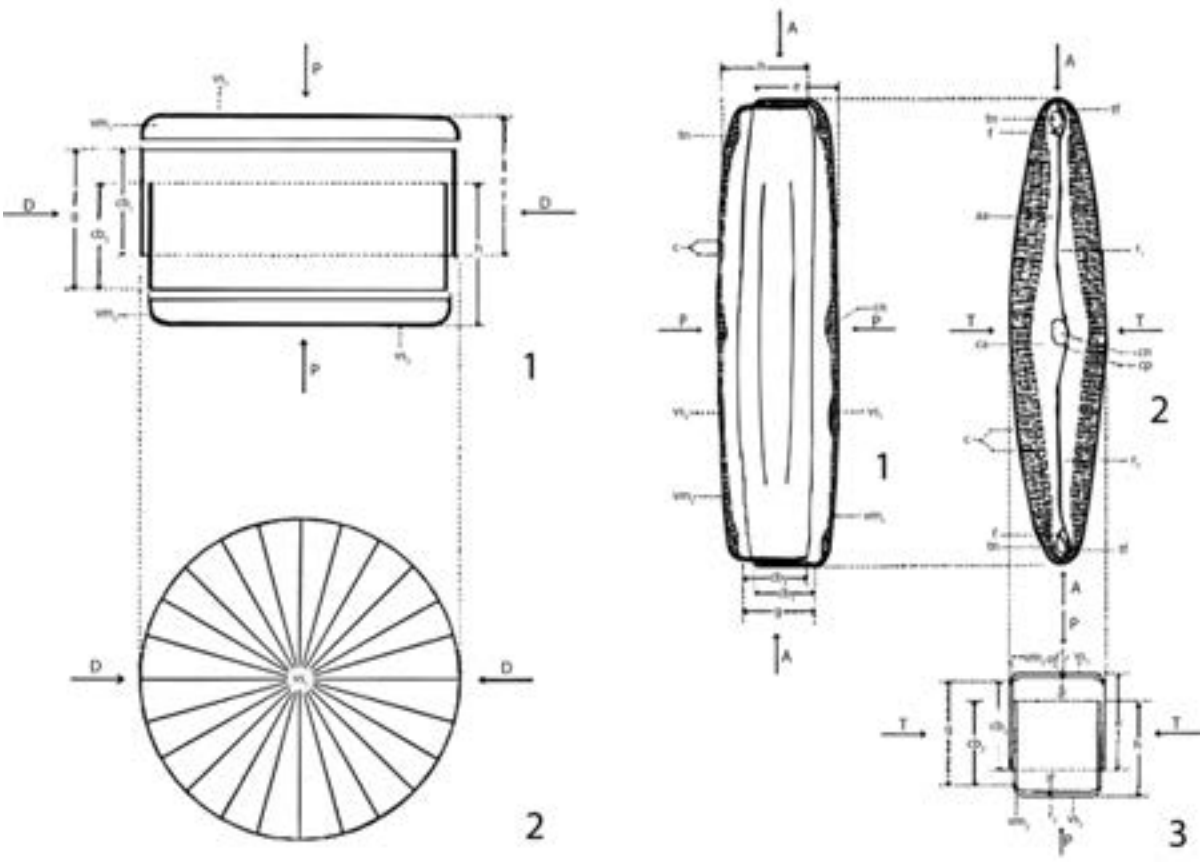


Figure 1. Diatom frustules. Left — centric diatom: (1) girdle view; (2) valve view. Structure: (e) epivalve; (h) hypovalve; (g) girdle consisting of connecting bands from epi- and hypo-valves; (vm) valve mantle; (D) diameter. Right — pennate diatom: (1) Broad girdle view; (2) valve view; (3) narrow girdle view (end). Axes: (A) apical; (P) pervalva; (T) transapical. Structure: (r) raphe. Redrawn from Cupp (1943).

Cells may be solitary (*Ditylum*, *Coscinodiscus*) or joined in colonies with cells linked by various processes such as:

1. Chitin secreted through strutted processes: *Thalassiosira*
2. Siliceous structures
   a. Hollow extensions of tubular processes: *Stephanopyxis* (labiate processes), *Skeletonema* (strutted processes)
   b. Solid spines or teeth: *Paralia*
   c. Interlocking setae: *Chaetoceros*
   d. Bipolar elevations: *Eucampia*
   e. Bipolar elevations with spines: *Cerataulina*
3. Abutting valve faces: *Leptocylindrus*, *Fragilariopsis*
4. Mucilage pads or stalks
   a. Mucilage pads at valve poles: *Thalassionema* (stars or zig-zags)
   b. Mucilage stalks: *Licmophora*
   c. Valve face: *Fragilariopsis* (ribbon chains)
   d. Part of valve: *Pseudo-nitzschia* (stepped colonies)
5. Mucilage tubes: *Nitzschia*, *Berkeleya*
6. Amorphous masses of mucilage: *Thalassiosira*

Movement in diatoms is limited to those pennate species that have slits called raphes and occurs only when a raphe contacts a solid surface. Mucopolysaccharide filaments are secreted into the raphe and become attached to the substrate. Actin filaments inside the cell move along the secreted filaments, and the cell glides. When the raphe passes over, the mucopolysaccharide detaches and remains behind as a sticky trail. The only flagellated cells occurring among diatoms are the male gametes of centric species.

Reproduction in diatoms is primarily via asexual cell division. Each daughter cell receives half of the parent cell's theca as an epitheca and forms a new hypotheca. As a result, a diatom population's average cell size decreases; it must recover or the population will die out. When cells are about one-third their maximum size, sexual reproduction occurs. Gametes emerge and fuse to form a zygote that sheds the silica theca and forms a large sphere, the auxospore, covered by an organic membrane. The initial cell, a new cell of maximum cell size, forms within the auxospore and begins a new generation (Fig. 2). Depending on the species, sexual reproduction may be infrequent, sometimes occurring only every 20 years or more.

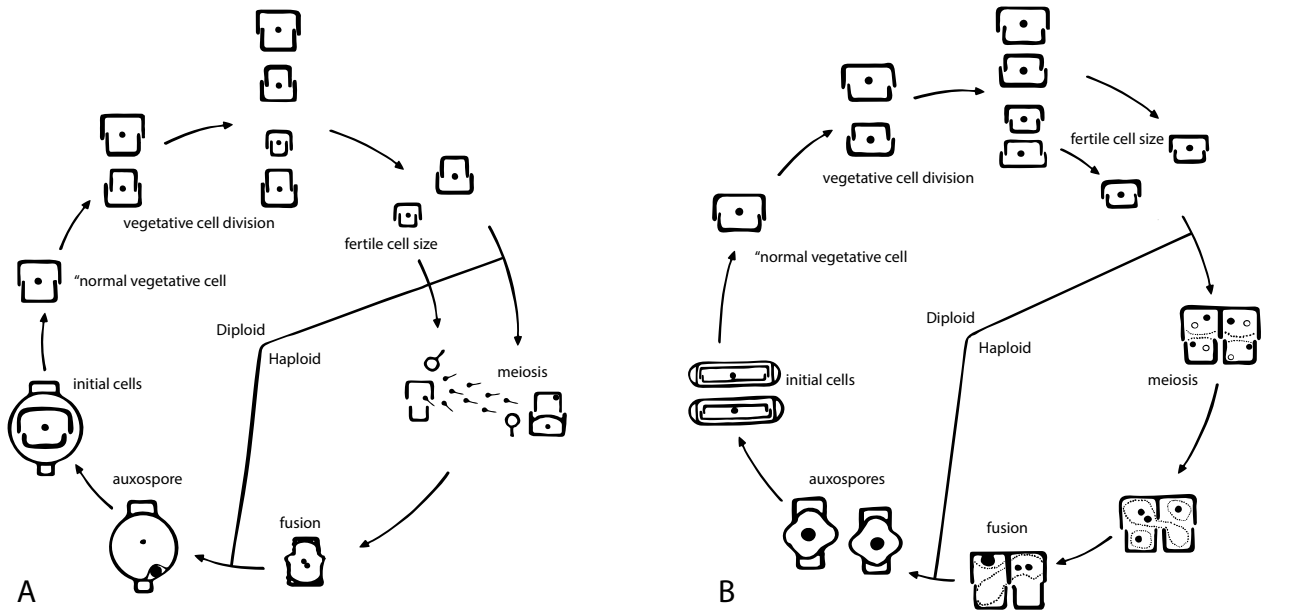


Figure 2. Diatom life cycles: (a) sexual reproduction of a centric diatom (oogamy) and (b) of a pennate diatom (morphological isogamy, physiological anisogamy). •= zygote, • = nucleus, ° = pyknotic nucleus. Redrawn from Hasle and Syvertsen (1996).

When environmental conditions become unfavorable, some diatoms, especially centric ones, produce resting spores. The resting spores may resemble the vegetative cells or may look so different, they have been described as different species (e.g., *Chaetoceros* spp.). Resting spores germinate when environmental conditions become favorable. They may require a dormancy period before germination, or may germinate within a few days.

Diatoms are primarily photosynthetic. A few are heterotrophs, drawing their nutrition from outside rather than producing it by photosynthesis, and some can live heterotrophically in the dark if an appropriate organic carbon source is available.

Healthy cells are golden brown in color. Unhealthy cells are greenish. Diatoms store food reserves as chrysolaminarin and lipids.

2. Dinoflagellates

Dinoflagellates are the second most important group of phytoplankton, comprising about 1,700 species (Sournia et al. 1991). They occur in all the oceans but are most successful in the tropics. They range in size from 2 µm to 2 mm.

A dinoflagellate's cell wall is complex, consisting of a layer of vesicles with or without cellulosic plates. If plates are present, the cells are said to be thecate, or armored; if plates are not present the cells are athecate, unarmored or naked. The arrangement of the plates is specific to each genus. The theca may have horns, spines or lists, and the plates may have ornaments of pores, depressions, spines, ridges and reticulations. Some taxa have delicate external scales or siliceous or calcareous skeletons. Most dinoflagellates are solitary, but a few genera, including *Alexandrium*, *Gymnodinium* and *Peridiniella*, contain species that produce chains.

Most dinoflagellate cells are divided into two parts by a horizontal groove or cingulum known as the girdle (Fig. 3). The anterior part is the epitheca (epicone if plates are not present), the posterior part is the hypotheca (hypocone without plates). The two parts may be equal or unequal in size; girdle placement helps define genera. A vertical groove, the sulcus, splits the hypotheca and may extend into the epitheca. Dinoflagellates have two dissimilar flagella that emerge through one or two pores on the ventral side of the cell near the ends of the girdle or from the anterior part of the cell (Fig. 3). The ribbon-like transverse flagellum, located in the girdle, beats with a helical or semi-helical motion, moving the cell forward. The longitudinal (trailing) flagellum, located in the sulcus, is cylindrical or flattened and beats with one or a few waves that cause the cell to rotate and move forward.

Reproduction is either asexual or sexual (Fig. 4). In asexual reproduction, cells divide by binary fission. In sexual reproduction, gametes are formed and fuse to form a zygote.

The zygote may be a nonmotile resting stage (hypnozygote) morphologically different from the vegetative cell that settles to the bottom and remains dormant for some period of time, or a motile zygote (planozygote) that is morphologically similar to the vegetative cell. Both may occur in a single life cycle (e.g., *Alexandrium*).Chloroplasts may or may not be

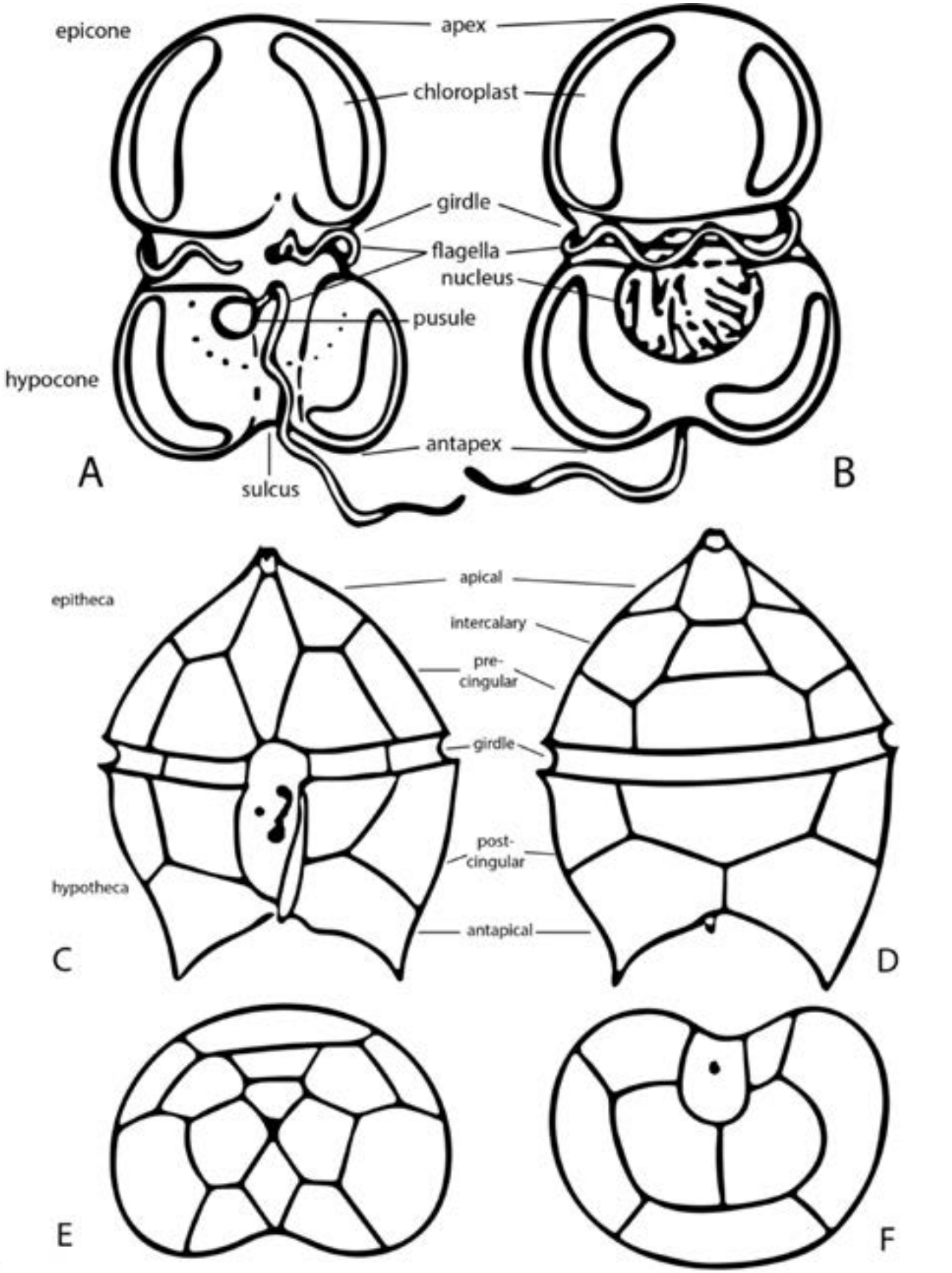


Figure 3. Top. Athecate (naked, unarmored) dinoflagellate: (A) ventral side with girdle and sulcus with flagella; (B) dorsal side. Bottom. Thecate (armored) dinoflagellate showing plate pattern: (C) ventral side with girdle and sulcus; (D) dorsal side; (E) cell apex; (F) cell antapex. Redrawn from Dodge (1982).

present. Dinoflagellates may be autotrophic or heterotrophic; some autotrophs are now known to be mixotrophs, combining photosynthesis and grazing. Some dinoflagellates are parasitic and some are symbiotic. Some have specialized structures such as feeding veils to trap food and peduncles to suck food from other organisms. Their reserve foods are starch and oil.

