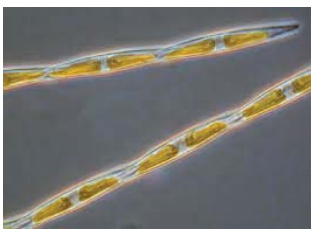
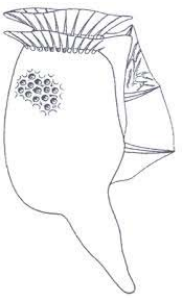
Southeast Alaska Tribal Toxins (SEATT)

A Partnership to Monitor
Harmful Algal Blooms

Sampling Manual





Southeast Alaska Tribal Toxins (SEATT)

Partners

SEATT partners include twelve of the Southeast Alaska Tribes that rely on subsistence shellfish for traditional use. The SEATT monitoring project is part of a large scale climate change research initiative that focuses on the marine environment called **Southeast Alaska Tribal Ocean Research** (www.SEATOR.org). Technical collaborators include the National Oceanic and Atmospheric Administration's (NOAA) Northwest Fisheries Science Center Marine Biotoxin Program and the National Centers for Coastal Ocean Science Marine Biotoxin Program, University of Alaska Fairbanks School of Fisheries and Ocean Science, Alaska Department of Conservation (ADEC), Southeast Alaska Regional Dive Fisheries Association (SARDFA), and Washington State Department of Health Marine Biotoxin Program.

Sampling

Required

- Sample collection is weekly from March through October and every other week November through February (weather and safety permitting).
- Seawater is tested for salinity and temperature.
- Seawater net tow sample is preserved and concentrated for identification and cell counts.
- Plankton diversity (on a relative abundance scale) is described.
- Whole water is collected and filtered for particulate toxin and sent to the Sitka Tribe's (STA) Biotoxin Lab for analysis.
- Data are entered into the SoundToxins database via SEATOR website on a weekly basis.

Optional

- Cell abundance (via cell counting) is determined for *Pseudo-nitzschia* spp., *Alexandrium* spp., *Dinophysis* spp. and *Heterosigma* spp.
- Shellfish samples are collected and sent to the Sitka Tribe's Biotoxin Lab for analysis.

***See training videos at www.seator.org/training Username: seator Password: katlian429**

Program Goal

The goal of SEATT is to use the data collected to asses each Tribe's vulnerability for human health risks associated with marine toxins and provide the necessary information to all Southeast Alaska Tribes to make adaptive management decisions regarding their cultural and natural resources. SEATT aims to provide a sufficient early warning of HAB events and sound regulatory data that can be used to develop subsistence management plans.

Funding

EPA Indian General Assistance Program, ANA Environmental Regulatory Enhancement, BIA Climate Change Program.

Southeast Alaska Tribal Toxin (SEATT) Contact Sheet

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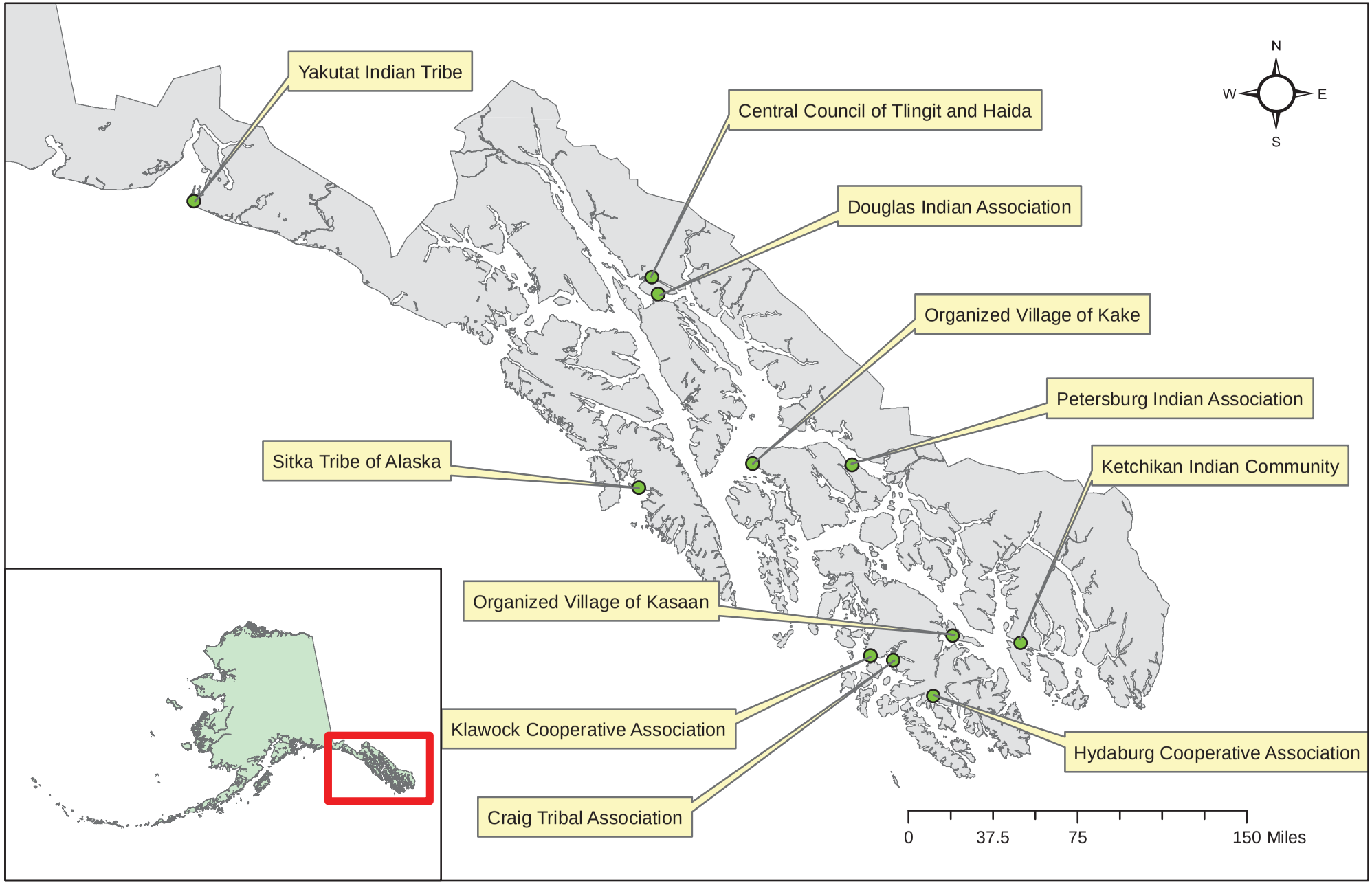
Southeast Alaska Tribal Toxins (SEATT) Partner Locations

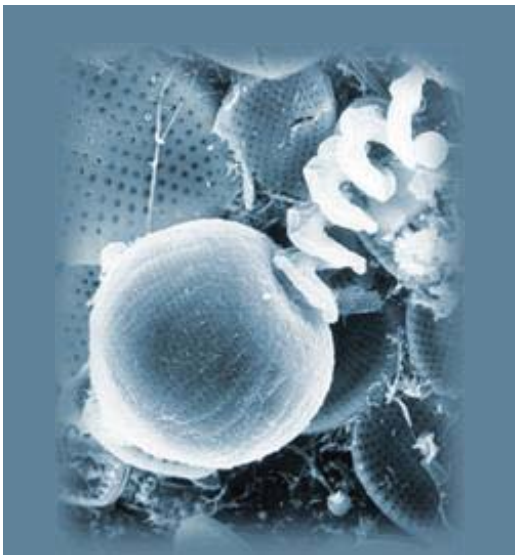


Phytoplankton- What are they?

Phytoplankton

Marine algae come in a variety of sizes and forms. They range from large sessile plants such as kelp to microscopic in single cells. The small, microscopic plants are often referred to as microalgae or phytoplankton. Phytoplankton literally means 'phyto' = plant and 'planktos' = made to wander. Like terrestrial plants, these organisms contain chlorophyll and need sunlight and inorganic nutrients to grow. Virtually all marine phytoplankton are buoyant and live in the upper part of the water column called the photic zone.

Most importantly, these marine microalgae or phytoplankton, similar to terrestrial plants, use inorganic nutrients, such as nitrate, phosphates, and sulfur, and convert them into the basic building blocks of living organisms--proteins, fats, and carbohydrates. Like other living organisms, they also need trace elements such as silicon, iron, and calcium.



Two broad classes of phytoplankton that are of interest to researchers at the NWFSC are dinoflagellates and diatoms. The dinoflagellates typically have a flagella or whip-like tail that can move them through the water column. They are composed of complex outer shells or armor plating (made of carbohydrate material) and come in a variety of shapes and sizes.

Perhaps the most varied, beautiful and geometrically intricate of all the phytoplankton are the diatoms (see the side bar to the right). Unlike most phytoplankton, these organisms have a rigid silica shell (and require silicate as an essential nutrient) composed of two interlocking parts. In contrast to the dinoflagellates, diatoms do not propel themselves in the water column, but are dependent on oceanic currents for transport. After diatoms die, their silica shells are either solubilized back into the seawater or sink to the bottom and eventually, given eons of time, become diatomaceous earth.

Harmful Algal Blooms (HABs) and Biotoxins

Phytoplankton, or algae, are normal components of all aquatic environments. When they bloom in significant numbers (approximately 1 million cells per liter of seawater equals a "bloom") and produce biotoxins, these events are termed harmful algal blooms or HABs. These blooms can have deleterious effects on both other aquatic life and on those who depend on that water for subsistence. How and why these blooms occur is a complex issue, depending on oceanographic currents, winds, and other factors.

In the marine environment these HABs produce some of the most toxic compounds known to man. In fact, the term Harmful Algal Blooms was initially coined to describe high concentrations

of algae that produce extremely potent poisons. During blooms, fish and shellfish consume these algae, then accumulate and concentrate the biotoxins without apparent harm. This renders the fish and shellfish extremely toxic to whomever consumes them, including marine mammals, sea birds, and humans. In places where HAB monitoring and surveillance programs do not exist, these blooms may go unnoticed until they cause illnesses and/or death in humans who consume products from the sea. The myriad of compounds, that marine phytoplankton can produce are known as marine biotoxins.

HABs can also have less lethal effects that range from noxious odors and aerosols to the production of slimes. In some circumstances, due to coastal wind and wave action, algal blooms will produce components that can be transported through the air, causing severe eye, nose, and throat irritation, much akin to pollen and other plant constituents on land. Many of these effects can have serious economic impacts on communities in coastal areas that depend on marine resources for their livelihood. Here on the west coast, we are plagued with several noxious HABs such as domoic acid, PSP, and Heterosigma fish poisoning

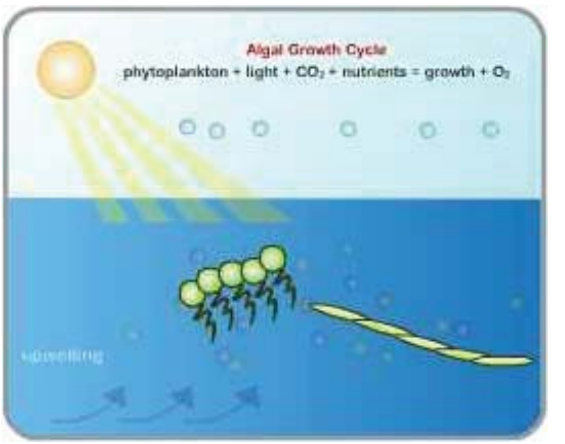
Algal Bloom Dynamics

Adapted from NOAA Northwest Fisheries Science Center
http://www.nwfsc.noaa.gov/hab/habs_toxins/phytoplankton/algal_dynamics.html

Most harmful algal blooms seem to appear from nowhere - it appears that they are suddenly there! In some ways this is true, but in other ways it isn't. First some basic biology about how algae grow.

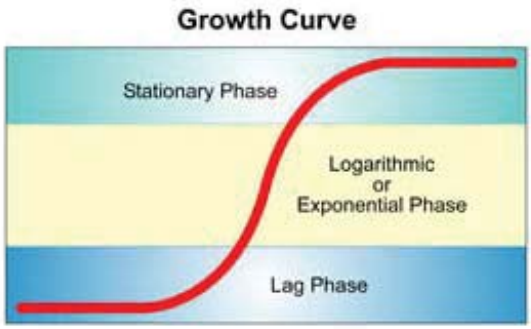
Cell Growth

What causes phytoplankton to grow? It is thought that when the environmental conditions are ideal for the particular organism, cells will begin to grow or divide. Phytoplankton are photosynthetic autotrophs. They only need light and inorganic nutrients such as phosphate (PO_4), nitrate (NO_3), ammonium (NH_4), carbon dioxide or carbonate (CO_2 or CO_4) to grow. They do this through their "chloroplasts", an internal structure that takes the energy of light and powers the synthesis of carbohydrates, proteins, and fats-all the building blocks of life. In addition, they also need very small amounts of certain trace metals such as iron (Fe), zinc (Zn), and perhaps a few others, such as silicate in the diatoms.



Algae, like other microscopic single celled organisms, grow by asexual reproduction (although there are instances where algae can also engage in sexual reproduction - this can have profound impacts on the ultimate survivability of the species). When these organisms divide, a duplicate copy of DNA from the mother cell is present in the daughter cell. Each resulting cell can then go on to divide again, and again, and again, and so on. This is exponential growth. Starting with only one cell, if the cell population from each generation increases by a factor of 2^n (where n is the number of generations), it is clear that after a relatively small number of generations, the number of cells will be very large. In the oceans a generation (doubling time) can range from hours to a few days. Most noticeable algal blooms in the aquatic environment range from 100,000 to 1,000,000 cells per liter.

If we count microorganisms under a microscope we will see that their numbers when plotted against time forms what appears to be an "S-shaped curve". In this curve, during the first stage, growth is slow and is referred to "lag phase"; in the second stage it appears to rapidly speed up and is called "logarithmic or exponential growth"; and then finally growth appears to slow and is said to enter "stationary phase". During stationary phase, it is thought that the number of dividing cells equals the number of dead or dying cells. For some marine phytoplankton that produce marine biotoxins, the bulk of the toxins seem to be produced during this stationary period. For others, toxin production sometimes coincides with log-phase growth.



What stops growth?

Within a confined area of sea water, there are only a finite amount of nutrients available for the phytoplankton. As they take in nutrients and grow, there is eventually a reduced amount of nutrients available to the resulting cells. As nutrients are used up and assimilated into cell tissue, the growth of the cells begins to slow in response to declining nutrients.

Another factor that may also inhibit cell growth is the presence of toxic components in the water. Some of these compounds can be man-made, for example, herbicides from land run-off, or they might be naturally derived compounds from other organisms (bacteria, fungi, other algae) in the water. These other organism may produce some of these compounds naturally.

The production of these control chemical compounds may confer a survival benefit to an organism, allowing it to have a small niche the overall scheme of the water column. As on land, it's a "jungle out there." Organisms are all competing for nutrients and survival. Every now and then, one organism is able to outcompete its neighbors and become the "top dog", which in the case of phytoplankton is what we call a "bloom".

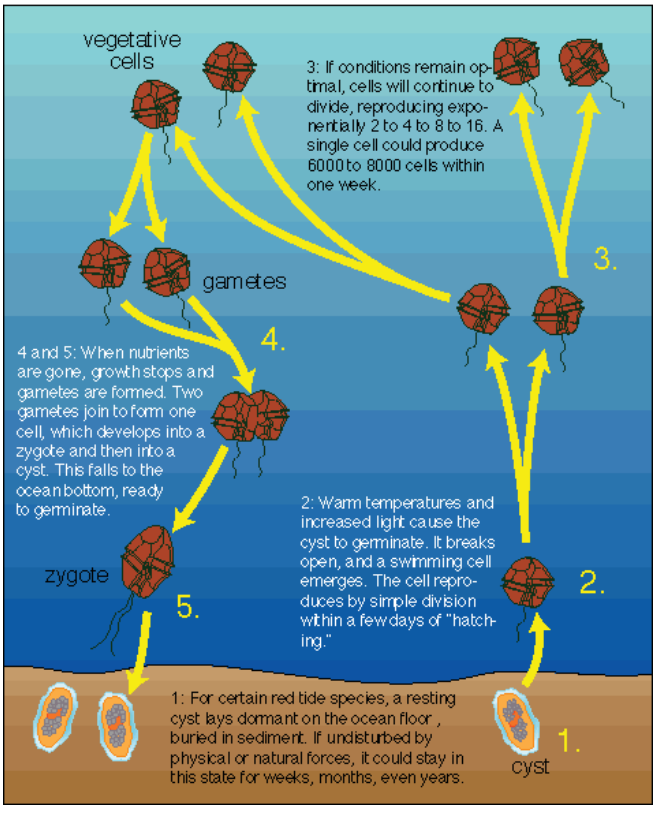
Phytoplankton also have another ability to respond to changes in their environment. When times get tough (nutrient levels are low or detrimental temperature change) they can form cysts. The cyst stage is similar to a "hibernation" or dormant state. When circumstances change, some trigger mechanism can cause the algae to come out of the cyst stage and return to a "vegetative" state, to begin the life cycle over again.

Bloom Movements or Spreading

Sometimes when algae forms cysts, they can be easily transported by both surface and deep currents over long distances. It is thought that this is how noxious phytoplankton have been spread from one location to another. In a special case, the cyst stage can cause worldwide distribution of HABs. For example, harmful algal cysts have been recovered from the bottom of ballast holds in tanks of oceanic freighter ships and shown to produce viable vegetative cells.