Dinoflagellates are divided into two groups depending on where their flagella emerge (Fig. 5). The group called desmokonts have two dissimilar flagella rising from the anterior of the cell (*Prorocentrum*), while the group called dinokonts have flagella inserted ventrally, with the transverse flagellum located in the cingulum and the longitudinal (trailing) flagellum in the sulcus (*Protoperidinium*).

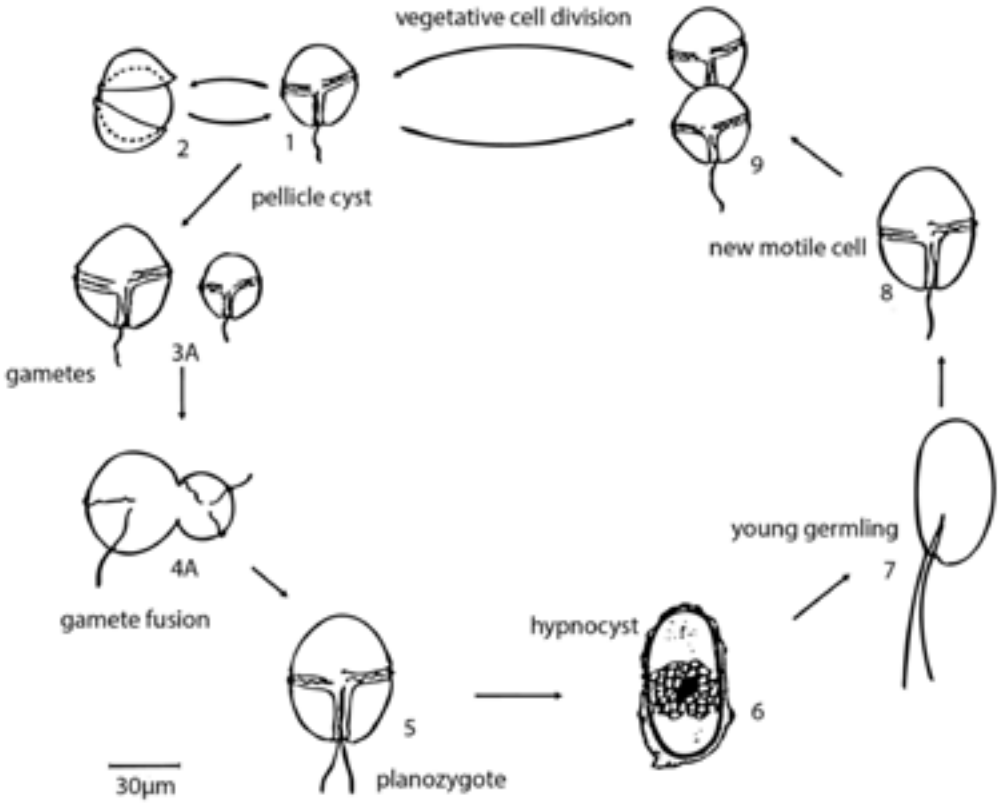


Figure 4. Dinoflagellate life cycle. Stages: (1) vegetative cell; (2) pellicle (temporary) cyst; (3) gametes; (4) fusing gametes; (5) planozygote (motile cell); (6) hypnozygote; (7) and (8) planomeiocyte (germinated cell); (9) pair of vegetative cells after division. Redrawn from Anderson (1998).

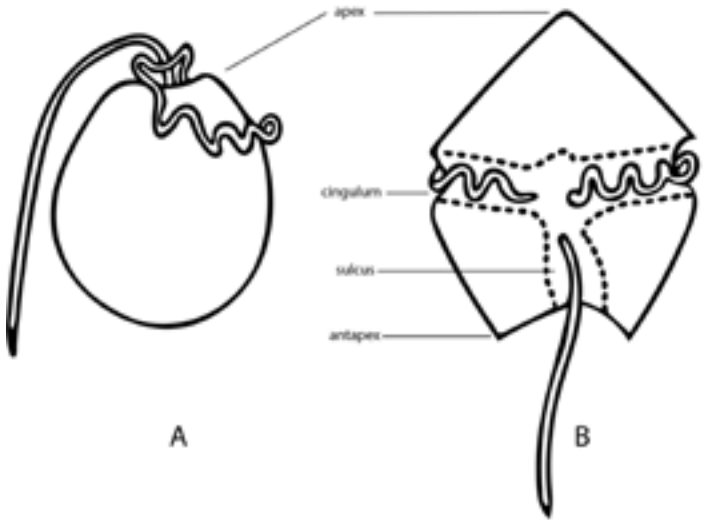


Figure 5. Schematic of dinoflagellates showing flagellar insertion: (A) desmokont; (B) dinokont. Redrawn from Taylor (1987).

3. Flagellates

Flagellates are a mixed bag of mostly unicellular motile cells that tend not to preserve well or to be very well known (Fig. 6). They occur in all oceans, lakes, rivers, streams and other bodies of water, from the poles to the tropics. They commonly range in size from about 2 to 20 µm but may be larger. When counting samples, flagellates are often grouped non-systematically into size classes to obtain numbers and/or biomass.

Cell coverings may be composed of organic strips (euglenoids), proteinaceous plates (cryptomonads), or silica (silicoflagellates). They may be smooth or covered with scales of one or more kind that may be organic, silicified or calcified. The scales have patterns that are used in taxonomy, but transmission electron microscopy is necessary to see them.

Cells may have 1, 2, 4, 8 or 16 flagella, which may be covered in hairs or scales. Flagellates use their flagella for swimming and/or attachment. One group, the Prymnesiophyceae (Haptophyceae), also have a thread-like appendage of variable length called the haptonema, located between the two flagella and used to anchor the cell to a substrate or capture food.

Reproduction is mostly by cell division. Most flagellates are known only as motile cells, the dominant stage in the life cycle, but some have nonmotile stages that may be described as different genera. Some have more than one motile stage or gelatinous stages (e.g., *Phaeocystis*). Some produce cysts that may degrade quickly or may fossilize (Chrysophyceae).

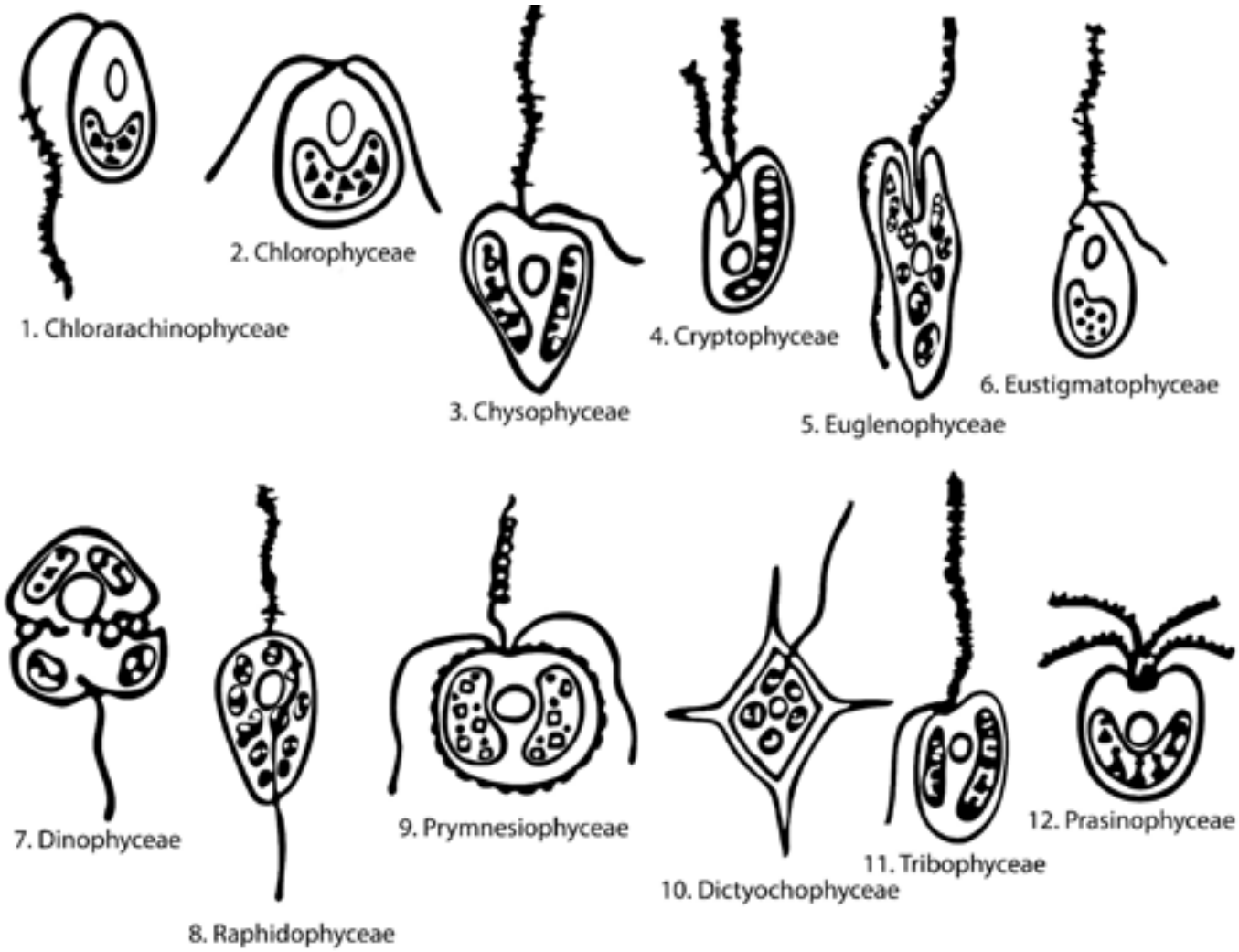


Figure 6. Line drawings with hair and scale patterns of various classes of flagellates. From Chrétiennot-Dinet (1990).

Chloroplasts may be present or absent. Pigment composition is variable depending on the taxonomic group and may sometimes serve to determine which classes of algae are present in a sample, using the program CHEMTAX. Cells may be autotrophs, heterotrophs or mixotrophs.

Flagellates are divided into taxonomic groups based on flagella (number, presence of scales or hairs), cell walls, skeletons, presence or absence of a haptonema, pigments, and other characteristics. The group that SoundToxins is most interested in is the Raphidophyceae, a relatively small group of freshwater green and marine golden-brown flagellates that includes *Heterosigma.*

Heterosigma range in diameter from about 10 to more than 25 µm. Their cells, naked and dorsoventrally flattened, can change shape. The cell surface has a glycocalyx composed of complex carbohydrates and glycoproteins. Mucocysts located near the chloroplasts produce large amounts of mucus. Two unequal heterodynamic flagella are present: one with hairs is directed forward and one smooth is directed posteriorly; cells rotate while swimming. There are many small, golden brown, discoid chloroplasts located along the periphery of the cells. *Heterosigma* is photosynthetic. Reserve food is lipids.

Reproduction is by longitudinal cell division with replication of the flagella indicating the onset of division. Cyst stages are present and germination can occur in the dark. Benthic masses of nonmotile cells enclosed in mucilage may be present. *Heterosigma* is a vertical migrator, descending at night and ascending in the day.

Raphidophycean flagellates produce hemolytic, neurotoxic, and hemagglutinatin compounds, as well as hydrogen peroxide and superoxide, hydroxyl radicals that may kill fish. Some raphidophyceans, including *Heterosigma*, produce toxins similar to brevetoxins. However, wild blooms of *Heterosigma* are not always toxic.

References

Anderson, D.M. 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: D.M. Anderson, A.D. Cembella and G.M.

Hallegraeff (eds.) Physiological Ecology of Harmful Algal Blooms. NATO ASI Series, Vol. G 41. Pp. 29–48.

Chrétiennot-Dinet, M.J. 1990. Chlorarachniophycées, Chlorophycées, Chrysophycées, Cryptophycées, Euglenophycées, Eustigmatophycées, Prasinophycées, Prymnesiophycées, Rhodophycées, Tribophycées. In: A. Sournia (ed.) Atlas du Phytoplancton Marin, Vol. 3. Editions du Centre National de la Recherche Scientifique, Paris. Pp. 1–261.

Cupp, E.E. 1943. Marine plankton diatoms of the west coast of North America. Bull. Scripps Inst. Oceanogr. 5:1–238.

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

Hasle, G.R. and E.E. Syvertsen. 1996. Marine diatoms. In: C.R. Tomas (ed.) Identifying Marine Diatoms and Dinoflagellates. Academic Press, San Diego. Pp. 5–385.

Sournia, A., M-J. Chretiennot-Dinet, and M. Ricard. 1991. Marine phytoplankton: how many species in the world ocean? J. Plankton Res. 13:1093–1099.

Taylor, F.J.R. 1987. Dinoflagellate morphology. In: F.J.R. Taylor (ed.) The Biology of the Dinoflagellates. Blackwell Scientific Publications, Oxford, pp. 24-91.

Phytoplankton Identification

As mentioned previously in Dr. Horner's "Brief Introduction to Marine Phytoplankton" (p. 3), important features for identifying phytoplankton include the shape of the cellwall (e.g., round, boat-shaped, triangular, square, or elliptical), patterns on the cell wall (pores, ribs, spines), cell movement, the presence or absence of chloroplasts, and the presence or absence of flagella. This section is dedicated to phytoplankton identification for a few key species. Guides are organized according to grouping (diatoms, dinoflagellates, flagellates and others). A genus-level quick reference guide is included in the appendix (p.40) as well as an illustrated guide on phytoplankton morphological terminology (p. 44).

In addition to the information SoundToxins participants need to identify plankton, the species-specific sheets contain information on toxicology, infection vectors, regional species of concern, action levels, and SoundToxins action. The toxic "action levels" represented are U.S. Food and Drug Administration (USFDA) thresholds for closing fisheries. A "SoundToxins action level" is one that should be reported to the SoundToxins alert line at soundtox@uw.edu.

Other phytoplankton identification resources include A Taxonomic Guide to Some Marine Phytoplankton (2002) by Dr. Rita Horner and Identifying Marine Plankton (1997) edited by Dr. Carmelo Tomas.

Species Specific Identification Sheets

A. Diatoms

Chaetoceros concavicornis

A centric diatom widely distributed in northern temperate and cold water areas, known to kill fish, especially salmon in net pens.

Identification

Cells joined in straight chains; valves unlike, with upper valve rounded with setae arising near the center and lower valve flat with setae arising inside the valve margin; apertures distinct; setae become wider away from the cell and are covered with small spines.

Toxicology

Mechanism for toxicity is not known, but cells thought to become trapped in gill filaments, causing irritation and mucus production by the gill tissue.

Regional species

Chaetoceros concavicornis. Similar species: *C. convolutus*.

Vectors

None.

Local distribution

Inland waters of Puget Sound and British Columbia, sometimes forming blooms.

Monitoring

Weekly from March through October and every other week from November through February.

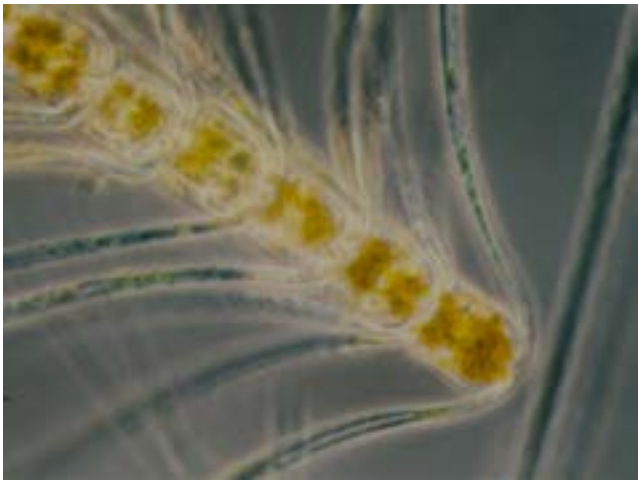
Daily by fish farmers during summer months when phytoplankton are most likely to occur.