Currents in the oceans can arise from different causes. On the surface, winds can move water layers. Slight differences in temperature can cause sea levels to rise or sink, carrying with these currents the phytoplankton. In some instances, phytoplankton in the water can be swept into areas where nutrients are high, for example near coastal upwelling areas, where growth can be stimulated. Most phytoplankton, at least the non-flagellated

How an Algal Bloom Occurs
The Life Cycle of a Single Cell



kind, spend most of their existence on or near the surface and basically drift with currents and tides. The diatom *Pseudo-nitzschia* is this kind of organism. However, some phytoplankton have flagella which allows them to move about in the water column. The phytoplankton *Alexandrium catenella* (the organism responsible for Paralytic Shellfish Poisoning) is such an organism. Many phytoplankton who have this ability also tend to respond to day/night cycles. Usually at night they tend to move down lower in the water column and then during the day they rise up near the surface.

Eventually many phytoplankton run out of nutrients, lose their buoyancy, and become part of oceanic "snow" that slowly falls into the benthic environment. In some cases, it is thought that this might be a way that marine biotoxins (produced perhaps during stationary phase) become introduced into the benthic environment. On the bottom, creatures can then consume this toxic "snow" and accumulate toxins. Either through the active uptake of live, vegetative cells or perhaps withered dead cells, biotoxins can enter the food web.

Bloom Initiation

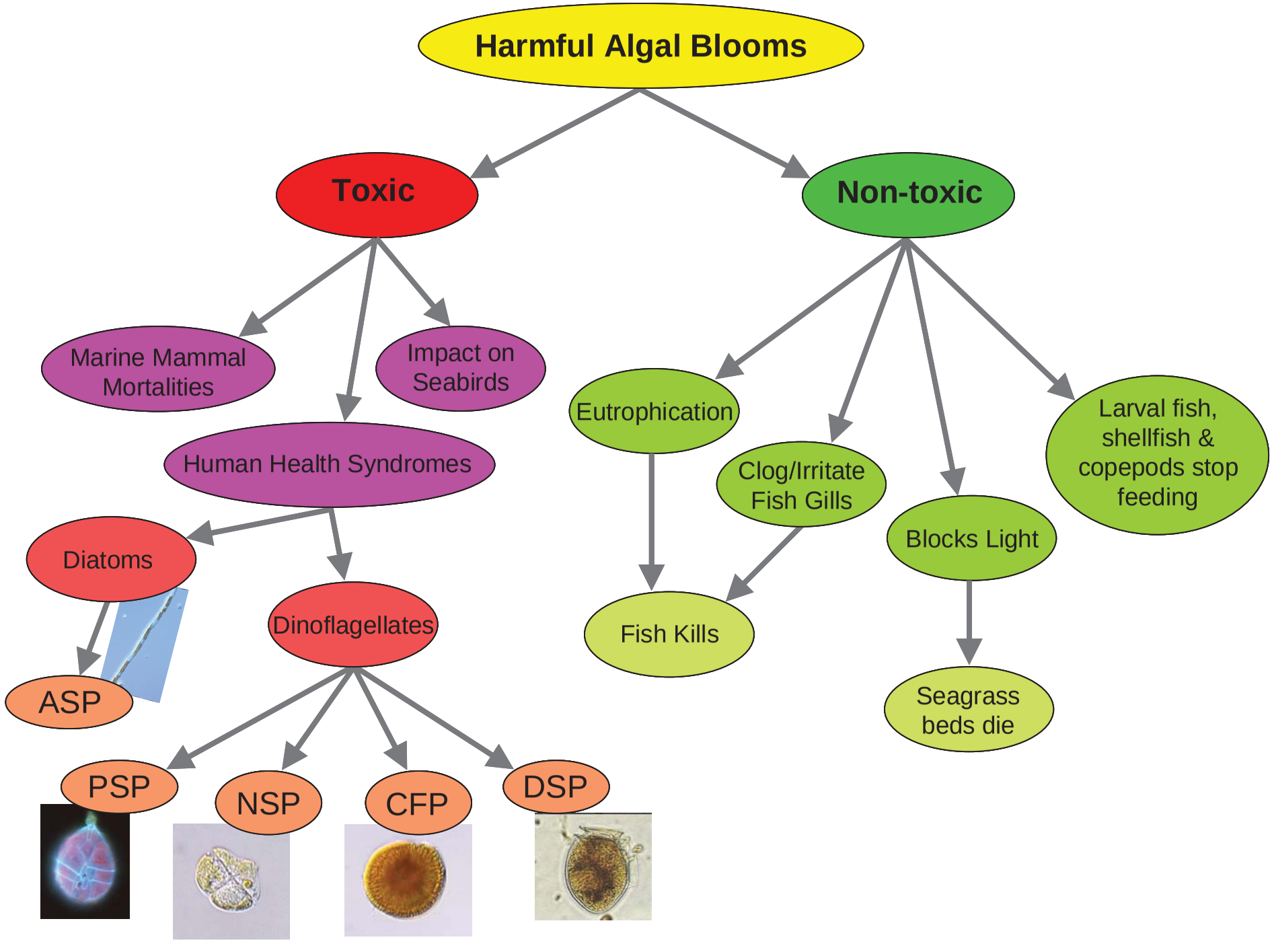
It is generally accepted that bloom initiation is caused by the right set of environmental conditions, i.e., nutrients or sunlight or temperature or a combination of these. These conditions can be provided on a local basis by natural run-off from the land or by human (anthropogenic) inputs (e.g., treated or untreated sewage, farming or urban gardening practices). These initiators appear possible for blooms in local estuarine areas but what drives oceanic blooms? We are now aware of large scale or "global" processes, such as El Nino-Southern Oscillation (ENSO) and Decadal Oscillations. These global processes can drive and cause huge weather and climatic occurrences such as higher than average rainfall (thus increasing runoff) and higher air and hence surface temperatures, all impacting surface and deep currents. These events may all impact the frequency and magnitude of oceanic HABs.

Harmful Algal Blooms

A phytoplankton bloom can be harmful in several ways. Some blooms can produce anoxic (without oxygen) or hypoxic (little oxygen) conditions in the water column. This occurs when one or two species is the dominant organism in a bloom and blocks the sunlight from other organisms in the lower water column. Other plankton begin to die and decompose. Through the process of decomposition, oxygen is used up. Fish die due to lack of oxygen and decomposition continues. Eventually, the massive bloom dies as well, removing any additional oxygen from the water column. This entire process can lead up to a fish and/or shellfish die-off, leading to a negative impact on our economy.

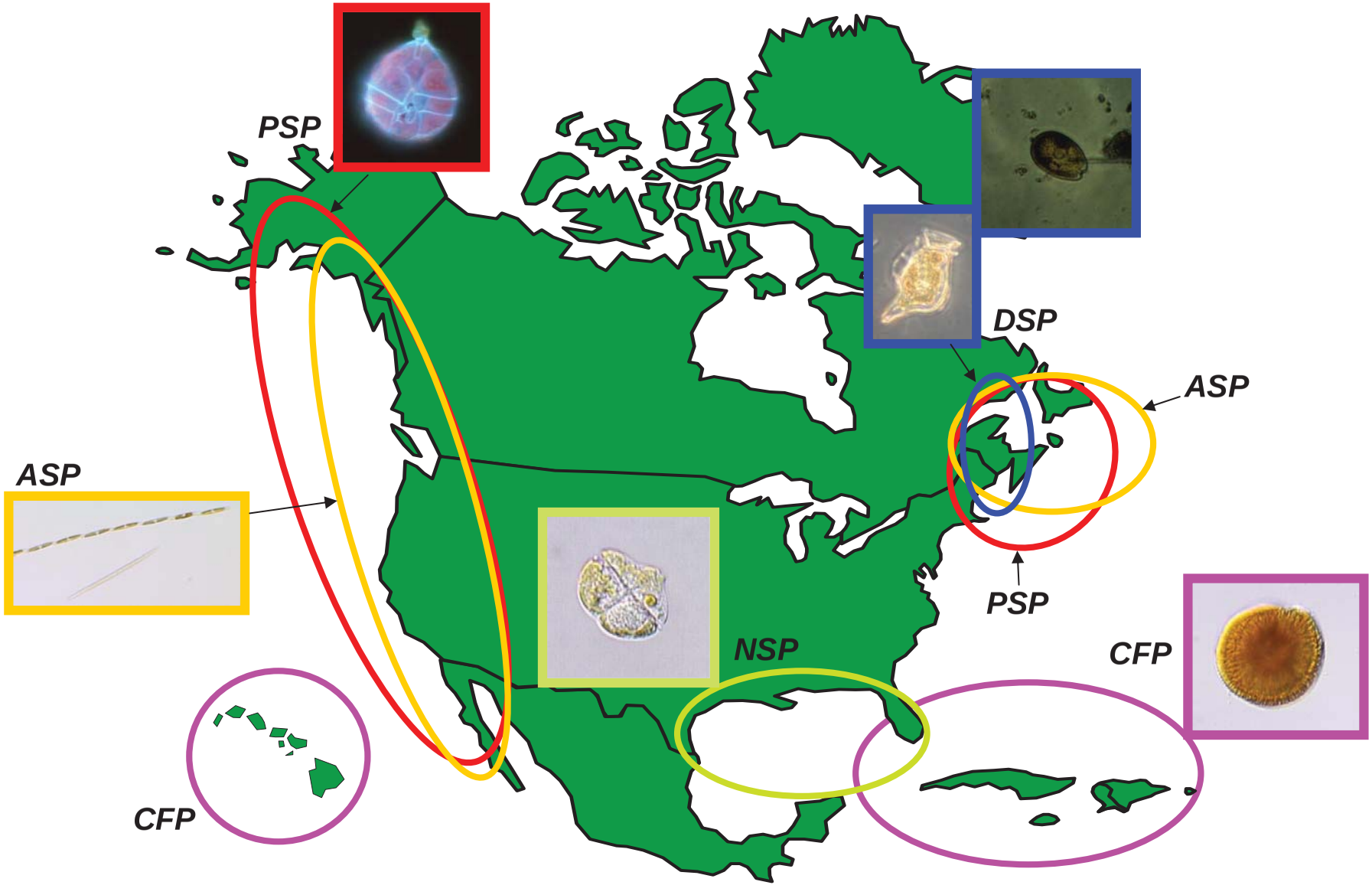
During harmful algal bloom events, there are closures of shellfish beds, lost production in fisheries, and reduction in tourism and associated service industries. Public illness, medical treatment, and advisories cost money. Fisheries-related business close and insurance and unemployment rates increase and public resources are redirected to monitoring programs.

Human health can also be impacted from harmful algal blooms. There are about 50 known species of phytoplankton that produce a toxin. As the toxin moves through the food web, it bioaccumulates in the tissue of large fish and marine mammals. Humans can contract illnesses from eating contaminated shellfish and fish.


Harmful Algal Blooms
Toxic
Non-toxic
Marine Mammal Mortalities
Impact on Seabirds
Human Health Syndromes
Diatoms
Dinoflagellates
ASP
PSP
NSP
CFP
DSP
Eutrophication
Clog/Irritate Fish Gills
Blocks Light
Larval fish, shellfish & copepods stop feeding
Fish Kills
Seagrass beds die

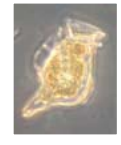
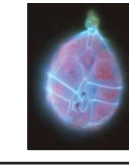
Human Health Syndromes

associated with phytoplankton



Human Health Syndromes

associated with phytoplankton

Name of Syndrome	Species and Toxin	Symptoms
Amnesic Shellfish Poisoning **(ASP)**	*Pseudo-nitzschia* **Domoic acid** 	Short term memory loss
Ciguatera *Fish* Poisoning **(CFP)**	 *Gambierdiscus toxicus* Ciguatoxin & Maitotoxin	Temperature Sensation Reversal
Diarrhetic Shellfish Poisoning **(DSP)**	 *Dinophysis* **Okadaic acid** *Prorocentrum lima* 	Diarrhea Nausea Vomiting
Neurotoxic Shellfish Poisoning **(NSP)**	 *Karenia brevis* Brevetoxin	Respiratory problems
Paralytic Shellfish Poisoning **(PSP)**	*Alexandrium* Saxitoxin 	Loss of motor control

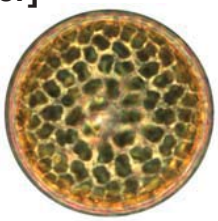
Basic Morphological Terminology of Phytoplankton

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

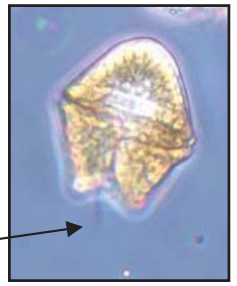
Phytoplankton –
organelles in the cytoplasm that contain cell pigments

Eyespot

Dinoflagellates –
red spot involved in light perception

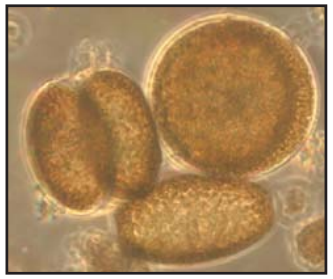
Flagella (p)

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

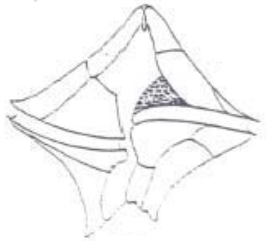
Heterotrophic Dinoflagellates –
mouth used for engulfing food

Pennate [Taxonomic Order]

Diatoms –
longitudinally symmetric

Plated

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



Phytoplankton Monitoring Network

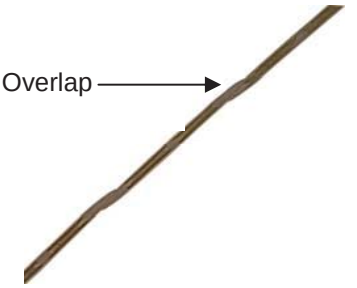
Promoting a better understanding of Harmful Algal Blooms by way of Volunteer Monitoring

Process

Diatoms -
an oriented projection of a silicate cell wall

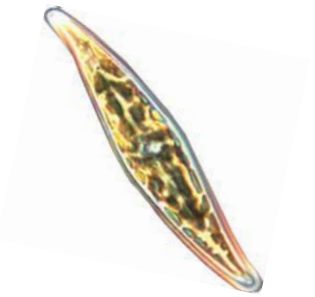
Stepped chain

Diatoms -
organism linked together to form a series of steps



Raphe

Pennate Diatoms -
longitudinal fissure associated with and involved in gliding locomotion



Theca

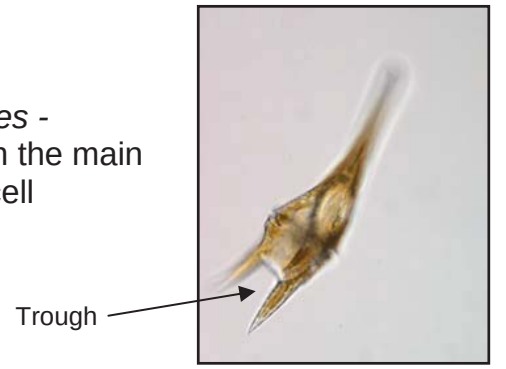
Dinoflagellates -
a multiple membrane complex with vesicles and some species with scales, composed of cellulose

Segmented

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

Trough

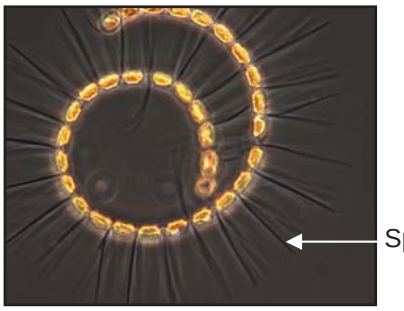
Dinoflagellates -
depression in the main body of the cell

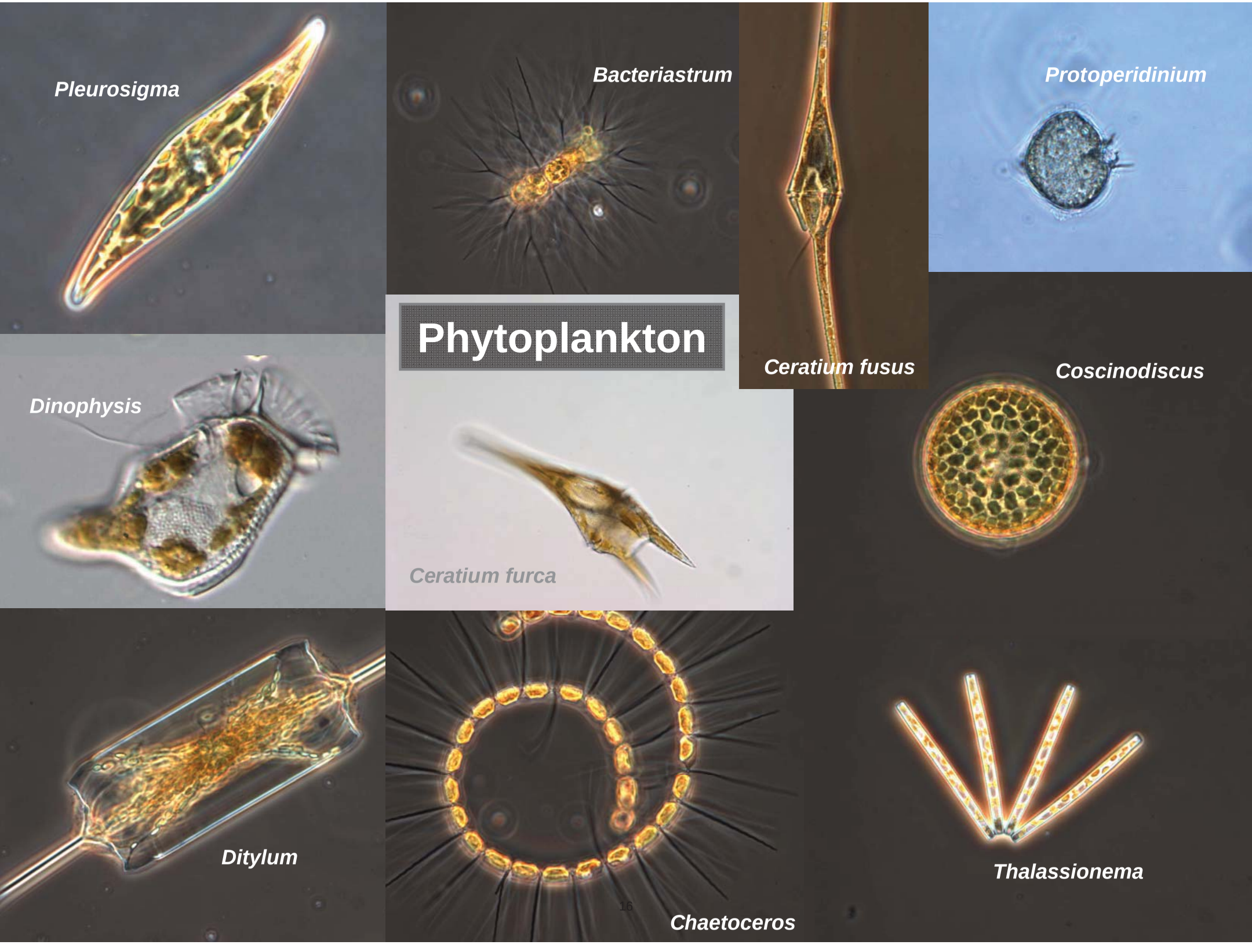


Spines

Diatoms -
closed or solid structures projecting from the cell wall

Dinoflagellates -
solid protuberances that usually taper to a point




Pleurosigma
Bacteriastrum
Protoperidinium
Phytoplankton
Ceratium fusus
Coscinodiscus
Dinophysis
Ceratium furca
Ditylum
Chaetoceros
Thalassionema


Gastropod Veliger


Copepod


Brittle Star Larvae


Starfish Larva


Barnacle Larva


Clam Larva


Portuguese Man of War


Tintinnid

Zooplankton


Crab Zoea


Polychaete Worm


Jellyfish

Alexandrium spp.