Analyses

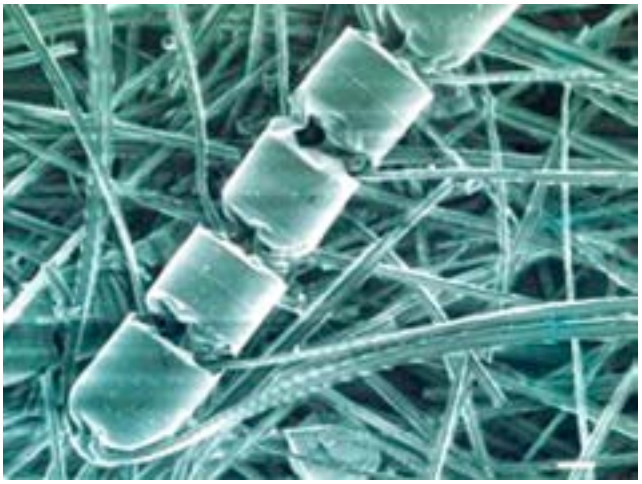
Cell counts in net tow.

SoundToxins action level

"Common," "Bloom," or >5,000 cells/ L.



A. Light microscope image. Note setae get larger away from cells. Photo from Horner (2002).



B. Scanning electron microscope image. Note small spines on setae. Photo from Horner, unpublished.

Pseudo-nitzschia spp.

A pennate diatom genus widely distributed in polar, temperate and subtropical waters worldwide.

Identification

Cells occur in stepped chains (cell ends overlap). Species difficult to identify in light microscopy. Need scanning and transmission electron microscopy for positive identification. Use size classes: long and wide (a/h/f), long and narrow (p/m), short and narrow (pd/d/c).

Toxicology

Some species are known to produce domoic acid (DA) associated with amnesic shellfish poisoning (ASP) that causes gastrointestinal and neurological problems in humans, marine mammals and sea birds.

Regional species

P. australis, P. heimii, P. fraudulenta, P. pungens, P. multiseries, P. cuspidata, P. delicatissima, P. pseudodelicatissima.

Vectors

Bivalve molluscs (mussels, razor clams), herbivorous fish (anchovies, sardines), Dungeness crab.

Local distribution

Pacific coast (California to Alaska) and inland waters of Puget Sound.

Monitoring

Weekly from March through October and every other week from November through February.

Analyses

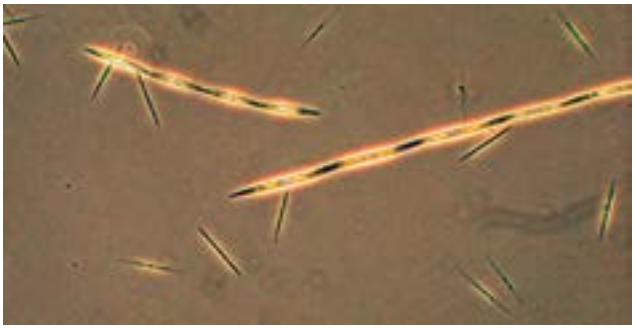
Cell counts in settled 10x whole water.

USFDA Action level: 20 parts per million of DA in shellfish tissue samples.

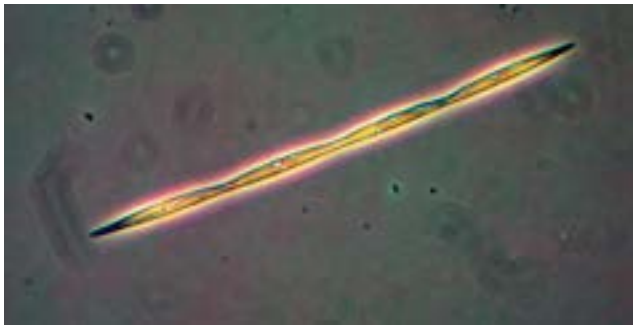
SoundToxins action level

Large cells (*P. australis, P. heimii, P. fraudulenta; P. pungens, P. multiseries* = a/h/f/p/m) > 50,000 cells/L, "Common," or "Bloom".

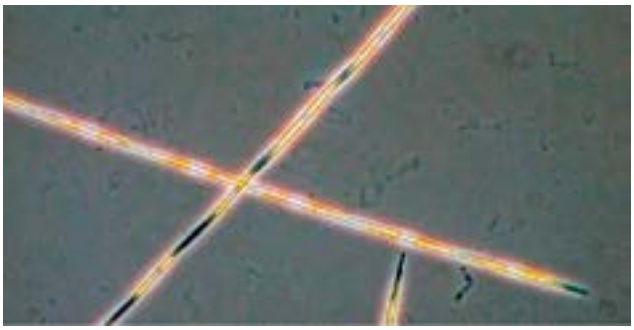
Small cells (*P. pseudodelicatissima, P. delicatissima, P. cuspidata* = pd/d/c) > 1,000,000 cells/L, "Common," or "Bloom".



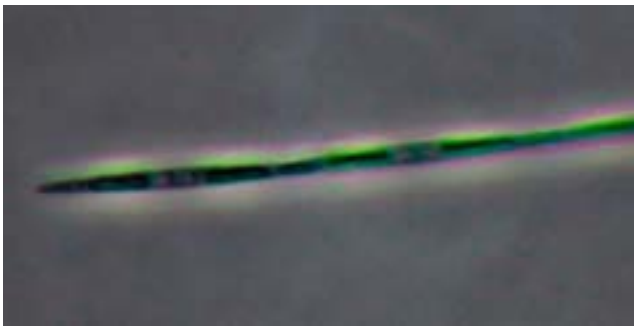
A. *Pseudo-nitzschia*: a/h/f = (large, broad cells); p/m = (large, narrow cells). Photo from Brian Bill, NOAA.



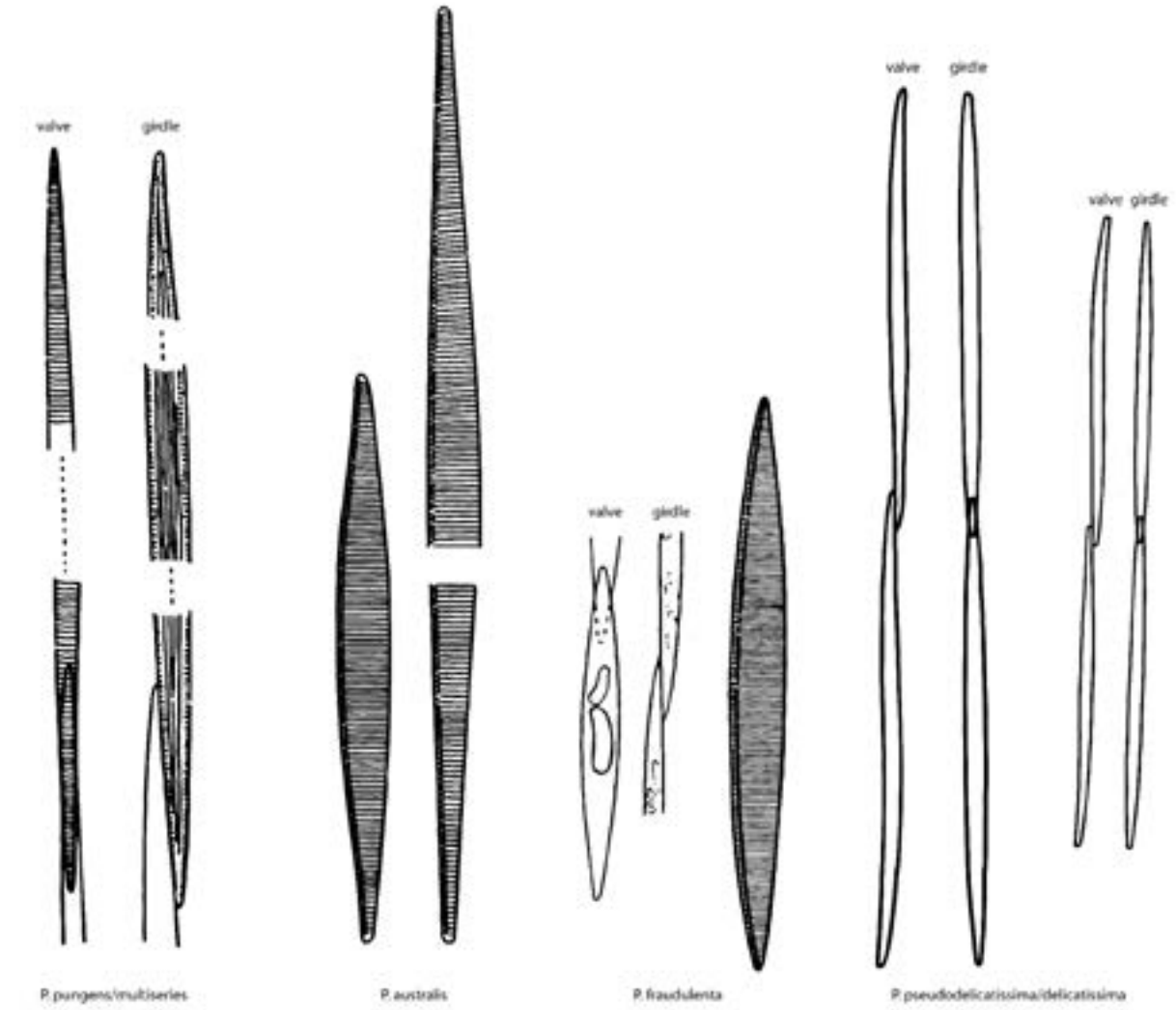
B. *Pseudo-nitzschia*: a/h/f (large, broad cells). Photo from Brian Bill, NOAA.



C. *Pseudo-nitzschia:* pm (large, narrow cells). Photo from Brian Bill, NOAA.



D. *Pseudo-nitzschia*: pd/d/c (small, narrow cells). Alan Sarich, Washington Department of Fish & Wildlife.



E. Diagram of *Pseudo-nitzchia* species. Figure from Hasle (1972).

B. Dinoflagellates

Akashiwo sanguinea

An athecate dinoflagellate genus with cosmopolitan distribution in temperate to tropical estuarine and coastal waters.

Identification

Small to medium (40-80 µm long) dorso-ventrally compressed cells, with broadly conical epitheca and bilobed hypotheca. Deeply notched sulcus in hypotheca that does not extend into the epitheca.

Toxicology

This species is associated with bird, fish and shellfish kills. This may be due to low oxygen and mucus production.

Regional Species

Akashiwo sanguinea; formerly known as *Gymnodinium splendens, G. nelsonii, G. sanguineum.*

Vectors

None known at this time.

Local distribution

Pacific coast (California to Alaska) and inland waters of Puget Sound.

Monitoring

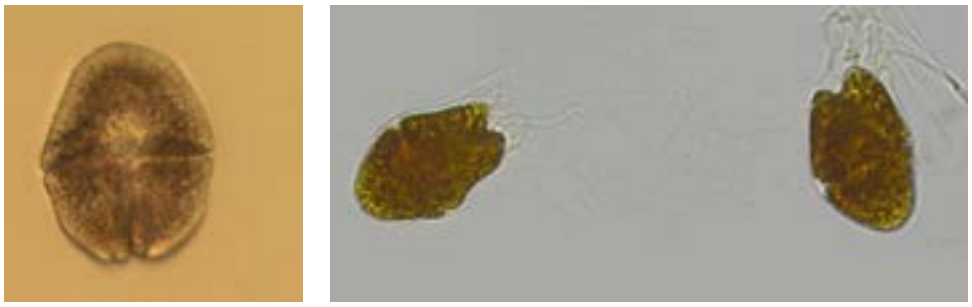
Weekly from March through October and every other week from November through February.

Analyses

Cell counts in net tow.

SoundToxins action level

Report any presence of this organism in the net tow.



A. Light microscope image of a single *Akashiwo sanguinea* cell and a set of two cells. SoundToxins, WSG.

B. Diagram of *A. sanguinea*

Alexandrium spp.

A thecate dinoflagellate genus widely distributed in temperate, subtropical and tropical oceans worldwide. Many species found primarily in coastal areas.

Identification

Cells are distinguished by cell size and shape, shape and position of the apical pore complex, presence and size of the ventral pore, and chain formation (cells solitary or forming chains, but chain formers can occur as single cells).

Toxicology

Some species known to produce paralytic shellfish toxins (PSTs), including saxitoxin, associated with paralytic shellfish poisoning that causes neurological symptoms and may lead to respiratory arrest in humans. May also affect marine mammals.

Regional species

A. catenella, A. tamarense.

Vectors

Bivalve molluscs, marine snails, barnacles, Dungeness crabs, anchovies, sardines.

Local distribution

Pacific coast (California to Alaska) and inland waters of Puget Sound.

Monitoring

Weekly from March through October and every other week from November through February.

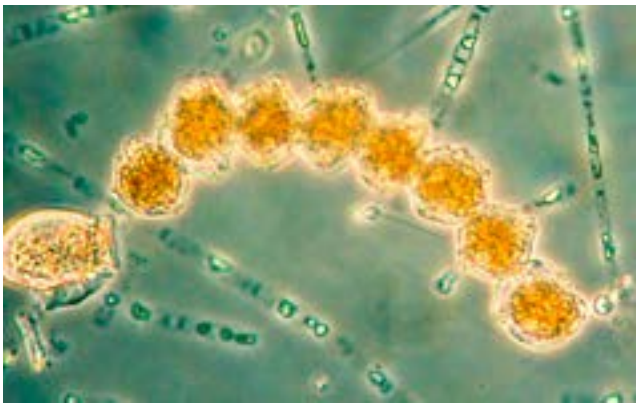
Analyses

Cell counts in net tow.

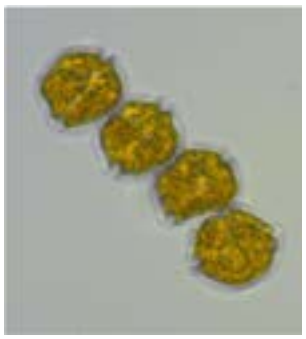
USFDA Action level: 80 µg/100 g shellfish tissue of toxins.

SoundToxins action level

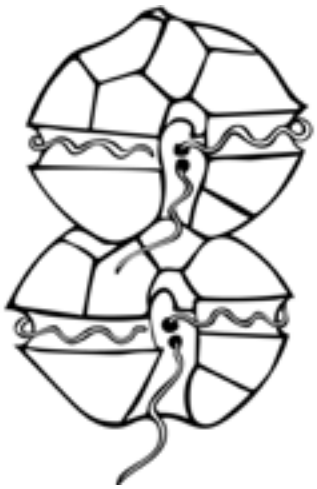
Report any presence of this organism in the net tow.



A. *Alexandrium* chain. From Horner (2002).



B. *Alexandrium* cells under light microscopy. SoundToxins, WSG.



C. Diagram of *Alexandrium* cells. SoundToxins, WSG.

Azadinium spp.

A small thecate dinoflagellate with global distribution.

Identification

Small cells between 12-16 µm long and about 5 µm wide. Because of their short width, they can sometimes slip through plankton nets. They are often mistaken for Heterocapsa or other small brown cells and may appear unarmored under light microscopy. Epitheca can be slightly pointy, and larger than hypotheca which is more rounded. Large descending cingulum displaced about 0.5x.

Toxicology

Some species produce azaspiracid (AZAs) which can lead to Azaspiracid poisoning (AZP), and are bloom-forming.

Regional species

A. cuneatum, *A. dalianense*, *A. cf. dalianense*, *A. obesum*, *A. poporum*, and *A. spinosum*.

Vectors

Bivalve shellfish.

Local distribution

Pacific coast (California to British Columbia) and inland waters of Puget Sound.

Monitoring

Weekly from March through October and every other week from November through February.

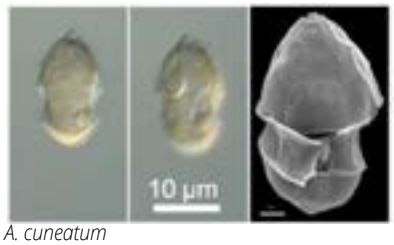
Analyses

Cell counts in net tow.

USFDA Action level: 160 µg per 100 g shellfish tissue of toxins.

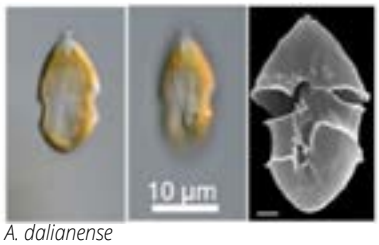
SoundToxins action level

Report any presence of this organism in the net tow.

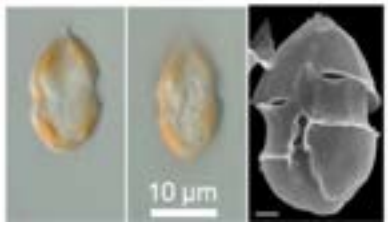


A. cuneatum

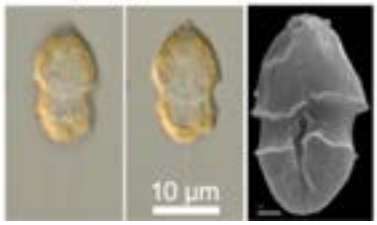
A. cf. dalianense



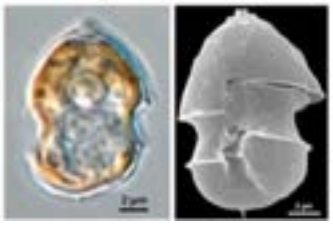
A. dalianense



A. obesum



A. poporum


A. spinsum

A-E Light microscopy and SEM images of several *Azadinium* species from Kim et al. (2017); F Light microscopy and SEM images of *A. spinosum* by Urban Tillman.

Dinophysis spp.

A thecate dinoflagellate genus widely distributed in tropical to cold temperate waters worldwide.

Identification

Cells laterally flattened; epitheca small, hypotheca large, girdle moderately wide and bordered by lists (wings), ventral sulcus bordered by lists; chloroplasts present or absent.

Key features used to differentiate between species include: overall roundness of the cell, where the widest section of the cell is in relation to the length, how pointy the bottom is, how long the lists/wings are, and whether or not the top of the epitheca is visible from the side.

Toxicology

Produces lipophilic toxins (diarrhetic shellfish toxins or DSP) that can accumulate and co-occur in shellfish. Toxins are okadaic acid (OA), a known tumor promoter; dinophysistoxins (DTX1-6), which are derivatives of OA; and pectenotoxins (PTX) harmful to the liver. Symptoms are nausea, vomiting, diarrhea; recovery in a few days without treatment.

Regional species

D. acuminata, D. acuta, D. fortii, D. norvegica, D. odiosa, D. parva, D. rotundata, D. tripos. The species *D. rotundata* has been reclassified as *Phalacroma rotundatum*, but we continue to use the *Dinophysis* classification within our program.

Vectors

Bivalve shellfish.

Local distribution

Pacific coast (California to Alaska) and inland waters of Puget Sound.

Monitoring

Weekly from March through October and every other week from November through February.

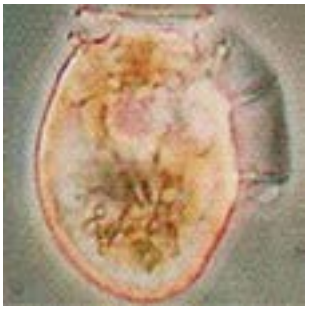
Analyses

Cell counts in net tow.

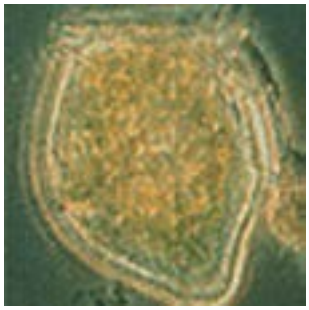
USFDA action level: 160 µg OA/kg shellfish edible parts.

SoundToxins action level

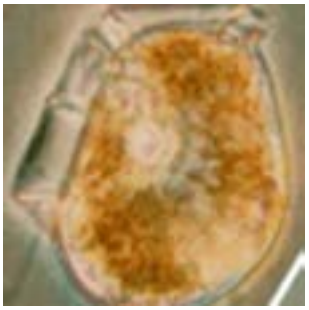
Report initial sighting, any increase from "Present" to "Common" or "Common" to "Bloom," and >1000 cells/L in the net tow.



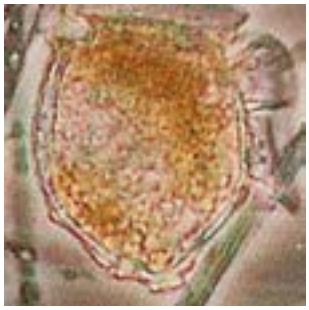
1. *D. acuminata*



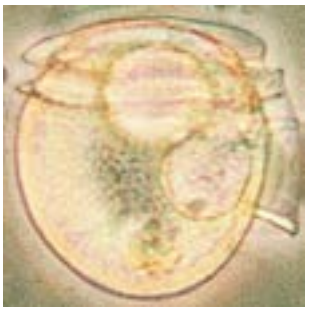
2. *D. acuta*



3. *D. fortii*



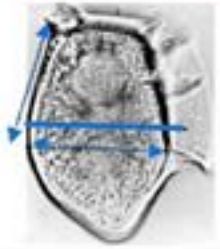
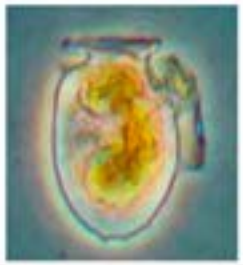
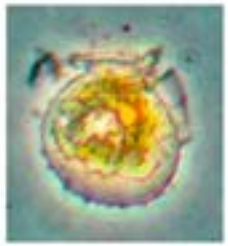
4. *D. norvegica*



5. *D. rotundata*

6. *D. tripos*

Regional *Dinophysis* species under light microscopy. A-E from Horner (2002); F from Port Townsend Marine Science Center.

Dinophysis norvegica	***Dinophysis acuta***
• Commonly associated with DSP • Length 48-67 µm x Width 39-53 µm • Curved edge • Widest at midpoint	• Associated with DSP • Length 54-94 µm x Width 43-60 µm • Straight edge • Widest below midpoint
	
Dinophysis acuminata	***Dinophysis rotundata***
• Commonly associated with DSP • Length 38-58 µm x Width 30-35 µm • Smaller and more elongated than *D. rotundata*	• Not associated with DSP in Puget Sound • Length 36-56 µm x Width 36-56 µm • Larger and rounder than *D. acuminata*
	
Dinophysis fortii	***Dinophysis parva***
• Associated with DSP • Length 62-66 µm x Width 43-58 µm • Eggplant-shaped	• Length 22 µm x Width 22 µm • Small and round
	
Dinophysis caudata	***Dinophysis tripos***
• Not very common in Puget Sound • Distinctive shape • Length 70-110 µm x Width 37-50 µm	• Not very common in Puget Sound • Distinctive shape • Length 90-125 µm x Width 50-60 µm
	

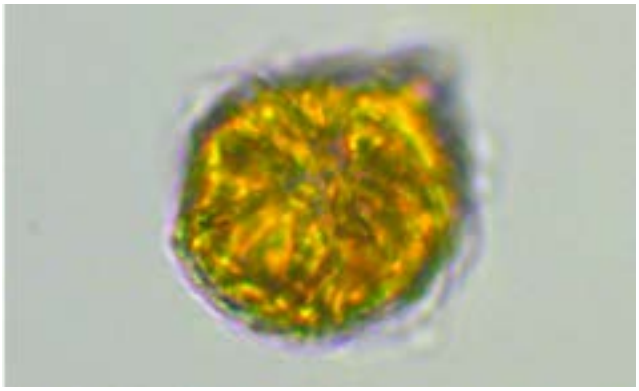
Gonyaulax spinifera

A thecate dinoflagellate distributed in estuarine, neritic, and cosmopolitan waters in the Pacific and Atlantic oceans, Gulf of Mexico and Mediterranean Sea.

Identification

Small to medium cells, 23–50 µm long and 30–40 µm wide. The cingulum is excavated and descending with the ends separated by at least two cingulum widths. The epitheca is conical with a short to moderate apical horn. Usually two, but possibly several, spines on the antapex. A single multilobed chloroplast is present.

Cells are often confused with other small, round brown dinoflagellates, especially with other *Gonyaulax* species (e.g., *G. digitale* and *G. diegensis*). Forms cysts. A number of cyst genera have been described and associated with various *Gonyaulax* species. *G. spinifera* is a species complex based on confusion arising from thecate stages identified as *G. spinifera* that have hatched from different cyst genera.



A. *Gonyaulax spinifera* cell under light microscopy.

B. *G. spinifera* shell under light microscopy.

Toxicology

Produces yessotoxins.

Regional species

Gonyaulax spinifera.

Vectors

Bivalve shellfish and abalone.

Local distribution

Pacific coast (California to British Columbia) and inland waters of Puget Sound. Blooms may form in summer.

Monitoring

Weekly from March through October and every other week from November through February.

Analyses

Cell counts in net tow.

SoundToxins action level

None.

C. Diagram of *G. spinifera* cell. SoundToxins, WSG.

Heterocapsa triquetra

A small thecate dinoflagellate with cosmopolitan distribution in neritic and estuarine waters

Identification

Small cells that might appear unarmored under light microscopy. Epitheca is rounded and larger than hypotheca, which is a bit pointier. Cingulum descending and slightly displaced. Nucleus in epitheca, and pyrenoid in hypotheca.

Toxicology

This species is not known to be toxic.

Regional species

Heterocapsa triquetra. May also be known as *Kryptoperidinium triquetrum*.

Vectors

None.

Local distribution

Pacific coast (Washington to British Columbia) and inland waters of Puget Sound.

Monitoring

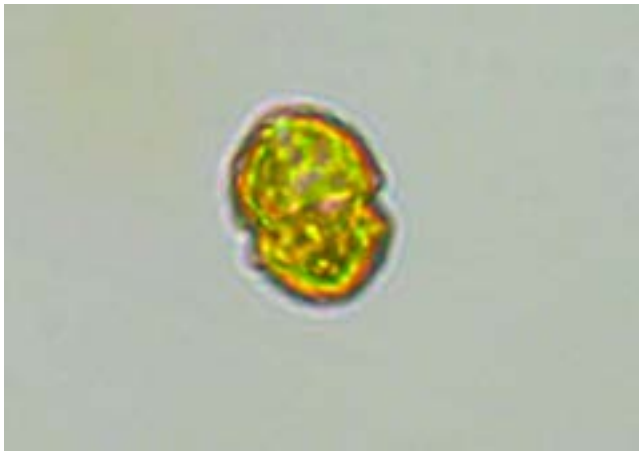
Weekly from March through October and every other week from November through February.

Analyses

Cell counts in net tow.

SoundToxins action level

None.



A. *Heterocapsa triquetra* cell under light microscopy.

B. Diagram of *Heterocapsa triquetra* cell showing general shape and locations of the nucleus (N) and pyrenoid (P). SoundToxins, WSG.

Karenia mikimotoi

An athecate dinoflagellate with cosmopolitan distribution in temperate to tropical neritic waters.

Identification

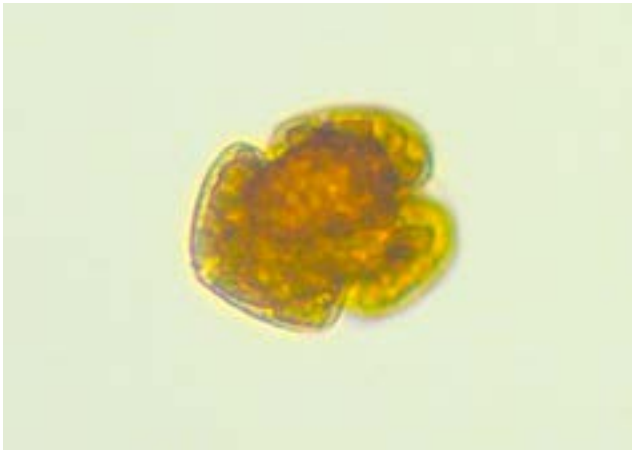
Cells 20–30 µm long, 16–30 µm wide. Shape dorsoventrally compressed to almost round. Nucleus oblong on left side of cell. Straight apical groove.

Toxicology

This species produces cytotoxic Gymnocin polyether toxins.

Regional species

Karenia mikimotoi. Formerly known as *Gymnodinium mikimotoi.*



A. *Karenia mikimotoi* cell

Vectors

None known at this time.

Local distribution

Found in coastal Alaska, and has potential to move down to Washington.

Monitoring

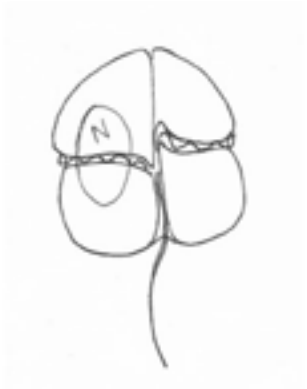
Weekly from March through October and every other week from November through February.

Analyses

Cell counts in net tow.

SoundToxins action level

Report any presence of this organism in the net tow.



B. Diagram of *K. mikimotoi* cell showing general shape and location of the nucleus (N). SoundToxins, WSG.

Lingulodinium polyedra

A thecate dinoflagellate found in neritic, warm temperate to tropical waters.

Identification

Cells 42-54 µm wide, polyedral with no spines or horns but strong ridges along plate sutures. Epitheca with straight sides, flattened or slightly curved apex; hypotheca with straight sides, flattened antapex. Cingulum displaced 1x. Often mistaken for other species including *Protoceratium reticulatum*, but has a distinctive flat bottom.

Toxicology

Produces yessotoxins.

Regional species

Lingulodinium polyedra, formerly known as *Gonyaulax polyedra*.

Vectors

Bivalve shellfish and abalone.

Local distribution

Pacific coast (California to Washington) and possibly the inland waters of Puget Sound.

Monitoring

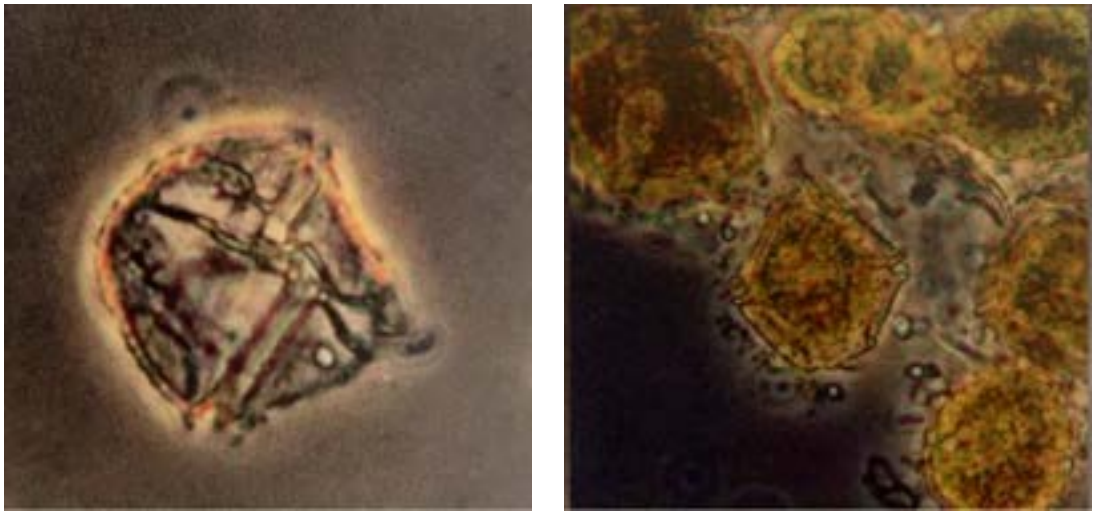
Weekly from March through October and every other week from November through February.