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

Identification: Cells 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, A. acatenella*

Vectors: Bivalve molluscs, marine snails, barnacles, Dungeness crabs, anchovies, sardines

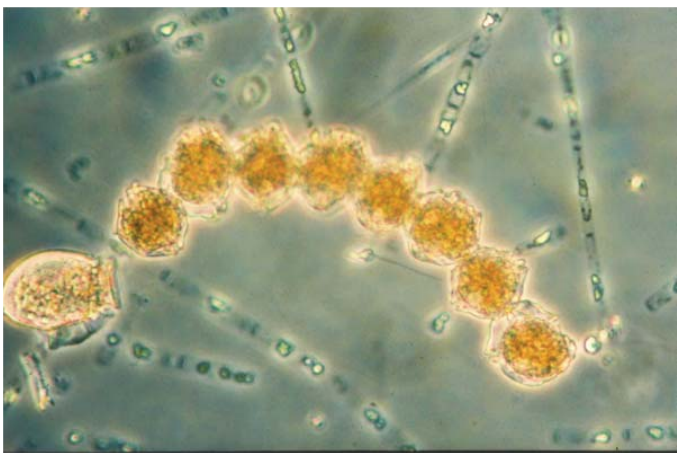
Local distribution: Pacific west coast (Alaska to California), all inland waters of Puget Sound except central and southern Hood Canal

Monitoring: Weekly at multiple coastal and inland marine waters
Water samples for presence, cell abundances (counts, estimates); assume toxin possible when cells are present
Shellfish collected and tested biweekly at ~ 70 sites (WA Department of Health)
SoundToxins action: report any presence of this organism

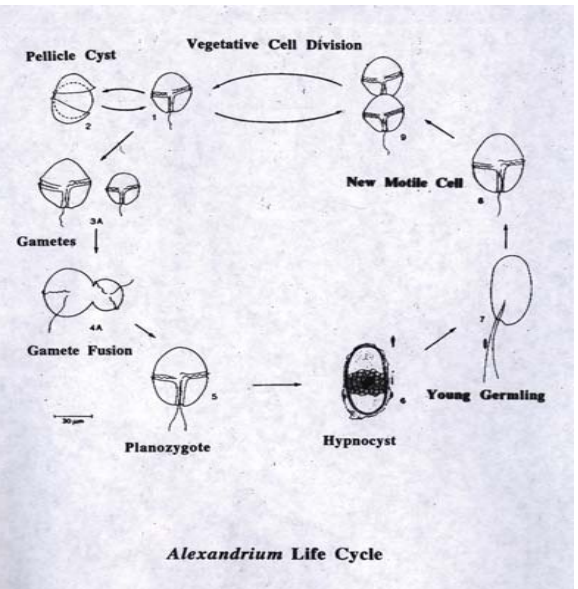
Analyses: Cell counts
Shellfish toxins

Action level: 80 µg/100 g shellfish tissue

Figures:



From Horner (2002)



From Anderson (1998)

Chaetoceros concavicornis

A centric diatom widely distributed in northern temperate and cold water areas and 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; lower valve flat with setae arising inside the valve margin; apertures distinct; setae become wider away from the cell and 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.

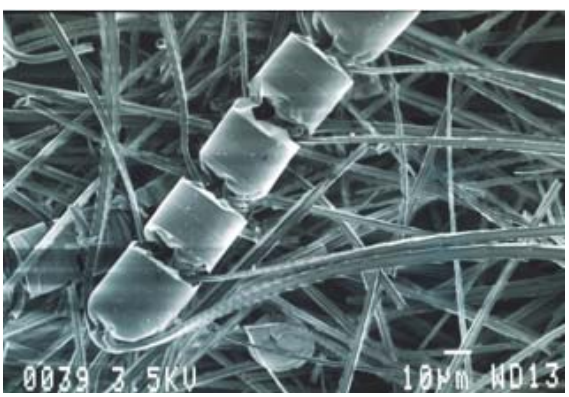
Monitoring: Daily by local fish farmers during summer months when phytoplankton most likely to occur.

Analyses: Cell counts
Action level: 5,000 cells/L
SoundToxins action: "Common," "Bloom," or >5,000 cells/L

Figures:

From Horner (2002)

Light microscope image. Note setae get larger away from cells.



Horner unpubl.

Scanning electron microscope image. Note small spines on setae

Dinophysis spp.

A thecate dinoflagellate genus widely distributed in cold temperate to tropical 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

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

Regional toxic species: *D. acuminata, D. acuta, D. fortii, D. norvegica, D. rotundata, D. tripos*

Vectors: Bivalve shellfish

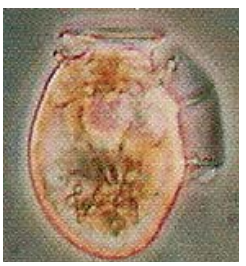
Local Distribution: Pacific west coast (Alaska to California), all inland waters of Puget Sound

Monitoring: Weekly at multiple coastal and inland marine sites
Water samples for presence, cell abundances (counts, estimates)
Shellfish when cells reach action level
SoundToxins action: report any increase from "Present" to "Common" or "Common" to "Bloom," > 20,000 cells/L or any increase from a few (5,000-10,000 cells/L) to > 10,000 cells/L

Analyses:
Cell counts
Shellfish toxins: LC-MS/MS, ELISA

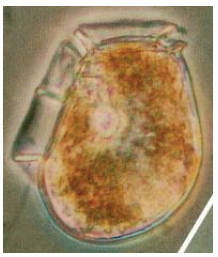
Action level: 160 µg OA/kg shellfish edible parts

Figures:



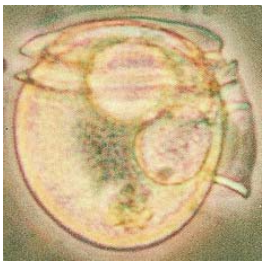
1. Dinophysis acuminata

2. Dinophysis acuta

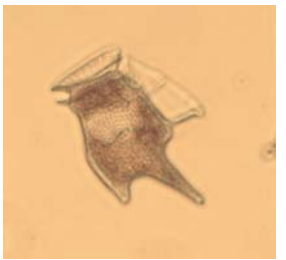


3. Dinophysis fortii

4. Dinophysis norvegica



5. Dinophysis rotundata



6. Dinophysis tripos

Figures 1-5: Horner 2002; Figure 6: Port Townsend Marine Science Center

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 therefore cells readily change shape; rapid swimmers.

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, but also wild fish and known for antagonistic effects on organisms ranging from bacteria to fish.

Regional toxic species: *Heterosigma akashiwo*

Vectors: None

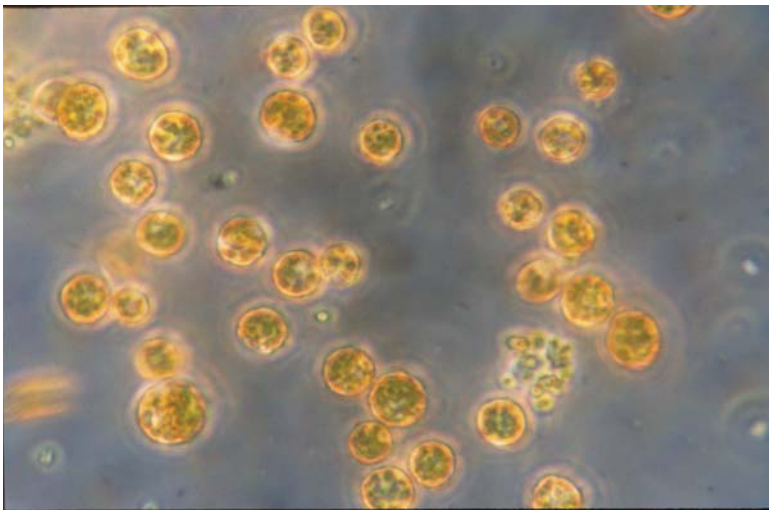
Local Distribution: Pacific coast from British Columbia to California, inland waters of British Columbia and Puget Sound especially in lower salinity waters.

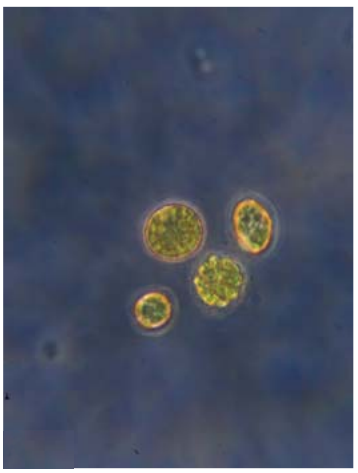
Monitoring: Daily by growers during spring, summer;
Included in weekly monitoring programs by volunteers
SoundToxins action: report presence of any cells

Analyses: Cell counts, estimates

Action level: >500,000 cells/L

Figures:





(All pictures, Horner 2002)

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 microscope (LM). Need scanning (SEM) and transmission (TEM) electron microscopy for positive identification. Use size classes: long and wide (ahf), long and narrow (pm), short and narrow (pddc)

Toxicology: Some species 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. mulitseries, P. cuspidata, P. delicatissima, P. pseudodelicatissima*

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

Local Distribution: Pacific west coast (Alaska to California), all inland waters of Puget Sound

Monitoring: Weekly at multiple coastal and inland marine sites
- Water samples for presence, cell abundances (counts, estimates)
- SoundToxins action: report
  - Large cells (*P. australis, P. heimii, P. fraudulenta; P. pungens, P. multiseries* = ahfpm) > 50,000 cells/L ("Common" or "Bloom")
  - Small cells (*P. pseudodelicatissima, P. delicatissima, P. cuspidata* = pddc) > 1,000,000 cells/L ("Common" or "Bloom")

Analyses:
- Cell counts
- Shellfish toxins

Action level: 20 ppm

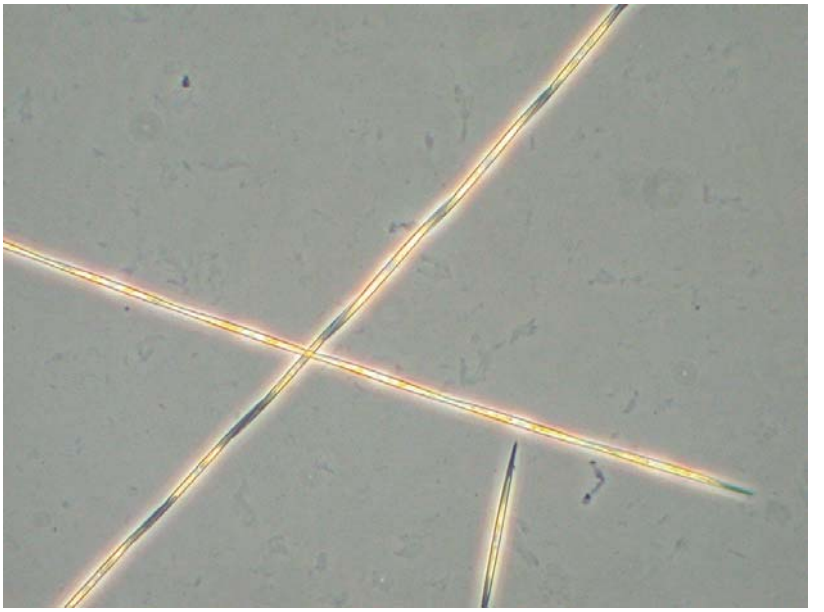
Figures:

Three-celled chain on left is ahf; four-celled chain on right is pm; small, single cells are pddc

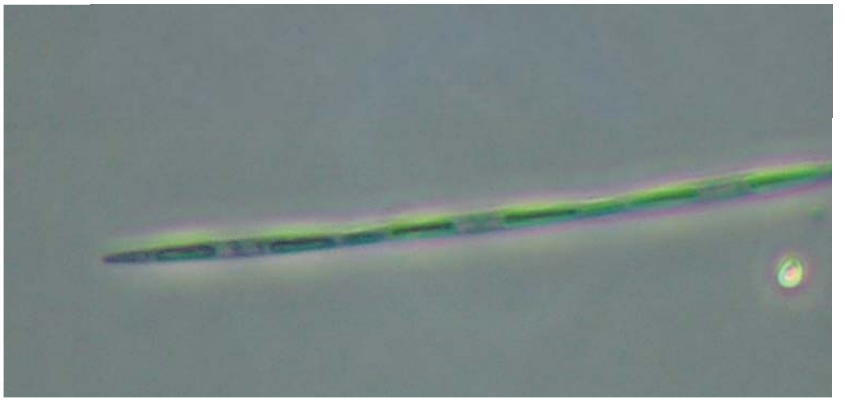
(Photo by Brian Bill, NOAA)

A. *Pseudo-nitzschia* ahf (large, broad cells)

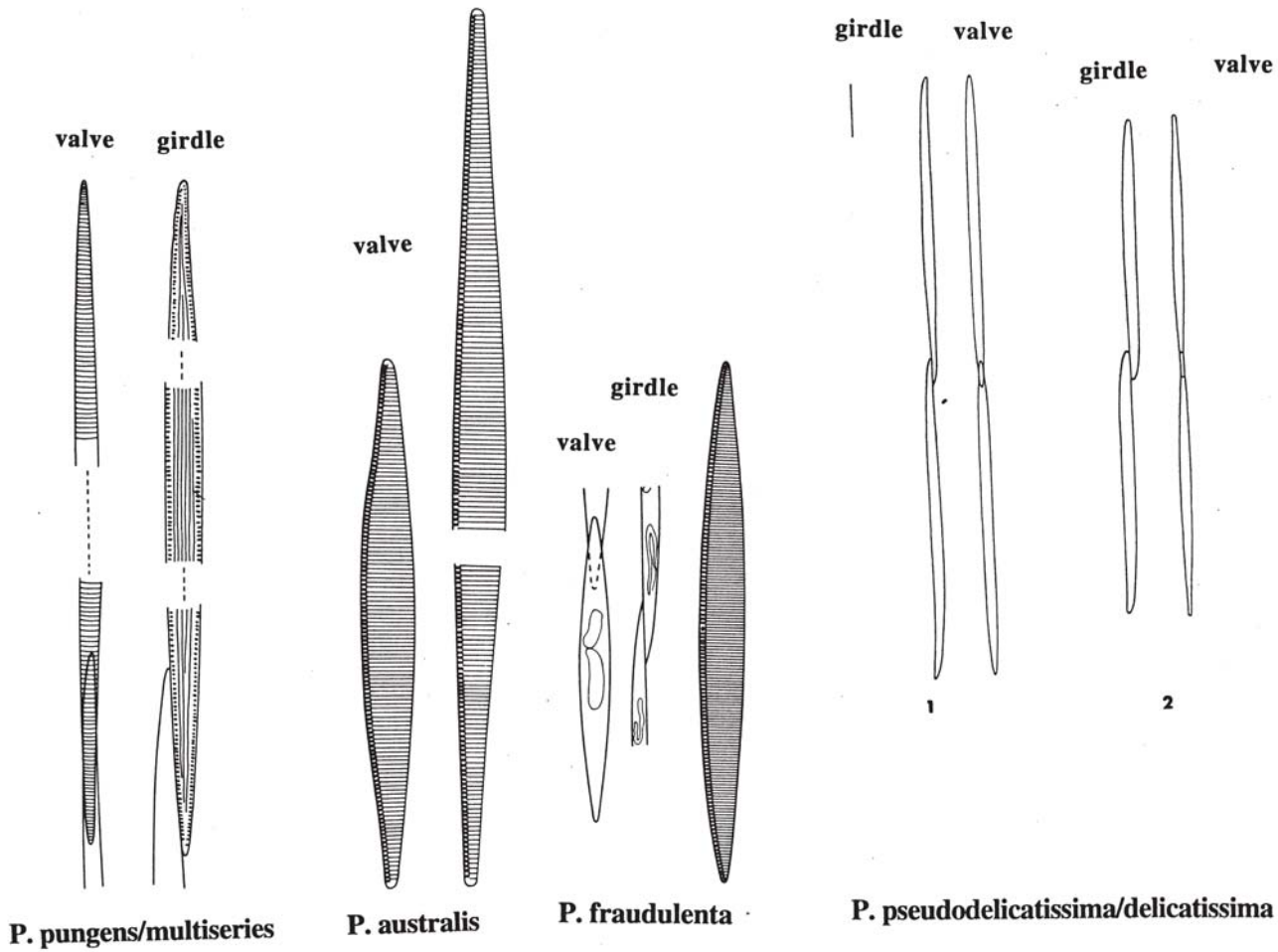
B. *Pseudo-nitzschia* pm (large, narrower cells)