Analyses

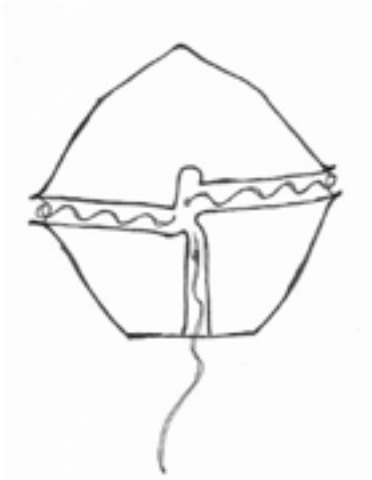
Cell counts in net tow.

SoundToxins action level

Report any presence of this organism in the net tow.



A-B. *Lingulodinium* polyedra cells under light microscopy. Horner (2002).



C. Diagram of *L. polyedra*. SoundToxins, WSG.

Margalefidinium polykrikoides

An athecate dinoflagellate found either solitary or chain forming.

Identification

Medium cells (<40 µm long) form chains up to 16 cells. Spiraling cingulum that turns 1.5x around the cell is a distinguishing feature, and the sulcus is immediately below. Cells are ellipsoidal, but can be compressed when in chains. Hypotheca is bilobed.

Toxicology

This species is associated with fish kills.

Regional species

Margalefidinium polykrikoides, genus formerly known as *Cochlodinium*.

Vectors

None known at this time.

Local distribution

Pacific coast (California to British Columbia) and inland waters of Puget Sound.

Monitoring

Weekly from March through October and every other week from November through February.

Analyses

Cell counts in net tow.

SoundToxins action level

Report any presence of this organism in the net tow.

A. *Margalefidinium polykrikoides* cells under light microscopy.

B. Diagram of *M. polykrikoides* cells. SoundToxins, WSG.

Prorocentrum spp.

A desmokont dinoflagellate genus distributed primarily in marine waters, planktonic or benthic/epiphytic.

Identification

Small to medium cells, laterally compressed, ranging in shape from spheroid to pyriform in valve view. They are made of two large lateral plates (valves). Two flagella emerge from an indented area in the anterior end of the right valve. Species are distinguished by general shape, presence and pattern of valves, pores, areolae and spines on plates.

Toxicology

Some species are toxic.

Regional species

P. gracile, *P. lima*, *P. micans*, and *P. compressum.*

Vectors

None known at this time.

Local distribution

Pacific coast (California to Alaska) and inland waters of Puget Sound.

Monitoring

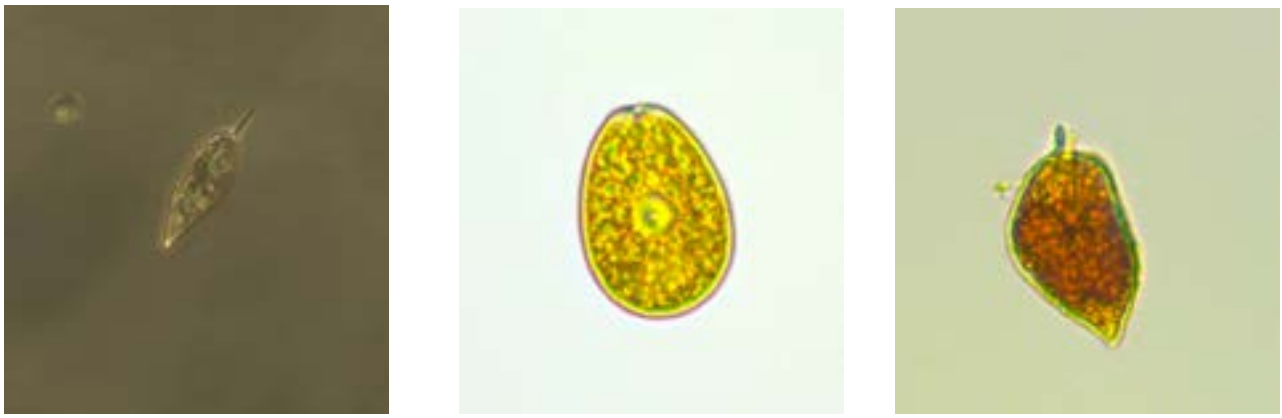
Weekly from March through October and every other week from November through February.

Analyses

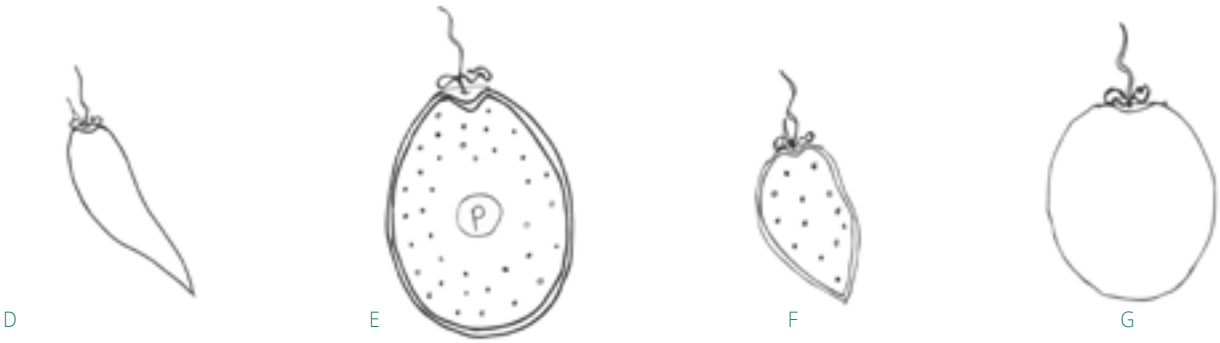
Cell counts form net tow.

SoundToxins action level

None.



A. *P. gracile* under light microscopy. B. *P. lima* cell under light microscopy. C. *P. micans* cell under light microscopy.



D E F G

D. Diagram of *P. gracile* cell. E. Diagram of *P. lima* cell. F. Diagram of *P. micans* cell. G. Diagram of *P. compressum* cell. SoundToxins, WSG.

Protoceratium reticulatum

A thecate dinoflagellate distributed in estuarine and neritic waters, cold temperate to sub-tropical oceans worldwide.

Identification

Cells are polyhedral in shape with strong reticulations that often mask the plates; cells small to medium, 25–55 µm long, 25–35 µm wide. Epitheca is a broad cone with straight sides, shorter than hypotheca; hypotheca has straight to convex sides, rounded to squarish antapex with no spines. Cingulum nearly medium, slightly descending. Chloroplasts are present, giving the cells a deep brown color.

Cells are easily confused with other smallish, round brown cells including solitary *Alexandrium* cells and some *Gonyaulax* species. Populations from South Africa were described as *Gonyaulax grindleyi*, which remains a synonym. Cysts, described as *Operculodinium centrocarpum* (also known as *Hys trichosphaeridium centrocarpum*), are spherical with dense ornamentation of tapering spines with hooked tips.

Toxicology

Produces yessotoxins.

Regional species

Protoceratium reticulatum.

Vectors

Bivalve shellfish and abalone.

Local distribution

Pacific coast (Washington to British Columbia) and inland waters of Puget Sound.

Monitoring

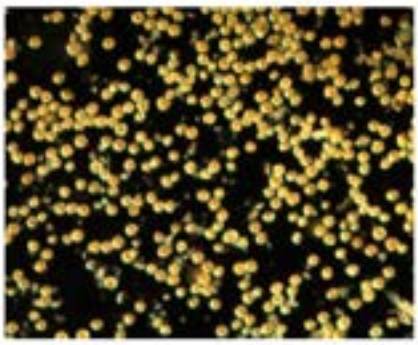
Weekly from March through October and every other week from November through February.

Analyses

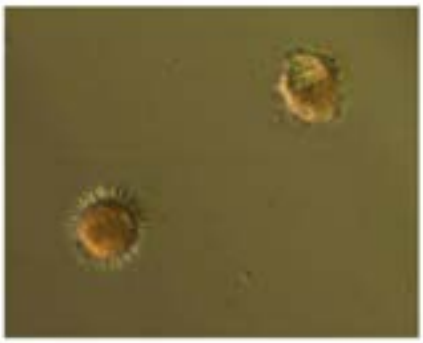
Cell counts in net tow.

SoundToxins action level

Report any presence of this organism in the net tow.

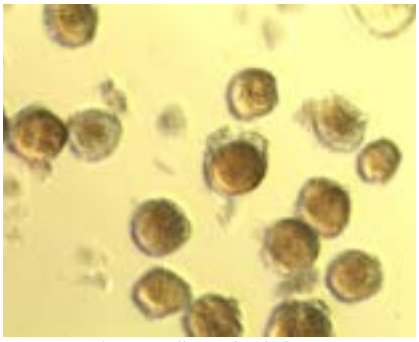


A. *P. reticulatum* cell



B. Vegetative cell (upper right) and cyst with spine

C. *P. reticulatum* shell.



D. *P. reticulatum* cells under light microscopy.

E. Diagram of *P. reticulatum* cell. SoundToxins, WSG.

Scrippsiella trochoidea

A thecate dinoflagellate distributed in estuarine and neritic waters, cosmopolitan in temperate waters.

Identification

Small to medium cells, 16–36 µm long, 20–23 µm wide, pear-shaped. Epitheca conical with apical process that is often clear; hypotheca round with no projections. Cingulum medium, excavated. Chloroplasts present.

Cells are often confused with other small, round, brown dinoflagellates including other *Scrippsiella species*, *Pentapharsodinium* and *Ensiculifera*; thus distribution records may be confusing. Forms ovoid calcareous cysts.

Toxicology

This species is not known to be toxic.

Regional species

Scrippsiella trochoidea.

Vectors

None.

Local distributions

Inland waters of Puget Sound; blooms may form in summer.

Monitoring

Weekly from March through October and every other week from November through February.

Analyses

Cell counts net tow.

SoundToxins action level

None.

A. *Scrippsiella* cell under light microscopy.

B. *Scrippsiella* diagram. SoundToxins, WSG.

C. Flagellates

Heterosigma akashiwo

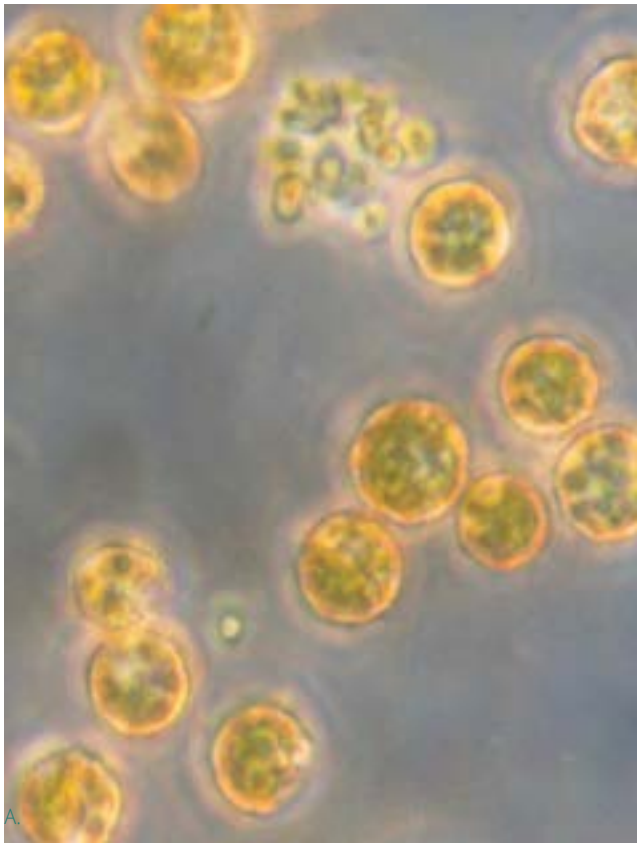
A photosynthetic, flagellated organism widely distributed in temperate coastal and brackish waters.

Identification

Cells slightly compressed with two unequal flagella that arise from a subapical, lateral groove; many golden brown chloroplasts; rigid cell wall absent, so cells readily change shape; rapid swimmers. When preserved, cells gain a distinctive cornflake- or raspberry-like appearance. At high population densities, they may form visible streaks in water samples.

Toxicology

Mechanism for toxicity not known, but suggestions include brevetoxin-like compounds, reactive oxygen species (hydrogen peroxide), hemaglutin or hemolysing compounds, mucus or lectin-like polysaccharides. Kills finfish, especially in net pens, as well as wild fish, and shellfish; known for antagonistic effects on organisms ranging from bacteria to fish.



A.

Heterosigma cells under light microscopy.Horner (2002).

Regional species

Heterosigma akashiwo.

Vectors

None known at this time.

Local distribution

Pacific coast (California to British Columbia) and inland waters of Puget Sound and British Columbia especially in lower salinity waters.

Monitoring

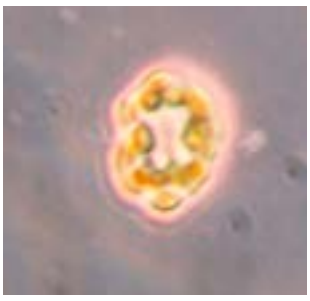
Daily by finfish growers during spring, summer; weekly by SoundToxins from March through October and every other week from November through February.

Analyses

Cell counts, estimates in whole water.

SoundToxins action level

Report any presence of this organism in whole water.



B. Single *Heterosigma* cell under light microscopy. Horner (2002).

C. Example of *Heterosigma* streaks in a jar of sample water. SoundToxins, WSG.

Phaeocystis globosa

A photosynthetic, flagellated organism found in cold to temperate coastal and oceanic waters.

Identification

Cells are 3–8 µm and can be solitary in motile stage, or in gelatinous colonies in nonmotile stage, Solitary cells have two short flagella and a short haptonema located between the flagella. Nonmotile cells form spherical colonies that can be up to 2 mm large. Cells in the colony are evenly distributed throughout the gelatinous matrix.

Toxicology

Associated with shellfish, finfish and bird deaths.

Regional species

Phaeocystis globosa.

Vectors

None known at this time.

Local distributions

Pacific coast of Washington and inland waters of Puget Sound

Monitoring

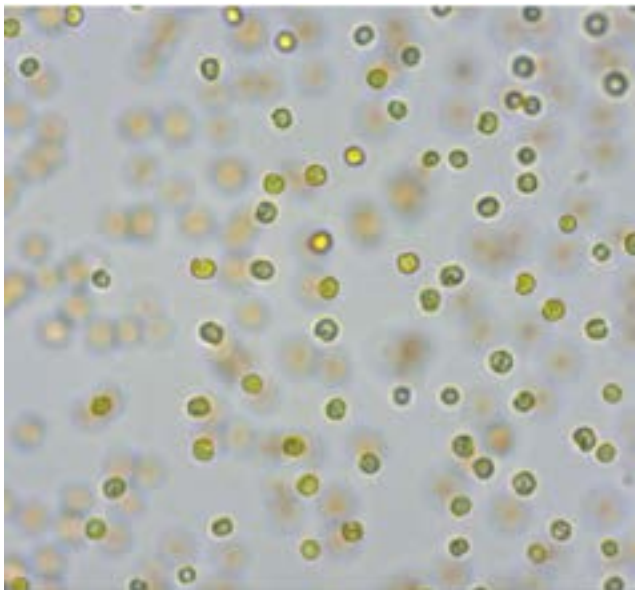
Weekly from March through October and every other week from November through February.