C. *Pseudo-nitzschia* pddc (small, narrow cells)



(A, B. Brian Bill NOAA;
C. Alan Sarich WDFW)



From Hasle (1972)

Protoceratium reticulatum (Claparède & Lachmann) Bütschli

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

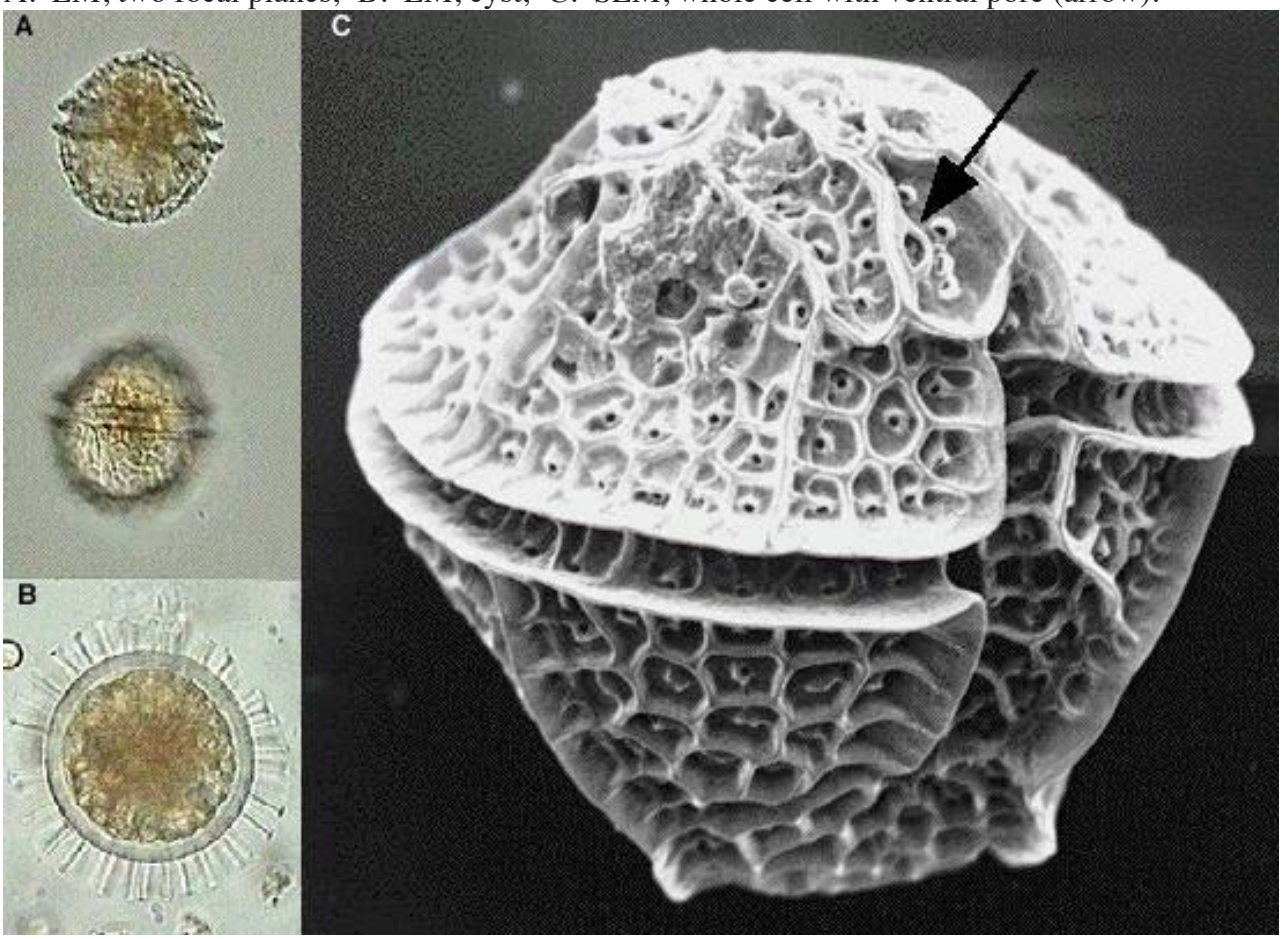
Cells are easily confused with other smallish, round, brown cells including solitary *Alexandrium* cells and some *Gonyaulax* species. Populations from South Africa were described as *Gonyaulax grindleyi* Reinecke which remains a synonym. Cysts, described as *Operculodinium centrocarpum* (Deflandre &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

Distribution: neritic, estuarine; cold temperate to subtropical waters; reported from all oceans.

Produces yessotoxins.

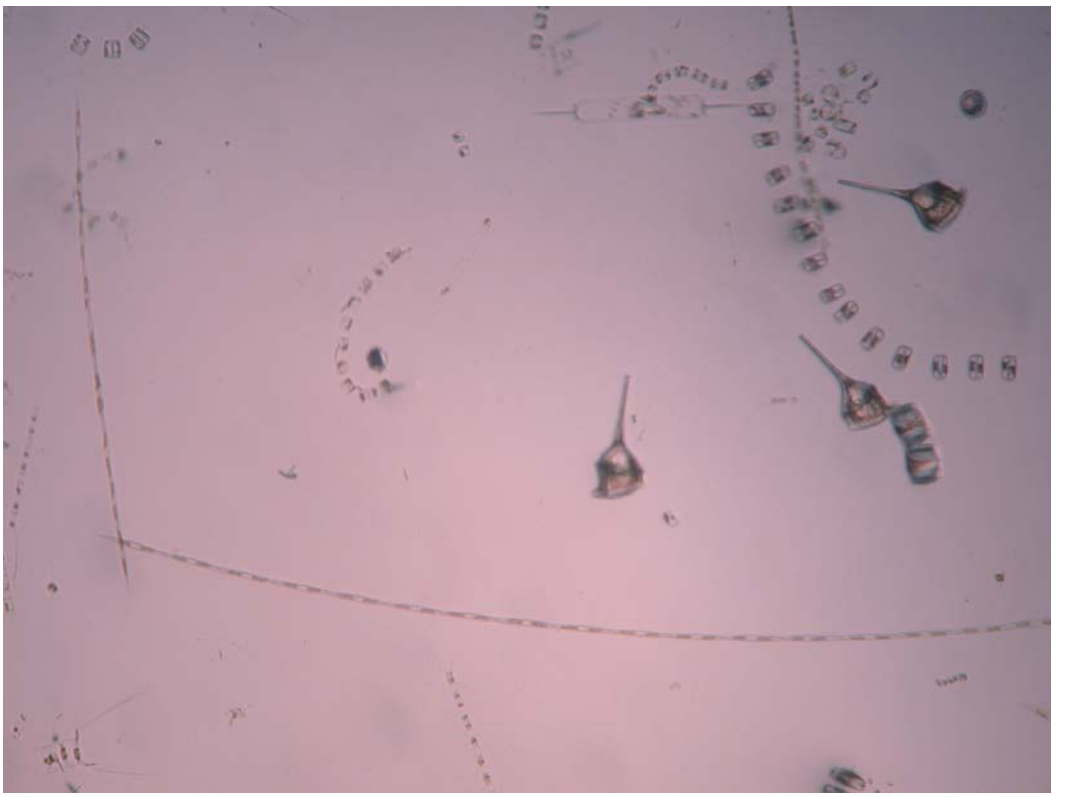
May form blooms in mid to late summer, often in the Sequim Bay/Port Townsend areas.

Photo from: http://media.nordicmicroalgae.org/large/Protoceratium%20reticulatum_2.jpg
A. LM, two focal planes; B. LM, cyst; C. SEM, whole cell with ventral pore (arrow).

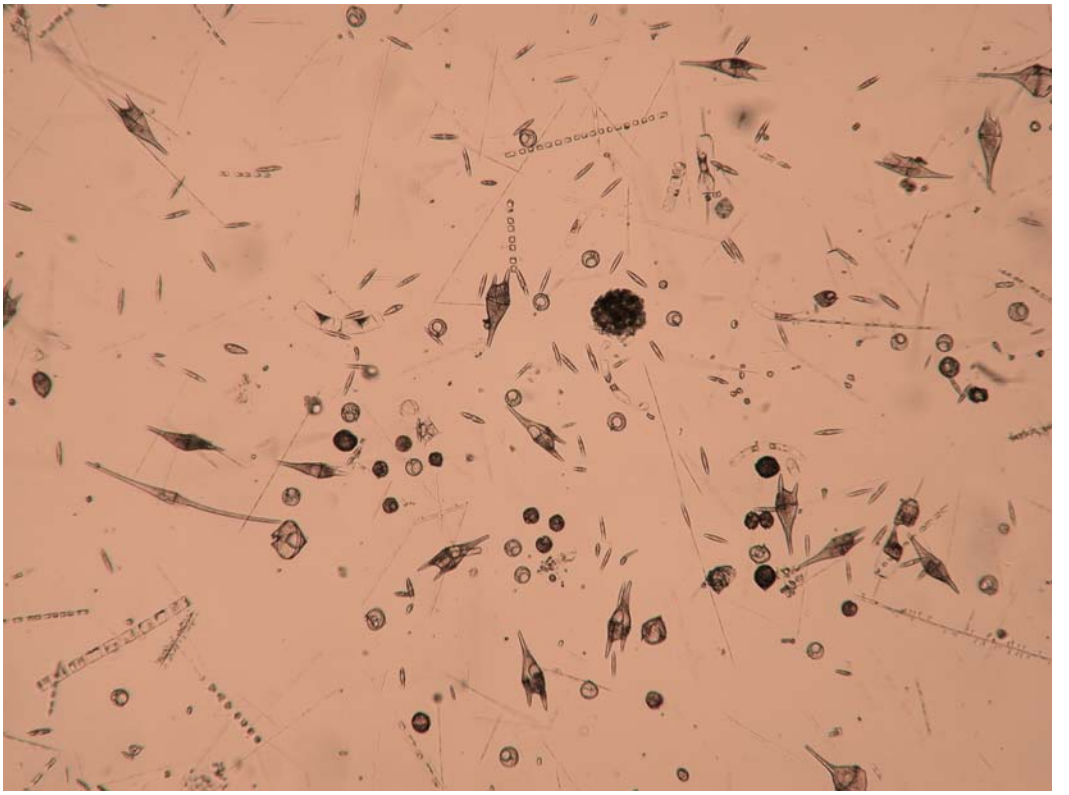


Relative Abundance Examples

Bloom: Ceratium
Present: Proboscia, Tiarina, Prorocentrum, un ID dinoflagellate



Common: Pseudo-nitzschia
Present: Ceratium, Thalassiosira, Chaetoceros, Ditylum, Skeletonema, Dactyliosolen

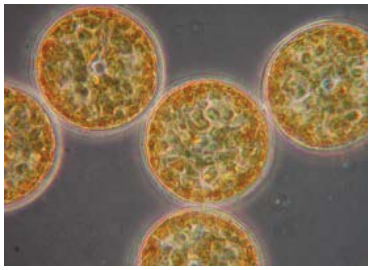


Bloom: Pseudo-nitzschia
Common: un ID pennate diatom, Ceratium, Protoperidinium
Present: Chaetoceros, Skeletonema, Ditylum, Guinardia, Alexandrium, Dictyocha, Prorocentrum, un ID dinoflagellate, Cylindrotheca

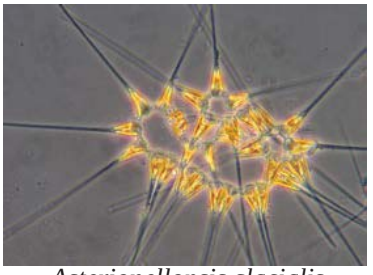
Common: Rhizosolenia
Present: Ditylum, Pseudo-nitzschia, Chaetoceros, Hemialus

Marine Phytoplankton of Alaska

Diatoms



Actinoptychus senarius
cells 20-150 µm in diameter

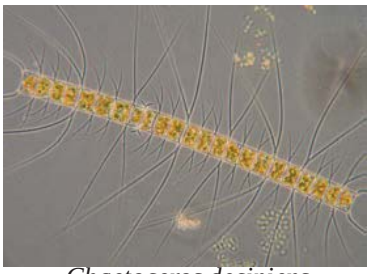


Asterionellopsis glacialis
cells 30-150 µm long

Asteromphalus heptactis
cells 42-125 µm in diameter

Chaetoceros concavicornis
cells 12-30 µm wide

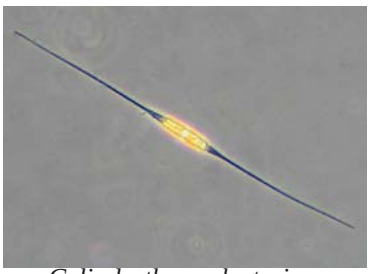
Chaetoceros debilis
cells 8-40 µm wide



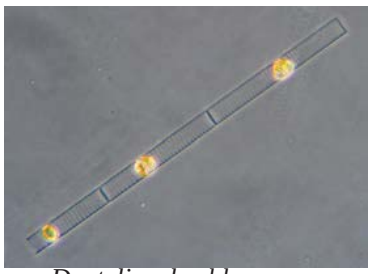
Chaetoceros decipiens
cells 9-84 µm wide

Corethron hystrix
cells 20-150 µm long

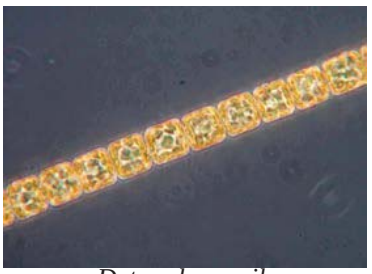
Coscinodiscus centralis
cells 100-300 µm in diameter



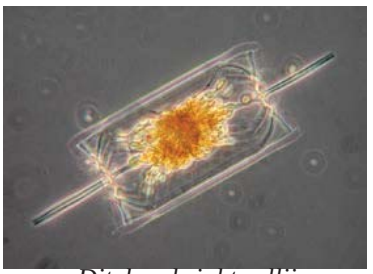
Cylindrotheca closterium
cells 30-400 µm long



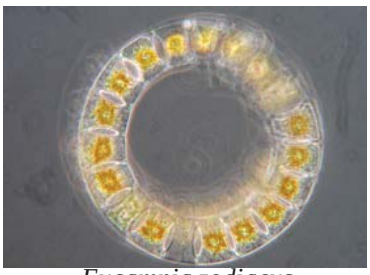
Dactyliosolen blavyanus
cells 6-38 µm in diameter



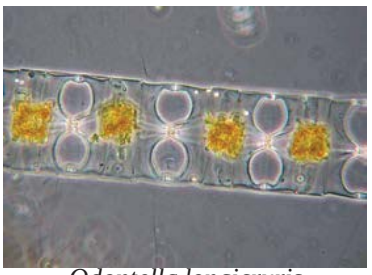
Detonula pumila
cells 16-42 µm in diameter



Ditylum brightwellii
cells 80-130 µm long



Eucampia zodiacus
cells 10-61 µm wide

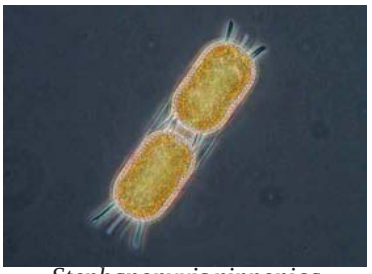


Odontella longicruris
cells 15-110 µm wide

Pseudo-nitzschia pungens
cells 74-174 µm long

Rhizosolenia setigera
cells 4-20 µm in diameter

Skeletonema costatum
cells 2-21 µm in diameter



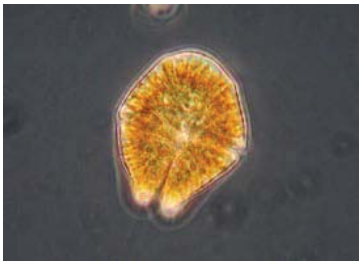
Stephanopyxis nipponica
cells 24-36 µm in diameter

Thalassionema nitzschioides
cells 10-80 µm long

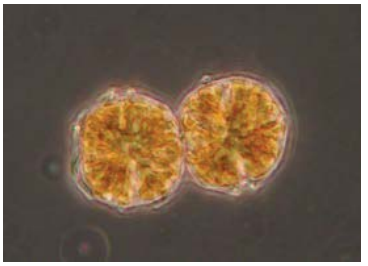
Thalassiosira spp.
cells 10-50 µm in diameter

Marine Phytoplankton of Alaska

Dinoflagellates



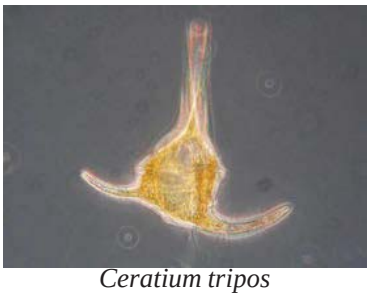
Akashiwo sanguinea
cells 40-80 µm long



Alexandrium catenella
cells 24-34 µm long

Ceratium fusus
cells 20-300 µm long

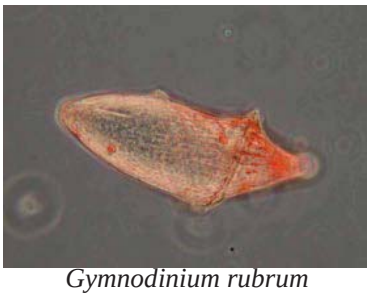
Ceratium lineatum
cells 30-60 µm long



Ceratium tripos
cells 70-90 µm long

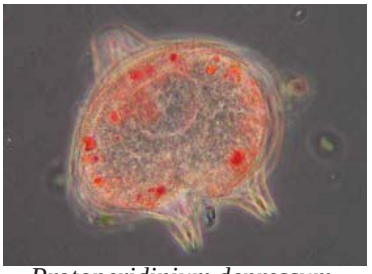
Dinophysis acuminata
cells 38-50 µm long

Dissodium pseudolunula
cells 23-28 µm long

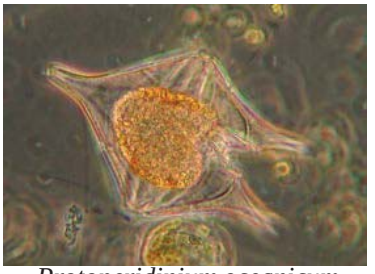


Gymnodinium rubrum
cells 100-145 µm long

Noctiluca scintillans
cells 200-2,000 µm in diameter



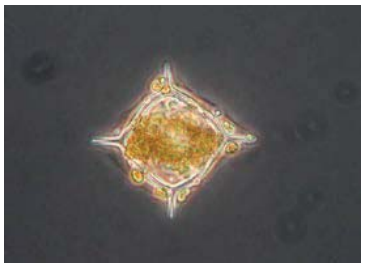
Protoperidinium depressum
cells 116-200 µm long



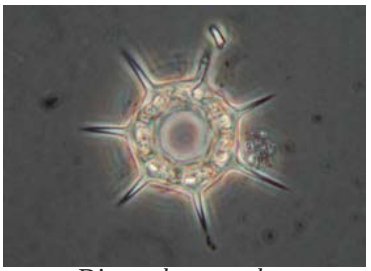
Protoperidinium oceanicum
cells 220-300 µm long

Polykrikos schwartzii
cells 100-150 µm long

Silicoflagellates



Dictyocha fibula
cells 10-45 µm in diameter



Dictyocha speculum
cells 19-34 µm in diameter

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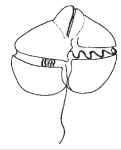
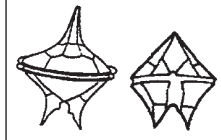
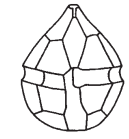
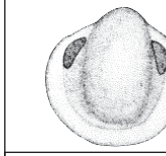
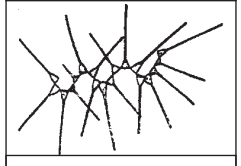
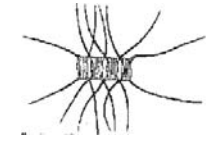
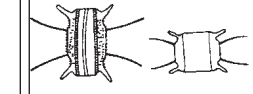
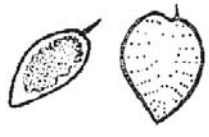
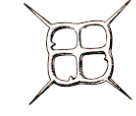
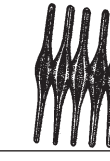
Target Species

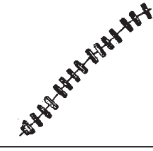
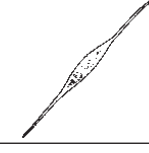
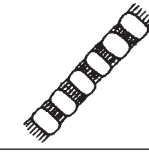
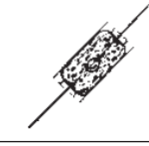
COMMON PHYTOPLANKTON KEY

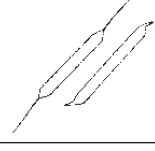
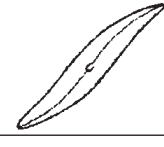
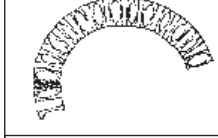
OTHER COMMON PLANKTON (non-phyto)

Alexandrium spp. **AL**	*Gymnodinium spp.* **GY**	*Gonyaulax spinifera* **GS**	*Protoperidinium spp.* **PT**	*Scripsiella spp.* **SC**	*Cocinodiscus spp.* **CO**	*Odontella spp.* **OD**	*Larval Clam* **LC**
							
25-46 µm	**24-50 µm**	**25-50 µm**	**50-95 µm**	**20-37 µm**	**40-500 µm**	**45-70 µm**	**Generally Large**

Dinophysis norvegica **DN**	*Dinophysis acuminata* **DA**	*Dinophysis tripos* **DT**	*Asterionellopsis spp.* **AS**	*Chaetoceros spp.* **CH**	*Chaetoceros socialis* **CS**	*Biddulphia spp.* **BD**	*Rotifer spp.* **RO**
							
48-80 µm	**40 - 50 µm**	**40 - 120 µm**	**30-150 µm**	**10 - 53 µm**	**3-15 µm**	**60 - 160 µm**	**Generally Large**

Prorocentrum lima **PL**	*Prorocentrum micans* **PM**	*Ceratium fusus* **CF**	*Ceratium lineatum* **CL**	*Ceratium longipes* **CP**	*Dictyocha spp.* **DO**	*Fragilaria spp.* **FR**	*Pollen Grain* **PG**
							
31-47 µm	**35-70 µm**	**200-540 µm**	**100-130 µm**	**150-250 µm**	**10-45 µm**	**10 - 70 µm**	**Generally Large**

Pseudonitzschia **PS**	*Thalassionema spp.* **TA**	*Thalassiosira spp.* **TL**	*Nitzschia spp.* **NZ**	*Skeletonema spp.* **SK**	*Ditylum spp.* **DM**	*Leptocylindrus spp.* **LP**	*Crab Zoea* **CZ**
							
64-117 µm	**16 - 90 µm**	**12-39 µm**	**60 - 125 µm**	**2-21 µm**	**80 - 130 µm**	**30 - 75 µm**	**Generally Large**

Species Name **CODE**	*Rhizosolenia spp.* **RH**	*Gyrosigma spp.* **GY**	*Navicula spp.* **NV**	*Melosira spp.* **ML**	*Guinardia spp.* **GN**	*Eucampia spp.* **EU**	*Tintinnid spp.* **TN**
(Guide to using key) *illustration of organism*							
Size Range (in µm)	**25-57 µm**	**110 - 175 µm**	**32-49 µm**	**10-50 µm**	**60 - 160 µm**	**10-33 µm**	**Generally Large**

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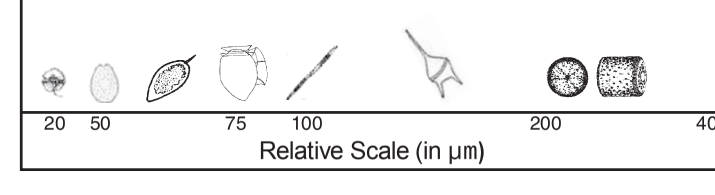
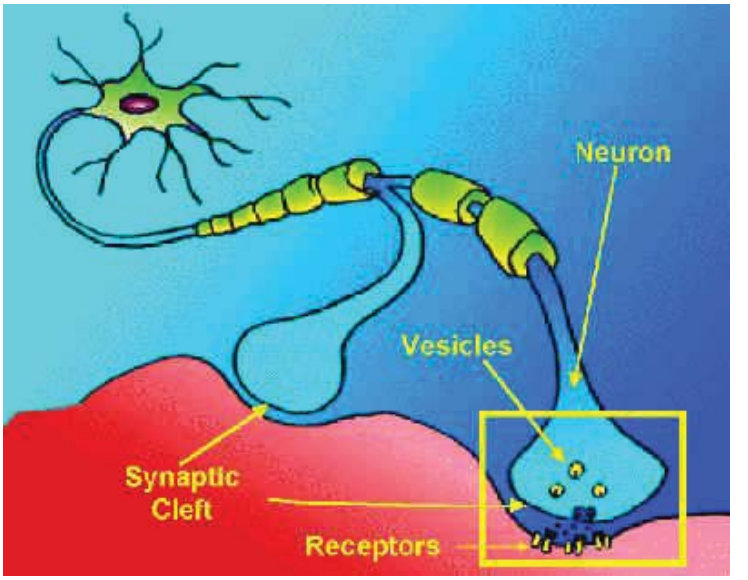


Receptor Binding Assay

First, a little background

Toxins exert their devastating affects in a variety of ways, but most commonly they interfere with the transmission of nerve impulses. The activation of a nerve impulse is an electrical phenomena in which a series of connected nerve fibers are sequentially polarized and depolarized. Actually these cells are not "connected" in the usual meaning of the word, but are "connected" through a space or junction called the "synaptic cleft" (see below). A nerve impulse is passed from one nerve cell to the other across the cleft by a "neurotransmitter". Usually, this is a small, low molecular weight chemical, such as acetylcholine or epinephrine (adrenaline). A number of chemicals have been identified as neurotransmitters in the past 20 years.

Nerve cells have an electrical potential between the inside and outside of the cell. This voltage comes about due to the differences between the ionic composition of inside the cell (where potassium ions, K^+, are in higher concentration) and outside the cell (where sodium ions, Na^+, are in higher concentration). When stimulus is applied to these cells, Na^+ ions flow into the cell, voltage increases, thereby causing K^+ to flow out of the cell. This change in voltage is referred to as depolarization. As K^+ flows out of the cell it releases a neuro-transmitter which crosses the synaptic cleft and goes to a receptor on the next nerve cell. When the neuro-transmitter binds to the receptor, a change occurs in the cell causing it to depolarize. When that cell depolarizes it also releases neuro-transmitters and the process goes on to the next cell.



What are receptors?

Propagation of nerve impulses can be a rather complicated process and there are plenty of opportunities for things to go wrong. In order for cells not to depolarize all the time, the receptor site must accept only very specific chemicals. By and large, the receptor site has a structure that is fairly specific for its designated neurotransmitter. Nerve Toxins are harmful in a number of ways, among them:

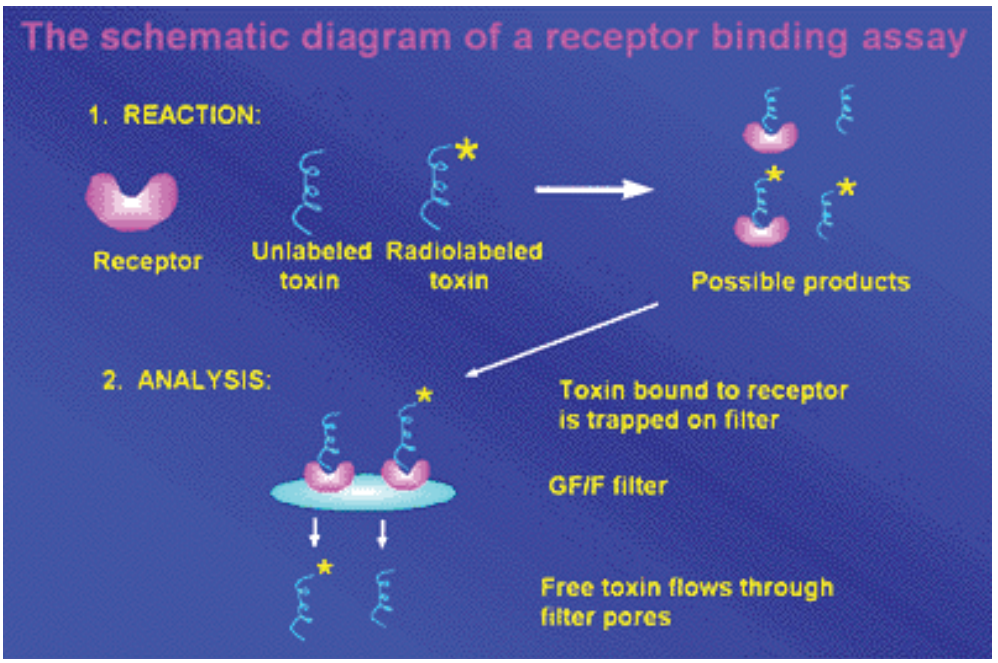
- They can mimic neurotransmitters (they have chemical structures very similar to the correct neurotransmitter.)
- They interfere with or block the release of the ions through the cell.

- They can block the action of a neurotransmitter at the receptor-- they don't let the neuro-transmitter attach allowing the cell to depolarize.

Assays

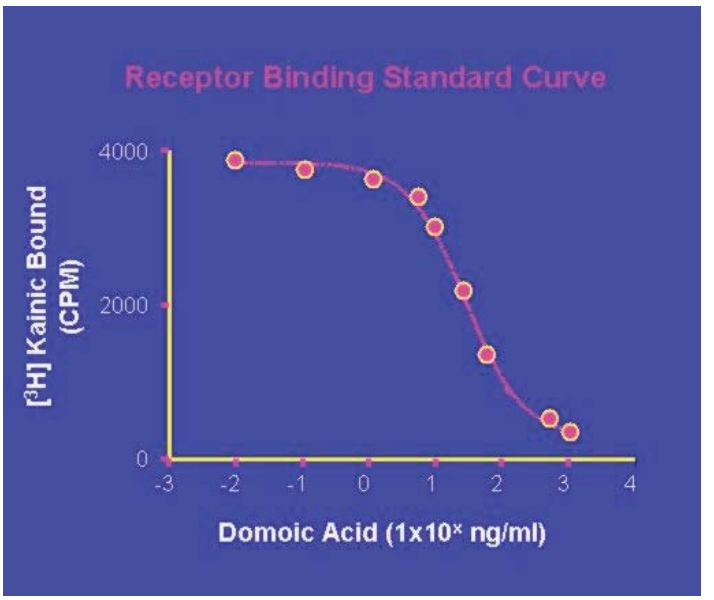
Very early on, researchers took advantage of toxin binding to nerves as the basis of assays for the toxins and/or neurotransmitters. However, these usually required very complex setups and the isolation of nerve receptors from a variety of animals (e.g., the giant squid axon). Needless to say these procedures were time consuming, very expensive, and required highly trained and skilled people. While they were used widely, and still are to some extent today, by medical and pharmacological researchers, recent advances in molecular biology have permitted much simpler methods. These new methods can be faster, cheaper, far more sensitive, and most importantly use very small amounts of toxic extracts. It is now possible to clone receptors from one species and have them reproduced in cells of another species. It is this method that we use to produce receptors used in our receptor binding assay for domoic acid determination.

Receptor binding assays measure the binding of a toxin to its nerve cell receptor by either marking the toxin or a suitable derivative of the toxin. This is most commonly done now using radioactive markers. In the procedure, the radioactive toxin will be displaced or "bumped off" its receptor by toxin present in an unknown sample, thereby reducing the total radioactivity. The amount of radioactively labelled toxin that is displaced is proportional to the amount of toxin in the unknown sample. The toxin present in an unknown sample can then be quantified by comparison to a standard curve obtained using pure toxin. The advantage of this technique is that it can be made highly specific and sensitive for a particular toxic activity. Currently, receptor binding assays have been developed for domoic acid and PSP toxins. Below is a general description of the receptor binding assay.



Domoic Acid Receptor Binding Assay

Domoic acid is a potent neurotoxin that binds very tightly to a glutamate receptor in the brain. Under normal circumstances, these cells use glutamic acid (a common amino acid) as a neurotransmitter. Unfortunately, this receptor also binds two other compounds even more tightly than glutamic acid. These two compounds, kainic acid and domoic acid, are at first glance not that similar to glutamic acid; nevertheless, they both bind to the glutamate receptor. Of the three compounds, domoic acid binds to the glutamate receptor most tightly and will displace both kainic acid and glutamate from the binding sites. We take advantage of this fact in designing this assay.



We can take advantage of the fact that kainic acid has a structure very similar to domoic acid. Furthermore, kainic acid is relatively easy to synthesize and is available from commercial sources. Kainic acid can be easily marked using tritium (a radioactive isotope of hydrogen). In addition, by using techniques developed in molecular biology, it is possible to grow cells in the laboratory that contain glutamate receptor sites. Using radiolabelled kainic acid we can then use the domoic acid to displace the radioactive kainic acid binding from the receptor. By measuring the radioactivity in counts per minute (CPM) of samples with known amounts of domoic acid we can create a "standard curve" such as the one shown here which relates added amounts of domoic acid and counts of radioactivity.