Analyses

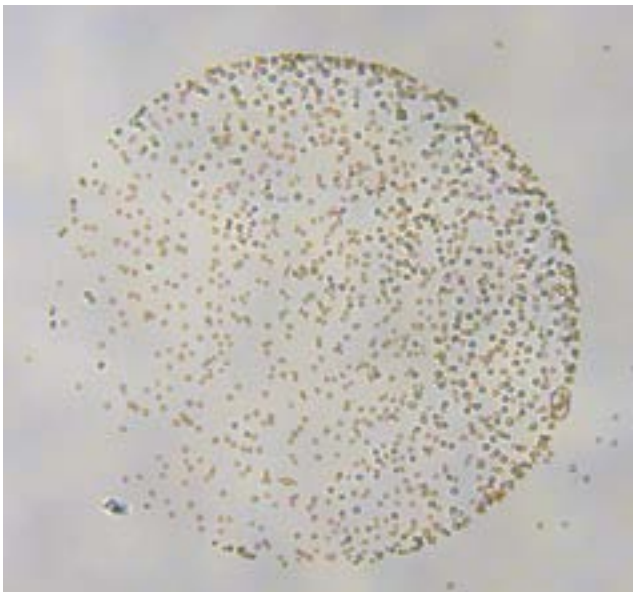
Cell counts, estimates in net tow.

SoundToxins action level

Report any presence of this organism in the net tow.



A. Non-motile stage *Phaeocystis globosa* cells under light microscopy.



B. *P. globosa* cells forming a spherical shaped colony. SoundToxins, WSG.

D. Other

Mesodinium rubrum

A photosynthetic, ciliate organism with cosmopolitan distribution from tropical to cold, brackish to marine waters.

Identification

Ovoid cells that are 10–70 µm long and 7–20 µm wide, with a constriction about midway through the cell from which many cilia emerge. From the side, two lobes are visible with the cilia merging from the middle constriction. From the top or bottom view, it will look circular with cilia forming a ring around the entire circumference. The cilia allow this organism to move very quickly, jumping up to 10–20 times its body length in one movement. It may also appear to spin from the top view. The cell appears red to brown due to its chloroplasts.

Toxicology

This species is not known to be toxic, but is bloom-forming and prey for some *Dinophysis* species.

Regional species

Mesodinium rubrum. Formerly known as *Myrionecta rubra*.

Vectors

None.

Local distribution

Pacific coast (California to Alaska) and inland waters of Puget Sound. In bloom form can turn the water a deep red color.

Monitoring

Weekly from March through October and every other week from November through February.

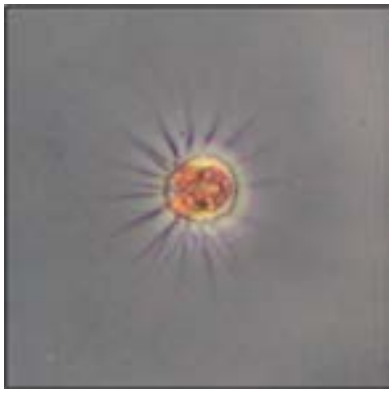
Analyses

Cell counts in net tow.

SoundToxins action level

Report any presence of this organism in the net tow.

A. *Mesodinium rubrum* under microscopy from lateral view.



B. *M. rubrum* under light microscopy from apical view. Brian Bill, NOAA

References

Anderson, D.M. 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. Nato ASI Series G Ecological Sciences, pp. 41, 29–48.

Hasle, G.R. 1972. The distribution of *Nitzchia seriata* Cleve and allied species. Nova Hedwigia, pp. 39, 171-190.

Horner, R.A. 2002. A Taxonomic Guide to Some Common Marine Phytoplankton, Biopress Ltd., pp. 69, 114–117, 120, 143, 168.

Kim, J.H., Tillmann, U., Adams, N.G., Krock, B., Stutts, W.L., Deeds, J.R., Han, M.S. and Trainer, V.L. 2017. Identification of Azadinium species and a new azaspiracid from Azadinium poporum in Puget Sound, Washington State, USA. Harmful Algae, 68, pp.152-167.

King, T.L., Nguyen, N., Doucette, G.J., Wang, Z., Bill, B.D., Peacock, M.B., Madera, S.L., Elston, R.A. and Trainer, V.L., 2021. Hiding in plain sight: Shellfish-killing phytoplankton in Washington State. Harmful Algae, 105, p.102032.

Tomas, C.R. 1997. Identifying Marine Phytoplankton, Elsevier.

Introduction to SoundToxins Protocol

This section provides step-by-step instructions for procedures ranging from the collection of your phytoplankton sample, sample archiving, alerting the SoundToxins notification system, and entering observations and data into the SoundToxins database. It also includes information on sample processing, phytoplankton calculations, Palmer-Maloney slide usage, and how to determine community composition. These protocols have been approved by the Washington State Department of Ecology under the quality assurance project plan "SoundToxins Partnership – HABs Monitoring." As always, if you need additional assistance, please email soundtox@uw.edu and we will be happy to help.

1. SoundToxins field sampling protocol

Field Measurements and Sample Collection

FIELD SUPPLIES

- Bucket
- 2-liter plastic bottle
- Glass jar
- Plankton net
- Refractometer
- Sampling data sheet
- Thermometer

AIR TEMPERATURE

- Hold the thermometer by the top in the open air for one minute.
- Record the temperature. If there is a weather station at your location, please refer to station data.

WIND

Use the wind scale on the data sheet to record how strong the wind is blowing (none, light, moderate or strong). If there is a weather station at your location, please refer to the station data.

TIDE

- Look at a local tide chart to determine what the tide level was at the time the plankton sample was taken.
- Record the time, date and sample location.

WHOLE-WATER COLLECTION,WATER TEMPERATURE, AND SALINITY

- Plunge the bucket through the surface of the water to fill to collect a whole water surface sample.
- Place the thermometer in the bucket of water for one minute. Record the temperature.
- Using a pipette, place a few drops of whole water in the viewing chamber of the refractometer. Close the viewing chamber. Look through the eyepiece to find where the blue and white colors meet. This color intersect is the salinity value.
- Fill the 2-liter plastic bottle to be used for cell counts, toxin, nutrients, and chlorophyll samples.
- Rinse the bucket, thermometer and refractometer with fresh water after each sampling event.

VERTICAL NET-TOW COLLECTION

- Let the net drop vertically through the water column, stopping when the cod end reaches near the bottom. Pull up at a steady pace (about 1 m/second) and note the depth of the sample by counting the meter marks on the net tow line from the top of the net to the point on the rope that reached the surface of the water.
- Pull the net through the water column up to three times. If color is seen after the first or second tow, stop. Note the total distance in meters that the net was towed (i.e., three tows at 4 m each equals a total distance of 12 m).
- Carefully transfer the cod end volume into a glass jar, being careful when swirling to mix contents for a complete transfer. You will need to measure this volume in the lab.
- Clean the net by rinsing it in freshwater. Hang the net up to dry, out of direct sunlight.

2. SoundToxins sample processing protocol and how-tos

Lab Supplies (All-Sites)

- Net tow sample
- Palmer-Maloney slide
- Formaldehyde fixative
- Glass test tube
- Whole water sample
- Pipette
- Scintillation vials
- Graduated cylinder

Additional Lab Supplies (Site-Specific)

- Forceps
- Swinnex filter holder
- Plastic bottle
- Pump
- 47 mm Millipore HA filters
- GF/F filters
- Syringe
- Aluminum foil
- Filter cup and flask
- Centrifuge tube

All-Site Net Tow Processing

PHYTOPLANKTON SAMPLE

(Species composition, cell counts, relative abundance)

- Use a graduated cylinder to measure the cod end contents.
- Invert the live net tow sample gently to mix. Pipette 0.1 mL into Palmer-Maloney cell.
- Record relative abundances of species on the data sheet.

SPECIES COMPOSITION, RELATIVE ABUNDANCE

(sample archive)

- Invert the live net tow sample to mix and pour an aliquot into a 20-mL scintillation vial.
- Add 1 mL of formaldehyde fixative, cap, and invert to mix.
- Label the cap with site name, date, and "NET."
- Store vials in a tray.

Use the net tow sample to count *Akashiwo*, *Alexandrium*, *Dinophysis*, *Phaeocystis*, and *Protoceratium.* Report any *Alexandrium* found. The first time any of the others appear in your sample, please report to soundtox@uw.edu as well as in your database entry. From then on report any population increases from the prior week. Also alert if any of the following appear in the net tow: *Chaetoceros concavicornis*, *Azadinium*, *Karenia mikimotoi*, *Lingulodinium polyedra, and Margalefidinium polykrikos*. Taking pictures of organisms from multiple angles is encouraged for identification confirmation, and can be emailed to soundtox@uw.edu

All-Site Whole-Water Processing

PHYTOPLANKTON SAMPLE

Invert 2-liter bottle to mix. Pipette 0.1 mL into Palmer- Maloney cell.

Use the whole-water sample to count *Heterosigma* levels. Report any *Heterosigma* found in your sample to *soundtox@uw.edu* and in your database entry.

10X PHYTOPLANKTON SAMPLE

(sample archive)

- Invert 2-liter bottle to mix and pour a 50 mL aliquot into the glass test tube.
- Add 1 mL formaldehyde fixative, cap, and invert to mix.
- After 24 hours or more of settling without disturbance, carefully aspirate off the top 45 mL of liquid using a pipette. Do not disturb the bottom.
- Transfer the remaining 5 mL of sample to a 20-mL scintillation vial.
- Label with site name, date, and "WW 10x."

Use the whole-water 10x sample to count *Pseudo-nitzschia* levels. If the large-celled species (*Pseudo-nitzschia australis, P. heimii, P. fraudulenta, P. pungens, P. multiseries*=a/h/f/ p/m) exceed 50,000 cells/L or if the small cells (*P. pseudodelicatissima, P. delicatissima, P. cuspidata*=pd/d/c) exceed 1 million cells/L, please report to soundtox@uw.edu and in your database entry.

Site-Specific Processing

CHLOROPHYLL/NUTRIENT SAMPLE

- Using forceps, place a GF/F filter into the Swinnex filter holder and tighten the parts together. Make sure the filter does not get bunched up in the threads.
- Take the plunger out of the syringe and screw the Swinnex filter holder onto the end of the syringe.
- Invert the 2-liter bottle of whole water to mix.
- Measure 50 mL of whole water from the 2-liter bottle with a graduated cylinder (or pour water into a syringe up to the 50 cc line).
- Rinse the nutrient bottle/tube by using the plunger. Filter a small amount of water into the plastic bottle/ tube to rinse. Repeat the rinse step two more times.
- Filter the remaining water into the rinsed plastic bottle/tube for nutrient sample (bottle/tube should be no more than ¾ full as it will expand when frozen).

- Record the nutrient bottle number and the amount of water filtered into the database.
- Place the nutrient sample bottle/tube upright in the freezer. After frozen, samples can be placed in large plastic bags labeled with site name, approximate date range, and "NUTRIENTS."
- Remove the GF/F filter from the Swinnex holder using forceps, fold the filter in half with plankton on the inside, place in a square of aluminum foil, and fold into a packet.
- Label with site name, date, and "CHL" to indicate chlorophyll.
- Put foil packet in a plastic bag labeled with site name, approximate date range and "CHLOROPHYLL."
- Store samples in a freezer. Extracted chlorophyll filters are shown to have high stability for three months (Wasmund et al. 2006). Water for nutrient analysis may be processed within one year of collection. Nutrient samples are shown to have the following percent difference values (versus freshly analyzed samples) per Macdonald & McLaughlin (1982): phosphate 2–7%, nitrate 1.5–3%, silicic acid 0.9–1.7%.

PARTICULATE TOXIN SAMPLE (CELLULAR TOXIN)

- Set up the pump, filtering flask, and filter cup with a 47 mm Millipore HA filter (be sure to remove the blue paper between the filters).
- Gently invert the 2-liter bottle to mix.
- Measure 1-liter of water with a graduated cylinder and pour into the filter cup to filter. Keep adding water to the filter cup until the entire 1-liter is filtered. If phytoplankton is dense and filtering is slow, you may use up to 3 HA filters for this sample.
- With forceps, remove the filter, fold in half, and place all filters into an aluminum foil packet.
- Record the volume of water filtered in the database.
- Label foil with site name, date, and "pTOX" to indicate particulate toxin.
- Put foil packet in a plastic bag labeled with site name, approximate date range, and "pTOX".
- Store samples in a freezer. When high numbers of Alexandrium or Pseudo-nitzschia are reported, filters may be analyzed within 1 month. Other samples preceding or following these HAB events may be analyzed within 1 month to a year to determine the timing of maximum toxin levels associated with the outbreak. Particulate domoic acid stored at −20°C is stable for up to 1 year, with less than 20% degradation. Ideally, however, stored samples should be analyzed within 2 months. Filters and dissolved extracts may be analyzed for saxitoxins using the method described in Lefebvre et al. (2008). Storage of samples containing saxitoxins at −20°C show stability for 6 months with ~20% loss at 1 year (Alfonso et al. 1994).

DISSOLVED TOXIN SAMPLE (TOXIN IN SEA WATER)

- Pipette 1-2 mL of filtered water from the filtering flask into a small centrifuge tube.
- Label the tube with site name, date, and "dTOX" to indicate dissolved toxin.
- Place in a centrifuge tube storage box labeled with site name, appropriate date range, and "dTOX."
- Store in a freezer. Dissolved domoic acid is stable for 1 year at −20°C with no detectable degradation (Baugh et al. 2005). Saxitoxins are water-soluble and very heat- stable, and so are not destroyed by cooking shellfish that contain them. Filters and dissolved extracts may be analyzed for saxitoxins using the method described in Lefebvre et al. (2008). Samples containing saxitoxins stored at −20°C show stability for 6 months with ~20% loss at 1 year (Alfonso et al. 1994).

CLEANING LAB GLASSWARE AND SAMPLING GEAR

- Rinse all lab glassware and sampling gear three times with freshwater.
- Set out to air-dry

References

Alfonso, A., Louzao, M.C., Vieytes, M.R., Botana, L.M. 1994. Comparative study of the stability of saxitoxin and neosaxitoxin in acidic solutions and lyophilized samples. Toxicon. 32(2):1593-1598.

Lefebvre, K.A., Bill, B.D., Erickson, A., Baugh, K.A., O'Rourke, L., Costa, P.R., Nance, S. and V.L. Trainer. 2008. Characterization of dissolved and particulate saxitoxin levels in both field and cultured *Alexandrium* samples from Sequim Bay, WA. Marine Drugs 6:103-116.

Macdonald, R.W., McLaughlin, F.A. 1982. The effect of storage by freezing on dissolved inorganic phosphate, nitrate and reactive silicate for samples from coastal and estuarine waters. Water Research 16:95-104.

Wasmund, N., Topp, I., Schories, D. 2006. Optimising the storage and extraction of chlorophyll samples. Oceanologia 48(1):125-144.

How to calculate the volume of water filtered through the net

To calculate the approximate volume of water filtered through a vertical net tow, we can use the equation for the volume of a cylinder, with the mouth of the net as the base, and the length of the net tow as the height.

The area of the mouth of the net is calculated with the equation for the area of a circle.

$$Area = \pi * r^2$$

Plankton nets are typically sized by their diameter, so we need to divide by 2 to get the radius. We will also convert all units to meters.

Example: Your net has a 25 cm diameter:

$$Net\ mouth\ diameter\ (d) = 25\ cm * \frac{1\ m}{100\ cm} = 0.25\ m$$

$$Radius\ (r) = \frac{d}{2} = \frac{0.25\ m}{2} = 0.125\ m$$

$$Area\ of\ mouth\ of\ net\ (A) = \pi r^2 = 3.14 * 0.125^2 = 0.049\ m^2$$

Note that nets have different mouth diameters. Measure your net to be sure and adjust the calculations if necessary.

Next, we calculate the volume filtered by multiplying the area of the mouth of the net by the total length of the tow. Make sure to multiply the length of the net tow by the number of times you towed your net for the total volume.

$$Total\ length\ (l) = tow\ length * number\ of\ tows$$

$$Volume\ filtered\ (V) = A * l$$

Example: You tow a 25 cm diameter net 2 times through 5 m of water.

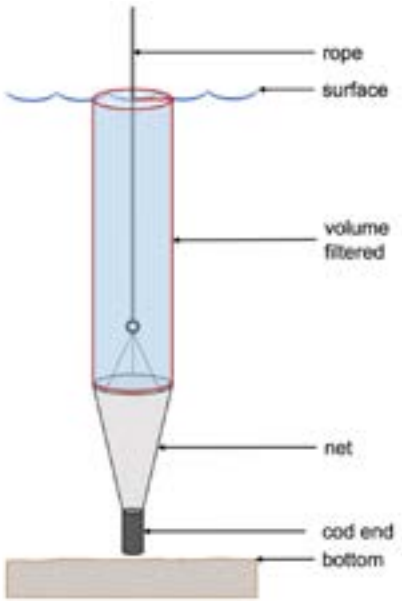
$$l = 5\ m * 2 = 10\ m$$

$$V = A * l = 0.049\ m^2 * 10\ m = 0.49\ m^3$$

Convert m^3 to liters (1 m^3 = 1,000 L).

$$V = 0.49\ m^3 * \frac{1000\ L}{1\ m^3} = 490\ L$$

Record total volume filtered on datasheet and use it to calculate semi-quantitative cell counts from net tow material.



How to calculate semi-quantitative cell concentration from net tow material

Our goal is to calculate the concentration of each type of plankton in the water column in terms of the number of cells per 1L of water from the number observed in the Palmer-Maloney counting chamber (see below). This semi-quantitative calculation requires that we know the volume of water filtered through the net, the number of cells counted, the volume of the Palmer-Maloney slide, and the volume of water in the cod end.

Start with the number of cells counted in the Palmer Maloney slide, the volume the slide chamber holds and the volume of the cod end to get the total number of cells in the cod end.

$$\frac{\#\ cells}{in\ cod\ end} = \frac{\#\ cells\ in\ slide}{slide\ volume(ml)} * cod\ end\ volume\ (ml)$$

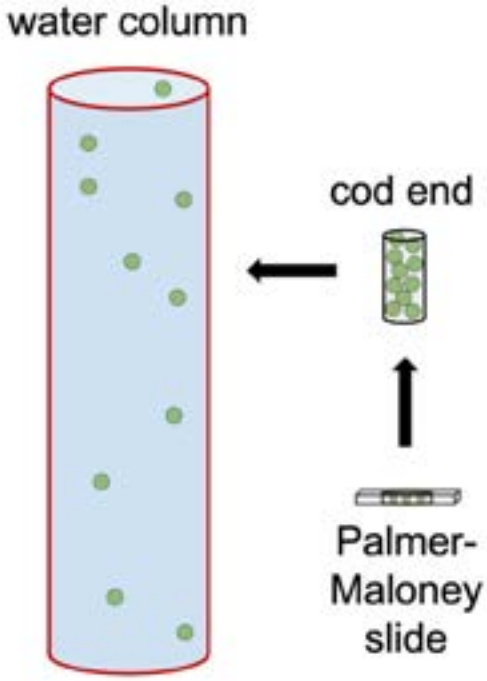
From the total number of cells collected in the cod end, we can find the concentration of the water column by dividing by the volume filtered through the net.

$$concentration = \frac{\#\ cells\ in\ cod\ end}{volume\ filtered\ (V)}$$

Example: You count 10 cells in a 0.1 mL Palmer-Maloney slide. The cod end volume was 250mL and the volume filtered was 490L.

$$\#\ cells\ in\ cod\ end = \frac{10\ cells}{0.1\ ml} * 250\ ml = 25000\ cells$$

$$concentration = \frac{25000\ cells}{490\ L} = 51\ \frac{cells}{L}$$



How to use a Palmer-Maloney slide

Adapted from A Taxonomic Guide to Some Common Marine Phytoplankton by Dr. Rita A. Horner

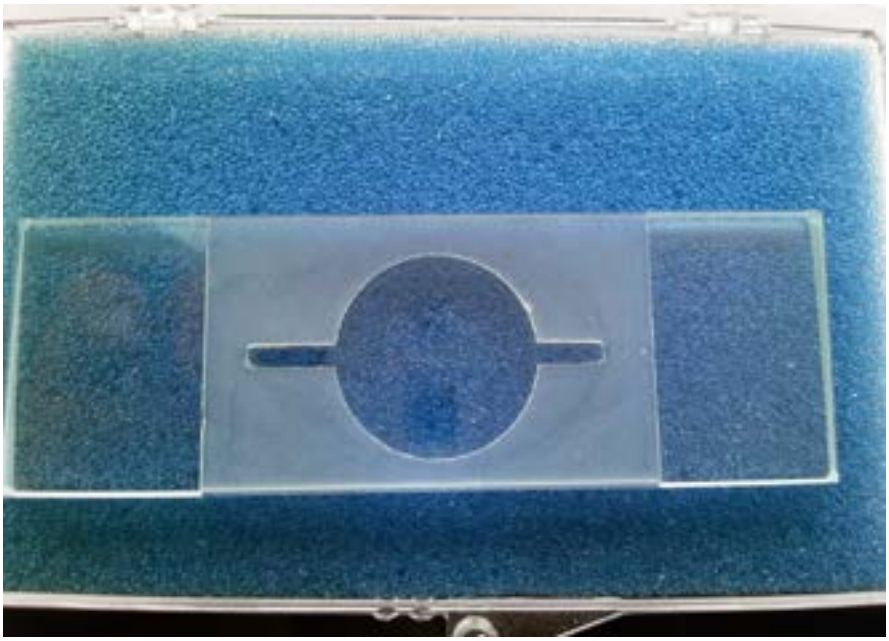
A typical Palmer-Maloney slide has a volume of 0.1 mL and a loading slot on each side. Some slides may have different volumes so be sure to double check the volume listed on your slide.

To use:

- Gently invert the sample bottle to mix before extracting the sample to count
- Put a cover slip (22 mm, No.1) over the ring and tilt the slide slightly while holding the coverslip in place.
- With a pipette, add the 0.1 mL sample to the lower of the two loading slots.
- Be sure there are no bubbles.
- To count cells, make sure to start at one "corner" of the slide and then move the slide horizontally.

A slightly longer cover slip, 22x30 mm, covers both loading slots and minimizes sample evaporation during counting.

This slide is thicker than a standard microscope slide and cover slip, but 40x objectives can be used with it.

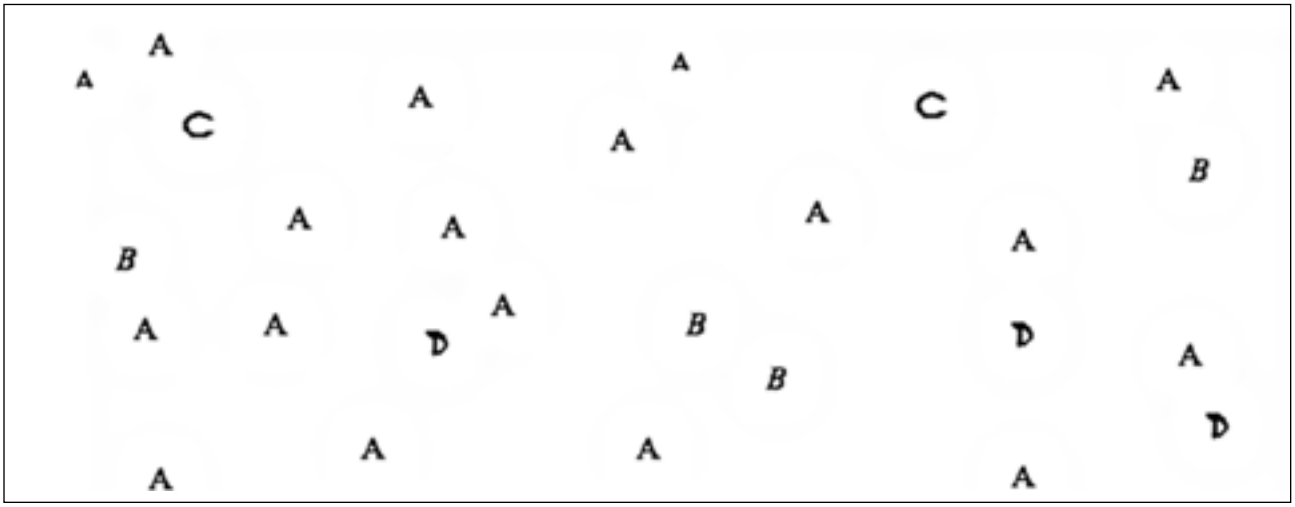


How to determine relative abundance

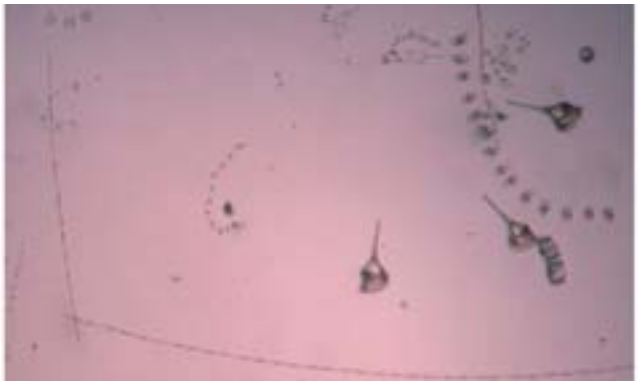
Relative abundance is a measurement of community composition. Understanding and recording community composition is important as the biodiversity of a system can tell us about its health, and the composition of species in a system can help us predict blooms. For example, a varied and well-balanced system may be less likely to have major blooms, as there is more competition for nutrients in the water column, and the abundance of an organism such as *Mesodinium rubrum*, which is a known prey item for some *Dinophysis* species, may indicate a future bloom of *Dinophysis*.

When determining relative abundance we are determining what is the dominant species present in the sample. Using the A, B, C, D example below, which letter is most abundant? There are a lot of A's, more than there are B, C, and Ds. Letter A is categorized as blooming because it is the dominant letter in the example. When you look at letters B, C and D, there are four B's, two C's and three D's. None of the B, C and D letters are considered common or abundant when the sample is looked at as a whole, so they are categorized as present. If the phytoplankton species seem uniform in low numbers or there is only one of them, they are categorized as "present."

Using the two images of plankton below and using the logic presented above, what would you call the photographs?

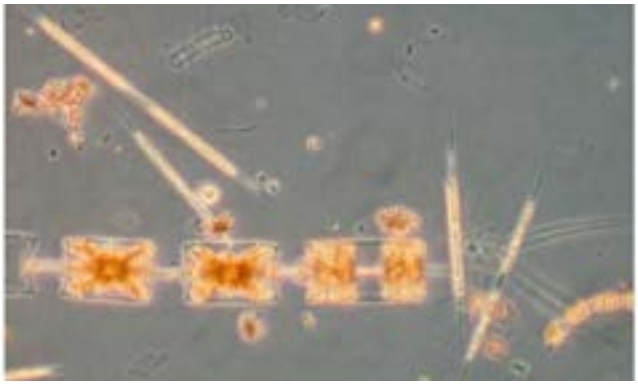


Bloom: Species A
Present: Species B, C, and D



Common: *Pseudo-nitzschia*

Present: *Ceratium, Thalassiosira, Chaetoceros, Ditylum, Skeletonema, Dactyliosolen*



Common: *Rhizosolenia*

Present: *Ditylum, Pseudo-nitzschia, Chaetoceros, Hemiaulus*

3. SoundToxins Data Entry Protocol

The SoundToxins database allows users to view/enter sampling events and species data. Each sampling event is considered a visit (date/time, water temperature, etc.). Every visit can be associated with multiple genera/species observations data (cell count, relative abundance, etc.). SoundToxins data is observed on a daily basis to track when and where harmful algal bloom species are occurring. Below are the steps to enter data in the application.

LOGIN

- Go to www.soundtoxins.org.
- Click "Data."
- Enter Username/Password.
- By entering the database you are agreeing to the database user agreements listed as follows:
  - I acknowledge that the data contained within this website is subject to change as people make edits.
  - I understand that the database, username and password are not to be distributed.
  - I understand that the data entered here is privately collected and are for partner use only.
  - As a partner, I shall contact the SoundToxins program director at Washington Sea Grant prior to publication of any analyses using any data from this site to review acceptable use of the data.
  - By signing into this database, you agree to the terms and conditions.
- Click "Login."
- To request or renew a password, send an email to soundtox@uw.edu.

SITES

A list of sampling sites is displayed along with a map of where the sites are located. The sites that you have permission to enter data for have an "Add/Update Visits" button to the left of the site name. If you do not see an "Add/Update Visits" button on any site, that means you only have view permissions for the application (meaning you can only see the data but not add to it or modify it).

For entering data purposes, click the "Add/Update Visits" button of the site where you want to enter data.

VISITS

A list of visits will be displayed for the chosen site. Each sampling event corresponds to a visit.

CREATING A NEW VISIT

- Click the "New Visit" button.
- Enter the visit data.
- Complete the Sampling Date/Time field.
- Click the "Create" button on the bottom right region of the page. Once a visit is created, you will be able to enter genus/ species observations associated with the particular visit. The seven required genera will now be visible in the Required Observations region in the middle (covered later). For additional genera/species observations, click the "Additional Observations'' button at the bottom right (covered later). The final section is for Bloom Observations. Please pick the genus from the drop-down menu and note what species is blooming at this site. If there is nothing blooming, please pick the genus "None." If the species blooming is one of the seven required species, do not complete the bloom section of the form.
- Between each page you navigate to, click the "Save" button on the bottom right of the page to ensure your data is recorded.

EDITING AN EXISTING VISIT

- Click the "Add/Update Visits" button of your site that you wish to edit.
- Click on the Date/Time in the first column of the visit you want to edit.
- Make any changes to the visit data.
- Click the "Save" button on the bottom right region of the page. NOTE: always click "Save" before navigating to another page to ensure that the data you entered was properly recorded.

REQUIRED OBSERVATIONS

- In the top box, enter the following required data: weather, air and water temperature, salinity, tide, wind, meters towed, and cod end volume.
- The second box is for plankton data. Seven genera — *Akashiwo, Alexandrium, Dinophysis, Heterosigma, Phaeocystis, Protoceratium,* and *Pseudo-nitzschia* — are listed as required. To add detailed data, click on the "Cell Counts" box for each genera one at a time.
- On the "Update Main Species Data" for each of the required genera, the species and cell counts are required and note the water source the count was derived from. You can also add the relative abundance. (To know which water source you should be counting from, check the species description pages earlier in this manual.)
- For the *Dinophysis* genus, please list the specific species observed and the percent abundance, if possible. For each additional species you would like to add, click the "Add Row" button and use the drop down menus to select the species. Please reach out if you need assistance recognizing species.
- For the *Pseudo-nitzschia* genus, approximate the percent of small to large cells observed.
- Click the "Save" button on the bottom right of each page before navigating away to ensure your data is saved.
- To go back to the main visit page, either select the "back" button after saving, or use the back arrow on your browser.

ADDITIONAL OBSERVATIONS

- Click the "Additional Observations" button to add the additional genera observed.
- Use the drop-down menus to select a "Genus," a "Species" (if possible), and the "Relative Abundance."
- You may also add cell counts or comments for the additional observations if you would like.
- To add additional observations, click the "Add Row" button and continue until you have added all your observations.
- Click the "Save" button before leaving the page or your observations will be lost.
- Note: the database does not like blank lines or duplicates and will give you an error message.
- If you accidentally added a row you do not need, click on the 3 horizontal lines in the second column of the row you wish to delete and click "delete row" from the pop-up menu that appears.

LOGGING OUT

- Click on your login email address on the top right of the site. From the drop-down menu that appears, click on "Log Out."

If there are any problems on the site, contact soundtox@uw.edu.

DATA SECURITY

These data are managed and viewed only by Sound-Toxins volunteers and staff according to our user agreement. You need to change your password every six months. If you do not change it ahead of time, the system will lock you out. Contact soundtox@uw.edu if you are locked out of the system

Marine Phytoplankton of Washington State Coastal Waters

This guide shows common genera of phytoplankton found in Washington's coastal waters, with approximate size ranges. Please note that some of these genera include many different species morphologies that may look different than the images shown.