Once we have established this "standard curve" we can then add our unknown samples to the radiolabelled kainic acid and receptor preparation. We can then compare the amount of radioactivity in our samples to our standard curve. This allows us to estimate very low concentrations of domoic acid in a variety of extracts, be they sea water, shellfish, or phytoplankton cells.

Southeast Alaska Tribal Toxins (SEATT)
Sampling Protocols

Lab Supplies
Net tow sample
Whole water sample
Forceps
Formaldehyde fixative
Pipette
Palmer-Maloney slide
Scintillation vials
GF/F filters
Glass test tube
Swinnex filter holder
Syringe
Graduated cylinder
Plastic bottle
Aluminum foil
Pump
Filter flask
Filter cup
47 mm Millipore HA
Centrifuge tube

Net Tow Processing

Net Tow Phytoplankton Sample: Sample Archive
• Invert net tow sample to mix and pour an aliquot into a 20 ml scintillation vial.
• Add 1 ml formaldehyde fixative, cap, invert to mix.
• Label cap with the following: site name, date, and "NET".
• Store vials in tray.

Net Tow: Species Composition, Relative Abundance
• Invert net tow sample gently to mix. Pipette 0.1 ml into Palmer-Maloney cell.
• Record relative abundances of species on data sheet.

Use net tow sample to count *Alexandrium* and *Dinophysis*. Report any *Alexandrium* found in your sample. The first time Dinophysis appears in your sample, please report to seator@sitkatribe-nsn.gov as well as in your database entry. From then on report if there has been an increase in the amount of *Dinophysis* seen from the prior week.

Whole Water Processing

Whole Water Phytoplankton Sample:
• Invert 2-liter bottle to mix. Pipette 0.1 ml into Palmer-Maloney cell.

Whole Water 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, 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 the following: site name, date, and "WW 10x".

Use the whole water 10x sample to count *Pseudo-nitzschia* levels. If the large celled species of *Pseudo-nitzschia* ((*P.australis, P.heimii, P.fraudulenta, P.pungens, P. multiseries*=ahfpm) are greater than 50,000 cells/L or if the small cells (*P.pseudodelicatissima, P.delicatissima, P.cuspidata*=pddc) are greater than 1,000,000 cells/L please report to seator@sitkatribe-nsn.gov **and in your database entry.**

Chlorophyll/Nutrient Sample:
• Using forceps, place GF/F filter into Swinnex filter holder and tighten parts together. Make sure filter does not get bunched up in threads.
• Take plunger out of syringe and screw Swinnex filter holder onto end of syringe.
• Invert 2-liter bottle of whole water to mix.
• Measure 50 ml whole water from 2-liter bottle with graduated cylinder (or pour water into syringe up to the 50cc line).
• Rinse nutrient bottle by using plunger. Filter a small amount of water into numbered plastic bottle to rinse. Repeat rinse step two more times.
• Filter remaining water into numbered plastic bottle for nutrient sample (bottle should be no more than 1/2-3/4 full).
• Record nutrient bottle number and the amount of water filtered into the database.
• Place nutrient sample bottle upright in freezer. After frozen, samples can be placed in large plastic bags labeled with the following: site name. approximate date range, and "NUTRIENTS".
• Remove GF/F filter from Swinnex holder using forceps, fold filter in half with plankton on the inside, place in a square of aluminum foil fold into a packet.
• Label with the following: site name, date, and "CHL" to indicate chlorophyll.
• Put foil packet in plastic bag labeled with the following: site name, approximate date range, and "CHLOROPHYLL".
• Store in freezer.

Particulate Toxin Sample (Cellular Toxin):
• Set up pump, filtering flask, and filter cup with 47 mm Millipore HA filter (be sure to remove the blue paper between the filters).
• Gently invert 2-liter bottle to mix.
• Measure 1-liter of water with graduated cylinder and pour into filter cup to filter. Keep adding water to filter cup until 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 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 in plastic bag labeled with the following: site name, approximate date range, and "pTOX".
• Store in freezer.

Dissolved Toxin Sample (Toxin in Sea Water):
• Pipette 1-2 ml of filtered water from filtering flask into small yellow centrifuge tube.
• Label tube with site name, date, and "dTOX" to indicate dissolved toxin.
• Place in storage box labeled with the following: site name, appropriate date range, and "dTOX".
• Store in freezer.

Cleaning Glassware
• Rinse all glassware three times with freshwater water.
• Set out to air dry

Shellfish Sample Collection

Mussel Cages may be used at SEATT sites for sentinel species analysis. Other species should follow the collection protocol below.

- Samples must yield at least 150 grams of meat for processing (approx. 5 oz.). Shellfish must be in the shell and fresh.
- Sample size is according to the following table:

Species	Number to Submit
Oysters	10 - 12
Butter Clams	10 - 12
Littleneck, Manila clams	35 - 45
Horse Clams	4 - 5
Eastern Softshells	12 - 14
Blue Mussels	80 -130
California Mussels	5 - 6
Rock Scallops	3 - 4
Pink Scallops	30 - 40
Geoduck	3 - 6
Crab	6

- These numbers are based on average-size specimens.
- Do not submit specimens with cracked or crushed shells.
- Place shellfish in a waterproof plastic bag.
- Do not mix species (only one species per bag).

Handling

- Do not hold shellfish in seawater or freshwater at any time after collection, however, rinsing with seawater or freshwater to remove sediment is recommended.
- If samples must be held prior to shipment, keep refrigerated. Putrid or decomposed samples will not be processed.

Data Forms

- Complete the form using black ink.
- Fill in dates collected and submitted, species and number of organisms.

Shipment

- Place samples in a labeled (date and location) plastic bag. The samples must then be put into a sealable

container or box, such as a plastic bucket. If plastic buckets are used then they must then be placed inside a cardboard box for shipping.

- Ice packs should be used to refrigerate samples during shipment.
- Include one Shellfish Biotoxin Sample Form for each sample submitted. Place form in a separate waterproof plastic bag.
- Submit samples as early in the week as possible.
- All PSP/ASP/DSP samples must be sent to the following address:
    - 456 Katlian Street
      Sitka Alaska 99835

Test Results

- Test results will be available from one to several days, depending upon laboratory workload.

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Depth Integrated Net Tow

Method -

1. Collect water as close to the shellfish site as possible.
2. Mark the line attached to the net at every 1.0 m interval with a black sharpie or electric tape.
3. Allow your net to drop to near the bottom. The cod end may need to be weighted if current is strong. The line should be kept as vertical as possible when towing.
4. Tow your net vertically to the surface at a steady speed (about 1m per second). Repeat until color is seen in the concentrated water in the cod end. Keep track of how many times net is pulled through water column (how many meters it is towed).
5. Record the **total** length of tow on data sheet (i.e 2 tows at 5m = 10m).
6. Ensure the water level is below the top of the cod end before removing it (minimize loss of water from cod end).
7. Measure the volume of water in the cod end and record cod end volume on data sheet. Measure volume with 1L graduated cylinder or similar graduated measuring device.
8. Swirl to mix net tow sample and pour into a glass jar.

Depth Integrated Net Tow
Calculation of volume filtered through net

Net mouth diameter = 25cm = 0.25m
(some nets have different mouth diameters, measure your net to be sure and adjust the calculations, if needed)

Radius (r) = 0.25m / 2 = 0.125m

Area of mouth = πr^2 = 3.14 x 0.125^2 = 0.049 m^2

Area of mouth of net **x** length of net tow **=** total volume filtered

Example: You tow your net 2 times through 5 m of water, so the total length of net tow is 2 x 5m = 10m. Area of mouth is 0.049 m^2.

Total volume filtered = 0.049 m^2 x 10m = 0.49 m^3. Convert m^3 to liters (1 m^3 = 1,000 L).
Total volume filtered in L = 0.49 m^3 x 1,000 L = **490 L**

Record total volume filtered on count sheet and use it to calculate semi-quantitative cell counts from net tow material (see below).

Calculate semi-quantitative cell concentration from net tow material.

Example: Total volume filtered was 490L and Cod end volume was 150ml, or **0.15L**.
Cell count from net tow was 250 cells/ml (or **250,000 cells/L in net tow**).

Cell concentration (cells/L) / Total volume filtered (L) X Cod end volume (L) = Cell concentration in sampling site water (whole water).

(250,000 x 0.15) / 490 = **77 cells/L** in sampling site water (whole water)

SEATT DATA ENTRY SHEET

Sample Location:	Starrigavan Dock
Collection Date and Time:	
Field Data Collected by:	
Data Entered by:	
Comments:	

Weather and Water
sunny partly cloudy mostly cloudy cloudy rain
Wind (none, light, moderate, strong):
Tide (incoming, outgoing, high, low):
Air Temperature (°C):
Water Temperature (°C): Salinity (ppm):

Cell Abundance

Genus	Species (if known)	Net Tow Relative Abundance N, P,C, B (none, present,common, bloom)
Pseudo-nitzschia		
Alexandrium		
Dinophysis		
Diatoms:		
Aterionellopsis		
Bacteriastrum		
Bacterosira		
Chaetoceros		
Corethron		
Coscinodiscus		
Cylindrotheca		
Dactyliosolen		
Ditylum		
Eucampia		
Fragilariopsis		
Guinardia		
Hemiaulus		
Leptocylindrus		
Licmophora		
Melosira		

***Pseudo-nitzchia* spp. size classification**	**% Small**	**% Large**
Shellfish Collected (#/species)		

Genus	Species	Abundance (N, P,C, B)
Diatoms (cont):		
Navicula		
Nitzschia		
Pleurosigma		
Rhizosolenia		
Skeletonema		
Stephanopyxsis		
Striatella		
Thalassionema		
Thalassiosira		
Dinoflagellates:		
Ceratium		
Cochlodinium		
Heterocapsa		
Noctiluca		
Prorocentrum		
Protoperidinium		
Haptophytes:		
Phaeocystis		

SoundToxins Basic Data Viewing/Entering Instructions

The SoundToxins application allows user to view/enter sampling event and species data. Each sampling event is considered a Visit (date/time, water temperature, etc). Every Visit can be associated to multiple genus/species observations data (cell count, relative abundance, etc).

Sound Toxins data is observed on a weekly basis to track when and where harmful algal bloom species are occurring.

Below are the steps to enter data in the application.

Login

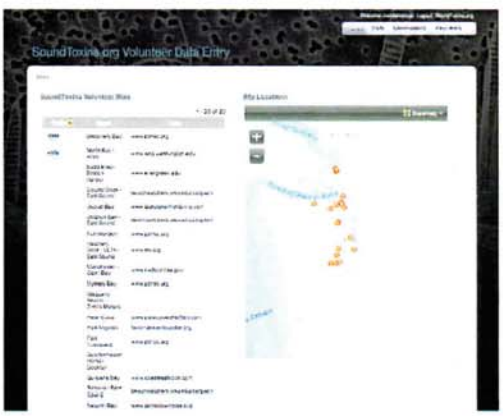
Use the Sidebar style for margin notes.

1) Go to www.soundtoxin.org and click Data
2) Enter Username/Password
3) Click on Login

To request a password, an e-mail must be sent to soundtox@uw.edu. A username and a temporary password will be emailed to your email address on file. The email will contain a link that will allow you to change your temporary password.

Sites

The map is only for viewing purposes, it does not provide any links to other pages.



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 visits image to the left of the site name. If you do not see an visits image on any site, that means you only have view permissions to the application (meaning you can only see the data but not modify it).

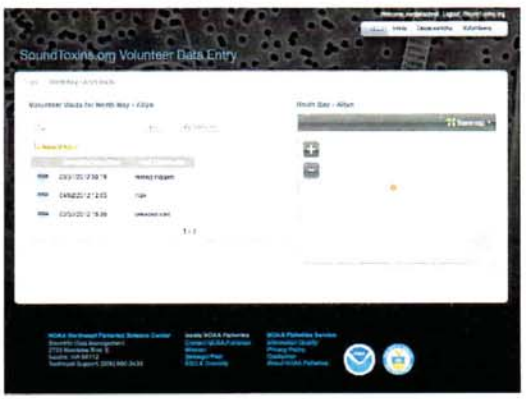
For entering data purposes, click on the visits image of the site you like to enter data for.

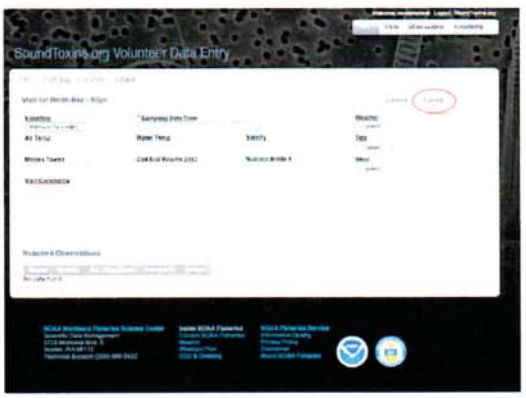
The map is only for viewing purposes, it does not provide any links to other pages.

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

For help on what each Visit field means, click on the field name.

Genus/Species observation data can only be entered AFTER you create a Visit.





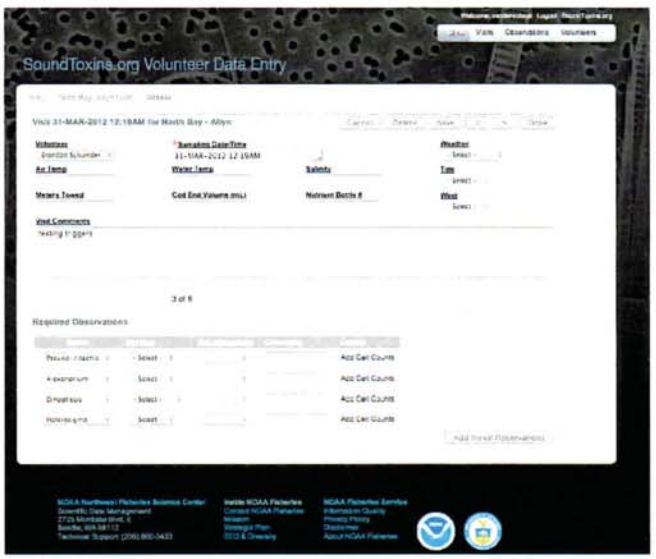
Create a new Visit

1) Click on Yellow "New Visit" button
2) Enter the Visit data
3) Click on the Create button on the upper right region of the page
4) Once a visit is created, now you will be able to enter genus/species observation associated to the particular visit. The four required genuses will be now visible in the Required Observations region at the bottom (covered later). For additionally genus/species observations, click on Additional Observations button at the bottom right (covered later).

Edit an existing Visit

1) Click on the visits image of your site that you wish to edit
2) Click on the data image to the left of the visit name
3) Make any changes to the Visit data
4) Click the Save button on top right region of the page. NOTE: always click on Save before navigating to another page to ensure that the data you entered was properly recorded.

The system will not allow you to enter the same genus and species observations twice for a particular visit. This applies to Main Observations and Additional Observations.



3) Click on Save

Required Observations

The Visit page (explained above) will also allow you to select Species, Relative Abundance and enter Comments for the four main genuses (*Pseudo-nitzchia*, *Alexandrium*, *Dinophysis* and *Heterosigma*). Please do not modify the Genus field for the main observations.

Entering high level data for required observations

1) Select the Species, Relative Abundance and enter Comments for the main observations.

2) Click on Save

Entering detail data/counts for required observations

1) Click on the Add Cell Counts link to the right of the Comments field.

2) Enter the detailed data for the main observation.

The system will not allow you to enter the same genus and species observations twice for a particular visit. This applies to Main Observations and Additional Observations.

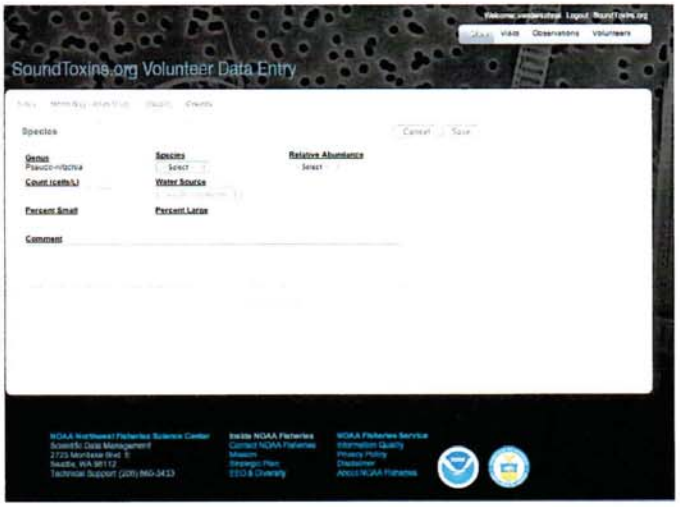
Additional Observations

When clicking on the Additional Observations button on the Visit page, you are taken to a page that displays a list of non-main genus/species associated with the chosen site. The page also allows you to add/associate multiple genus/species to the chosen.

Add new genus/species

1) Click on Add Species

2) Select the Genus, Species (if possible), Relative Abundance and enter Comments for the additional observations.



Entering detail data/counts for additional observations

1) Click on the Add Cell Counts link to the right of the Comments field.

2) Enter the detailed data for the main observation.

3) Click on Save

Viewing Data from Other Sampled Locations

1) Click on the site you wish to view

2) Click on the date you would like to view data from

Logging Out

1) Click Log Out, which is located in the top right corner of the site

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

Data Security

This data is managed and viewed only by Sound Toxins volunteers and staff. You need to change your password every three 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 database.

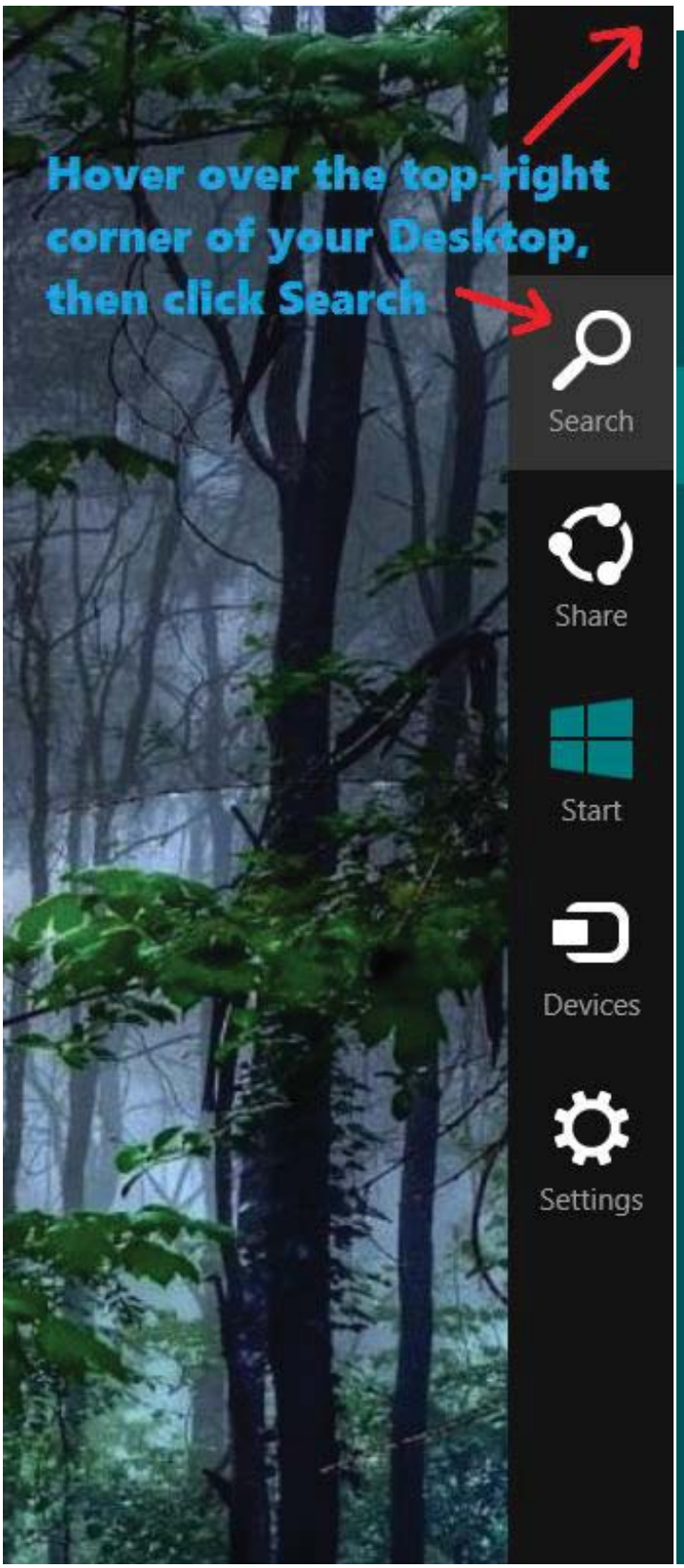
Equipment & Supplies List -
Updated May 2015

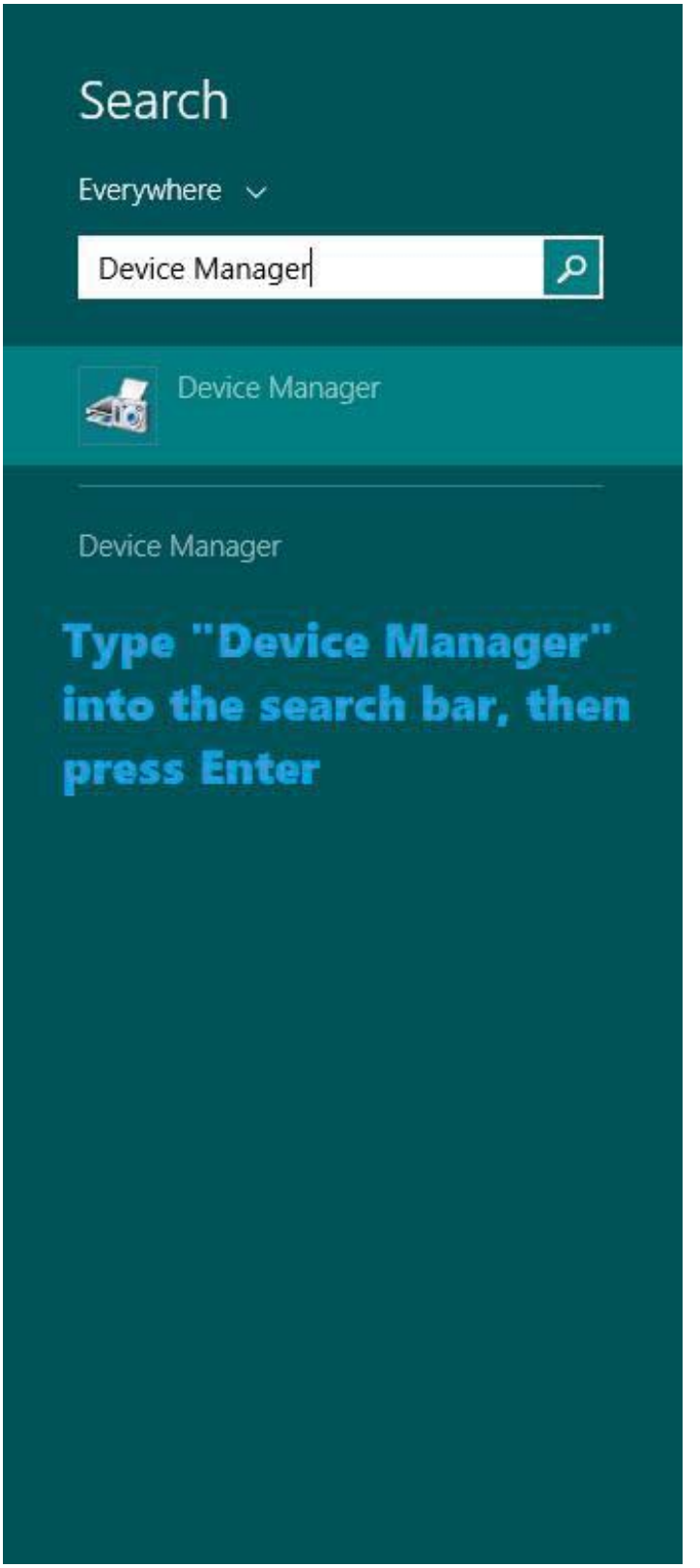
SEATT SUPPLY LIST

Equipment	Function	Parameter	Part #	Supplier	Contact info	Quantity	List Price	Total Price	NOTES
PSP ELISA kits	Toxin analysis of filters and/or shellfish to be analyzed by STA in our lab	Toxin	Abraxis 52255B	Abraxis LLC 54 Steamwhistle Dr., Warmister, PA 18974 USA	(215) 357-3911 www.abraxiskits.com	5	$570	$2,850	Have it shipped to CHRIS WHITEHEAD at :429 Katlian Street Sitka AK 99835
Filter funnels (47mm, 300mL capacity)	Filtering sample	Toxin	# 4242	Pall/Gelman Scientific	http://www.pall.com/main/laboratory/product.page?id=20098	3	$250	$750	
Filter rig 3-place filter manifold	Filtering sample	Toxin	# 09-753-39A	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1	$1,125	$1,125	Set up business account with Fisher Scientific, this will allow you to order everything on the list. (some items they will only ship if you are a buisness member)
Oilless Vacuum pump	Filtering sample	Toxin	# S63086	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1	$400	$400	
In Line vacu guard	Filtering sample	Toxin	09-744-75	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1 pack	$180	$180	
Stoppers #8	Filtering sample	Toxin	14-130M	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1 pack	$73	$73	
1 Liter Collection Bottle	Filtering sample	Toxin	03-311-1D	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10653	1 case	$119	$119	Check with your school system and ask if they have any of these items they would be willing to let you borrow or have. You really only need one of each, but Fisher sells them in cases.
2 Liter Collection Bottle (Field sampl)	Filtering sample	Toxin	03-309	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10654	1 case	$219	$219	
1 Liter graduated Cylinder	Filtering sample	Toxin	03-007-44	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10655	1	$63	$63	
Filter Forcepeps	Filtering sample	Toxin	XX62 000 06P	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10656	1 pack	$53	$53	
1/4 I.D. and 1/8 inch wall Nalgene clear tubing.	Filtering sample	Toxin	14-176-28	Fisher Scientific or Local hardware store	http://www.fishersci.com/ecomm/servlet/home?storeId=10657	1 pack	$236	$236	You can also find this at a local hardware store. You only need 8' of each size
Tubing 3/8 ID 1/8 wall	Filtering sample	Toxin	14-169-7H	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1 pack	$155	$155	
Carboy Heavy Duty 10L PP	Filtering sample	Toxin	02-960-14	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1	$231	$231	
Carboy Quick Filling/Venting Closures	Filtering sample	Toxin	02-923-19	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1	$139	$139	
Nucleopore HA (mixed cellulose ester)	Filtering sample	Toxin	HAWP04700	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1	$135	$135	
ID Book - Horner, Rita "A taxonomic guide..."	ID Book	ID	ISB #0-948737-65-4	Balogh International 1911 N. Duncan Rd., Champaign, IL 61822 USA	(217) 355-9331 www.balogh.com	1	$200	$200	
Secchi Disk	Turbidity measurement	Turbidity	Make	Local	Local	1	$100	$100	

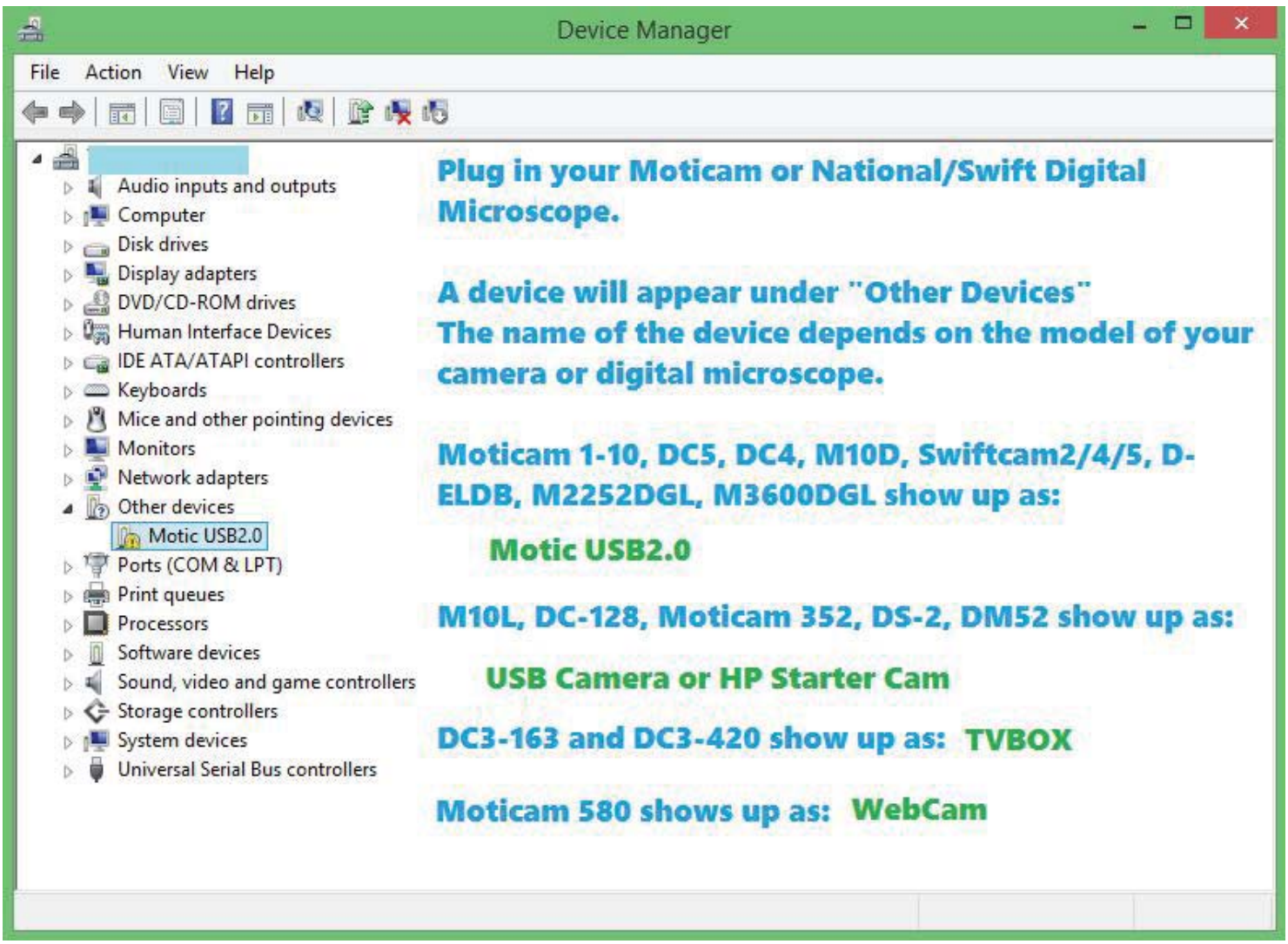
Plankton Net & Sample Bottles	Collect phyto sample. Student Plankton Net, 20 cm. dia. 3:1 #20 micron mesh, with cod end collar, hose clamp and 2 of the 8oz cod end bottles.	Phyto ID	#9120-20	Sea-Gear Corporation	http://www.sea-gear.net/student-nets.html	1	$150	$150	
Refractometer	Dual scale salt refractometer may be used to determine either the parts per thousand of salt dissolved in water or the specific gravity of salt water.	Salinity	#76273	Forestry Suppliers	http://www.forestry-suppliers.com	1	$108	$108	
Thermometer	Enviro-Safe® "Easy Read" Armor Case Thermometers.	Temperature	#89108	Forestry Suppliers	http://www.forestry-suppliers.com	1	$12	$12	
Ruled Microscope Slides	Gridwork on this slide allows you to easily keep track of the contents. The specially lined slides feature sixty-four 2mm squares.	microscopy	#S66585	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	5	$10	$50	
Swift M10-LMS Digital Microscope	Digital microscope that allows you to ID phyto species and transfer pictures directly to PC	microscopy	M10LM-S	Swift	http://www.swiftoptical.com/p-160-m10lm-s.aspx	1	$1,450	$1,450	
Glass or Plastic Cover Slips (pack of 100)	To cover slides	microscopy	S175211A	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/ho	2	$6	$12	
Transfer Pipets (1.5mL, 400count/pack)	Transfer sample bottle to slide	microscopy	13-711-25	Fisher Scientific	http://www.fishersci.com/ecomm/servlet/home?storeId=10652	1	80	$80	
Nylon Rope for net	25' to allow for net to swim	Field net tow	Local			1	5	$5	
String for thermometer	3' + to sink thermometer below surface	Field temp	Local			1	2	$2	
							TOTAL	$8,897	