Diatoms

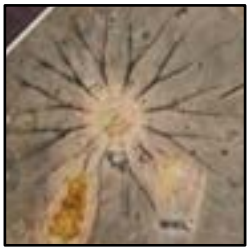
Centric Diatoms

Achtinoptychus
20-150 µm

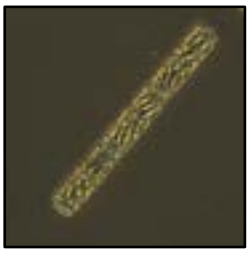
Asteromphalus
42-175 µm

Attheya[1]
18-64 µm

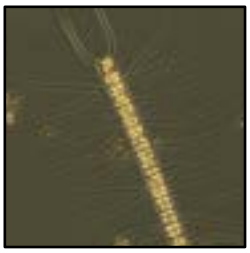
Aulacodiscus[1]
40-100 µm



Bacteriastrum[1]
6-15 µm



Cerataulina
11-36 µm



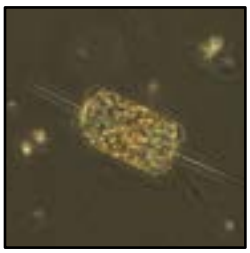
Chaetoceros
8-80 µm

Corethron[1]
20-38 µm

Coscinodiscus
100-500 µm

Dactyliosolen[1]
6-38 µm

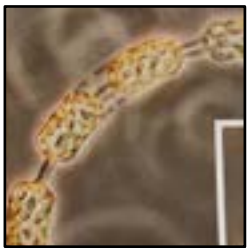
Detonula
16-42 µm



Ditylum
80-300 µm

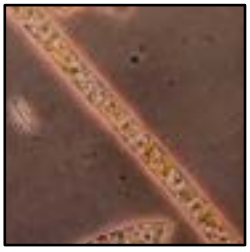
Eucampia
10-61 µm

Guinardia
6-45 µm



Hemiaulus[1]
15-35 µm

Lauderia
24-75 µm

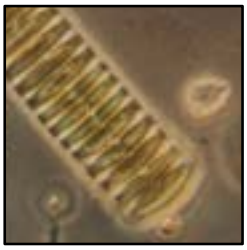


Leptocylindrus[1]
1.5-16 µm

Melosira[1]
17-70 µm

Odontella[1]
10-110 µm

Paralia[1]
8-30 µm



Plagiogrammopsis[1]
3-50 µm

Proboscia[1]
7-18 µm

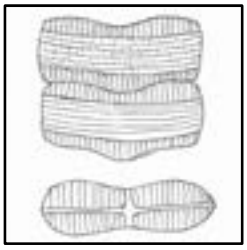
Rhizosolenia
4-70 µm

Skeletonema
2-21 µm

Stephanopyxis
24-71 µm

Thalassiosira
10-186 µm

Pennate Diatoms



Achnanthes
15-70 µm

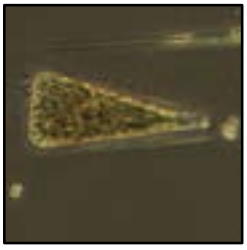
Asterionella
60-85 µm

Asterionellopsis
30-150 µm

Bacillaria
70-150 µm

Cylindrotheca
30-400 µm

Fragilariopsis
50-174 µm



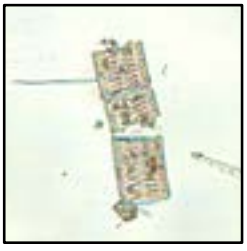
Licmophora
20-60 µm

Navicula
10-65 µm

Nitzschia
3-375 µm

Pleurosigma
90-600 µm

Psuedo-nitzschia
Size



Striatella
35-125 µm

Thalassionema
10-80 µm

Tropidoneis[1]
160-350 µm

Dinoflagellates

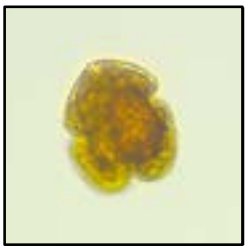
Unarmored Dinoflagellates

Akashiwo
40-80 µm

Amphidinium
10-100 µm

Gymnodinium[1]
75-145 µm

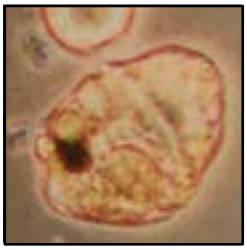
Gyrodinium
40-200 µm



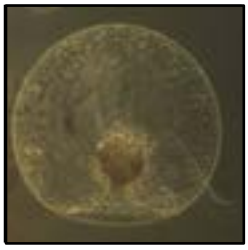
Karenia
100-300 µm

Kofoidinium[1]
100-300 µm

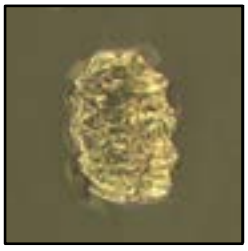
Margalefidinium
26-60 µm



Nematodinium[1]
30-100 µm



Noctiluca
200-2000 µm

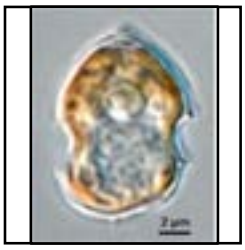


Polykrikos
100-150 µm

Armored Dinoflagellates

Alexandrium
24-50 µm

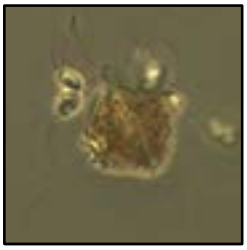
Amylax[1]
42-60 µm



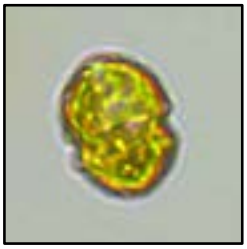
Azadinium
16-20 µm

Ceratium
30-300 µm

Dissodinium[1]
100-140 µm



Gonyaulax
40-75 µm

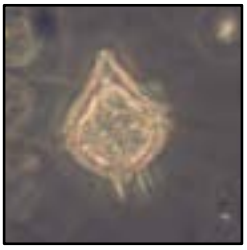


Heterocapsa
16-30 µm

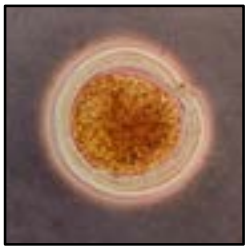
Lingulodinium[1]
42-54 µm

Oxyphysis
60-68 µm

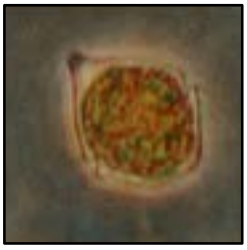
Protoceratium
28-43 µm



Protoperidinium
18-300 µm

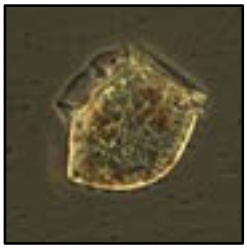


Pyrophacus[1]
35-136 µm



Scripsiella[1]
16-36 µm

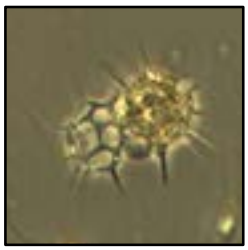
Prorocentrum
35-70 µm



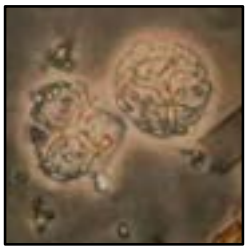
Dinophysis
38-105 µm

Other

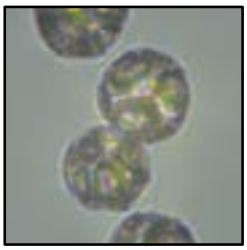
Flagellates



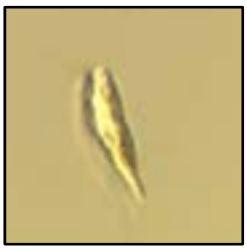
Dictyocha
10-45 µm



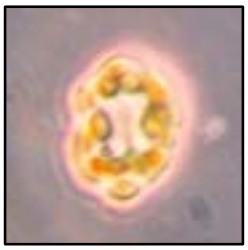
Ebria
30-40 µm



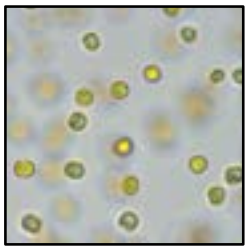
Emiliania
5-10 µm



Euglenoids
12-115 µm



Heterosigma
<25 µm



Phaeocystis
3-8 µm

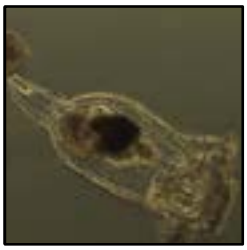
Pyramimonas
4-35 µm

Ciliates

Mesodinium
10-100 µm

Strombidinium
50-100 µm

Tiarina
~100 µm



Tintinnid
~200 µm

Microscopy images and hand drawn images were provided by Teri King and Michelle Lepori-Bui from SoundToxins, Washington Sea Grant unless other wise noted. Images marked with superscript [1] are from A Taxonomic Guide to Some Marine Phytoplankton (2002) by Dr. Rita Horner.

Basic Morphological Terminology of Phytoplankton

BILOBED
Dinoflagellates –
divided into two lobes

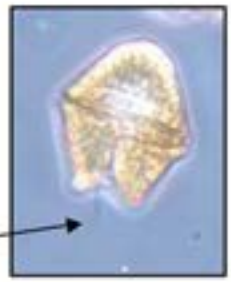
CENTRIC
[TAXONOMIC ORDER]
Diatoms –
valve striae arranged in relation to a point or central areola; often round or circular

CHAIN
Phytoplankton –
of the same species linked together

CHLOROPLASTS
Phytoplankton –
organelles in the cytoplasm that contain cell pigments

EYESPOT
Dinoflagellates –
red spot involved in light perception

FLAGELLA (P)
Dinoflagellates –
whip-like structures used primarily for locomotion



FRUSTULE
Diatoms –
siliceous parts of the cell wall or skeleton

NUCLEUS
Phytoplankton –
organelle in eukaryotic cells containing most of the cell's genetic material

PEDUNCLE
Heterotrophic Dinoflagellates –
mouth used for engulfing food

PENNATE
[TAXONOMIC ORDER]
Diatoms –
longitudinally symmetric

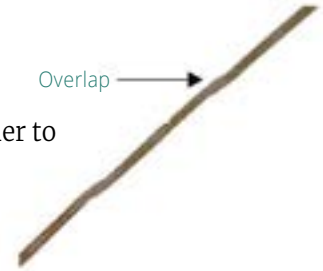
PLATED
Some Dinoflagellates –
armored plates composed of cellulose found in the cell wall

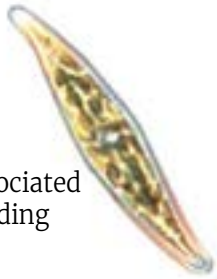
PROCESS

Diatoms –
an oriented projection of a silicate cell wall

STEPPED CHAIN

Diatoms –
organism linked together to form a series of steps





RAPHE

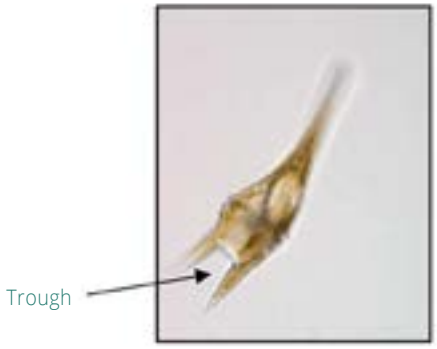
Pennate Diatoms –
longitudinal fissure associated with and involved in gliding locomtion

THECA

Dinoflagellates –
a multiple membrane complex with vesicles and some species with scales, composed of cellulose

SEGMENTED

Diatoms –
separation of the main body into sections, may be equal or unequal



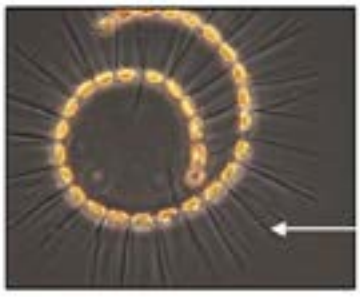
TROUGH

Dinoflagellates –
depression in the main body of the cell

SPINES

Diatoms –
closed or solid structures projecting from the cell wall

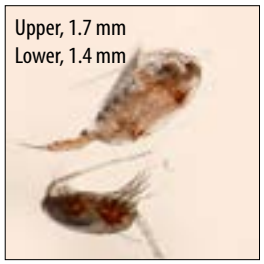
Dinoflagellates –
solid protuberances that usually taper to a point



This identification card includes most groups of marine zooplankton found in Puget Sound. Size ranges provided are approximate - some specimens may be outside of the stated ranges. Exact measurements for specimens in photos are provided where available.

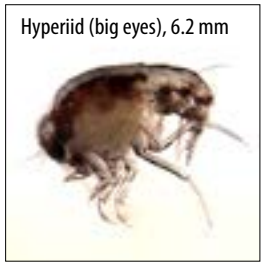
Marine Zooplankton of Puget Sound

Copepods 0.5-5 mm



Cladocerans (water fleas) ~1 mm

Amphipods 0.5-2 mm

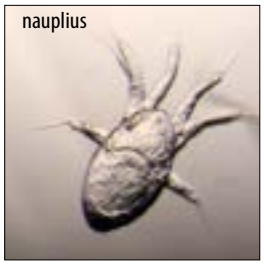


Ostracods 0.5-2 mm

Crab larvae 2-5 mm

Pagurus larvae 1-4 mm

Euphausiids (krill) nauplius ~ 0.5 mm; calyptopis 0.5 mm-1.5 mm; furcilia 2-5 mm; adults 8-15 mm



All photos by Audrey Djunaedi, except *Calanus* provided by Julie Keister; larvacean provided by NOAA Photo Library; fish larva provided by Sarah Norberg, NWFSC, NOAA. Special thanks to Julie Keister and Audrey Djunaedi for advising on content and providing size information.

Barnacles (larvae and adult molt) larvae 0.5-1 mm; molt size varies

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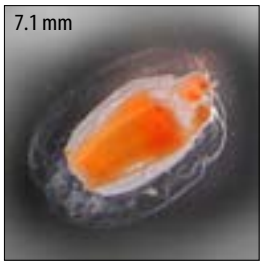
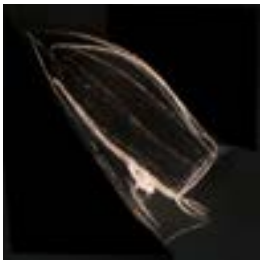
Jellies



Siphonophores 4-8+ mm



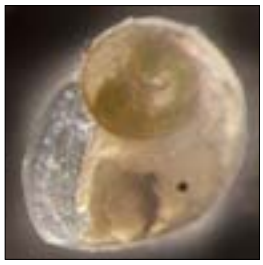
Ctenophores

Polychaete worms 1 mm-50 cm

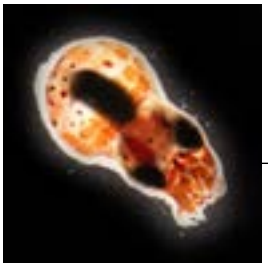
Chaetognaths (arrow worms) 3-40 mm

Pteropods 0.1-3 mm

Gastropod larvae 0.1-0.8 mm

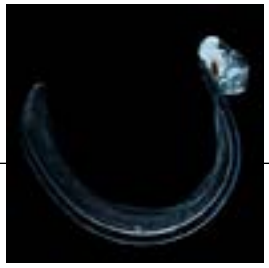


Octopus larvae 3-10 mm



Cyphonautes height ~0.6 mm

Larvaceans 2-4 mm



Fish larvae

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