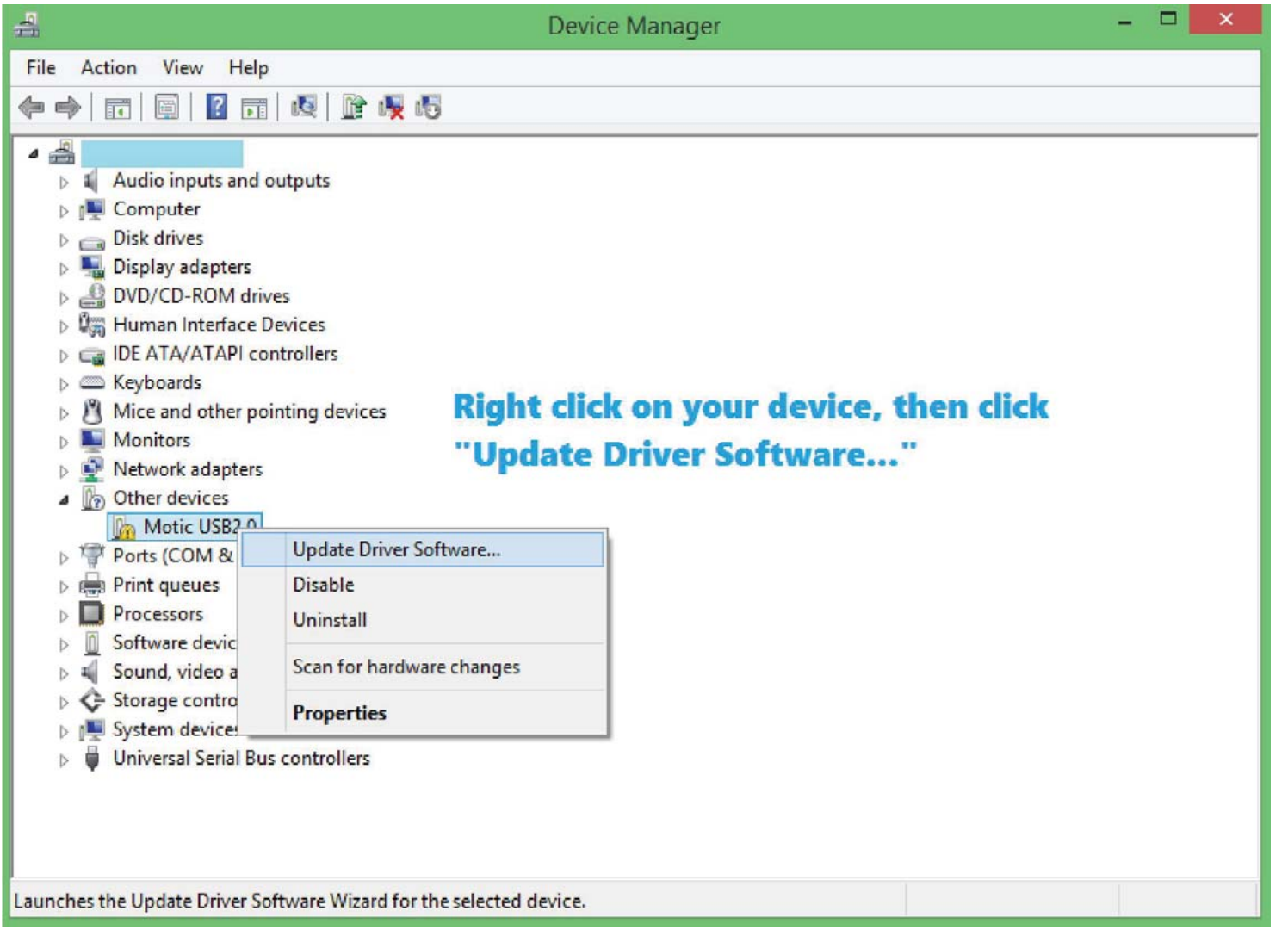
Windows 8 – Motic Driver Installation

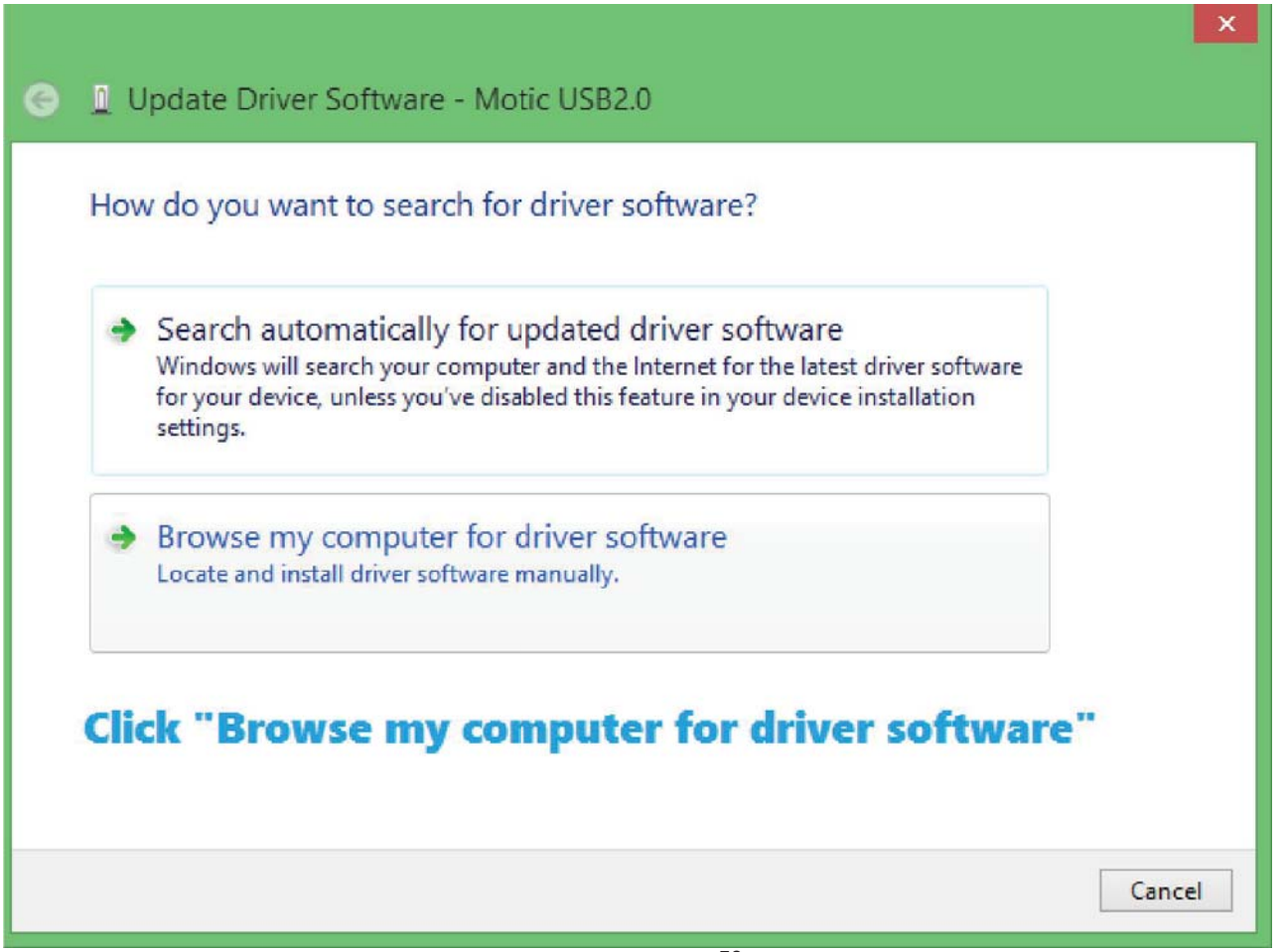


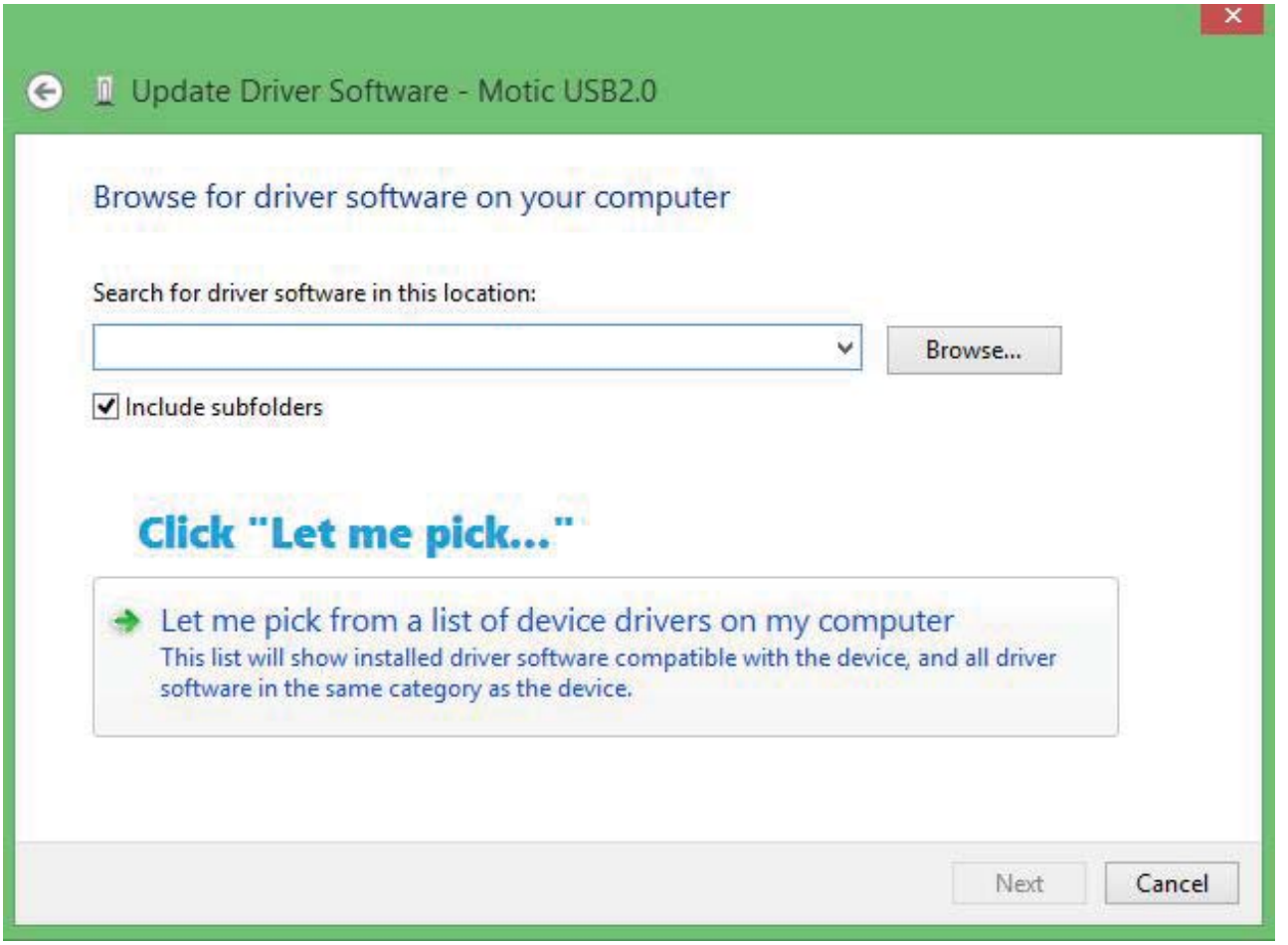


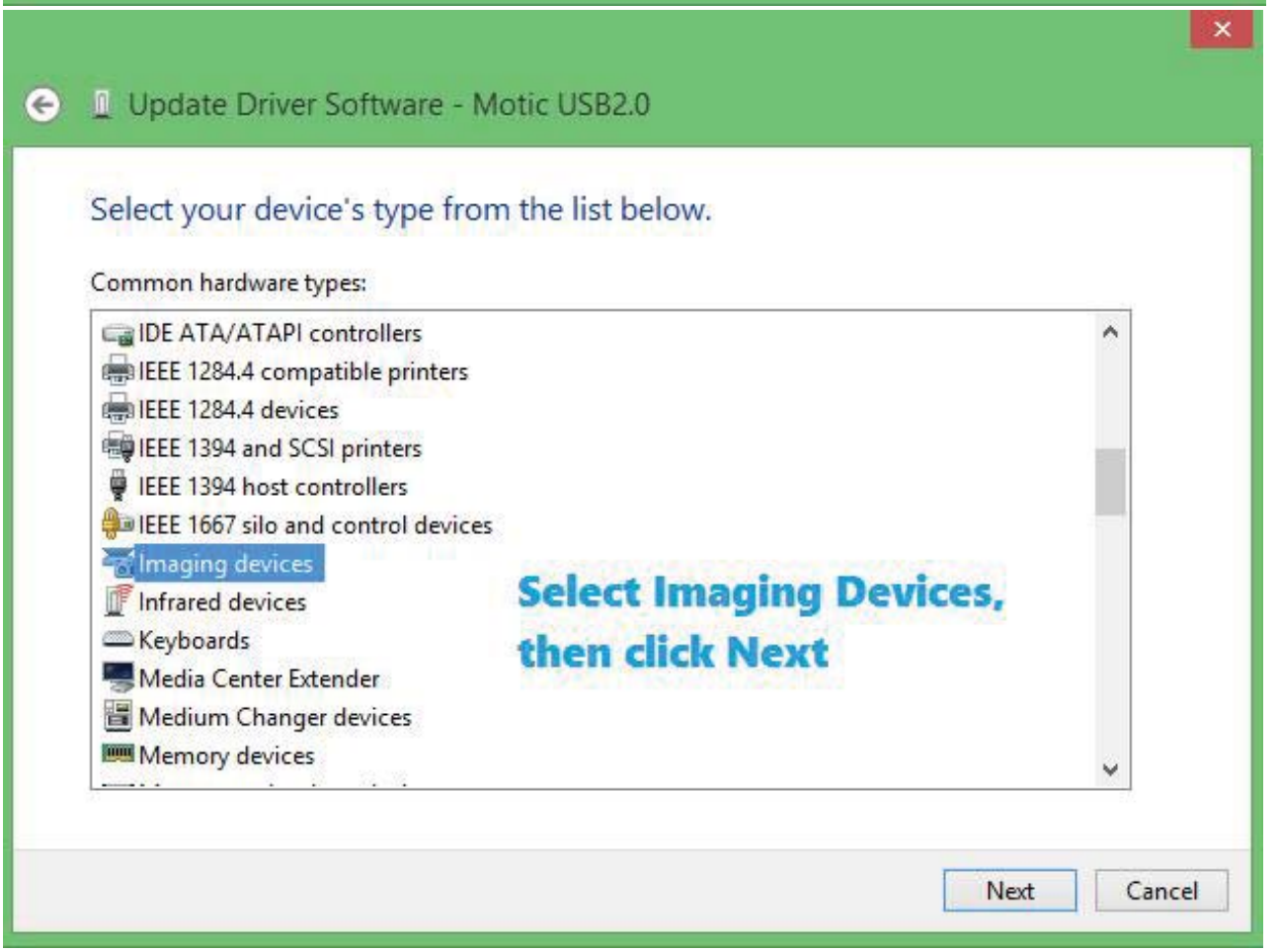
NOTE: If you are using Windows 7, click the Start button at the bottom left, then type Device Manager into the search bar located directly above the Start button, then press Enter.

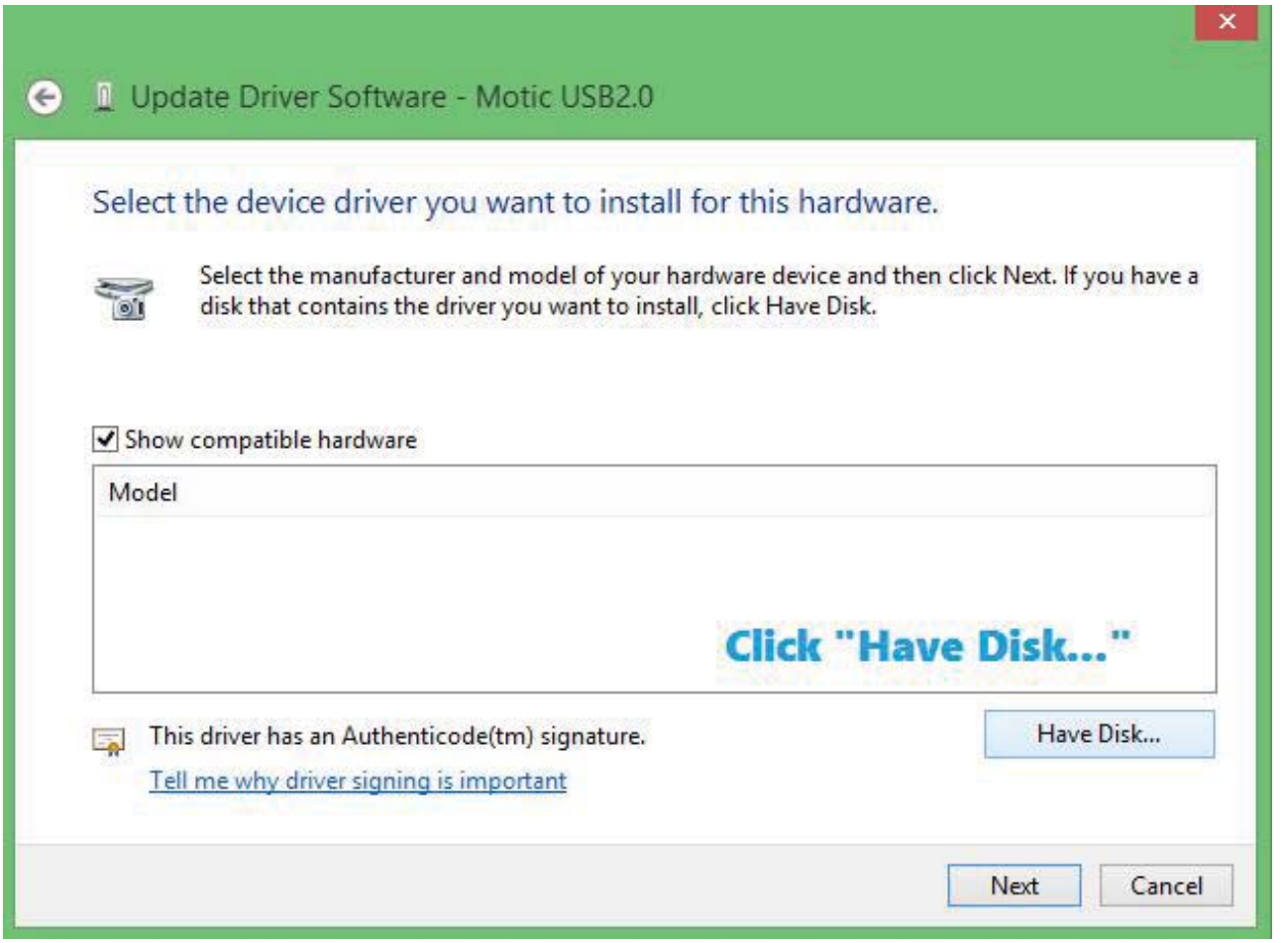

Device Manager
File Action View Help
Audio inputs and outputs
Computer
Disk drives
Display adapters
DVD/CD-ROM drives
Human Interface Devices
IDE ATA/ATAPI controllers
Keyboards
Mice and other pointing devices
Monitors
Network adapters
Other devices
Motic USB2.0
Ports (COM & LPT)
Print queues
Processors
Software devices
Sound, video and game controllers
Storage controllers
System devices
Universal Serial Bus controllers
Plug in your Moticam or National/Swift Digital Microscope.
A device will appear under "Other Devices"
The name of the device depends on the model of your camera or digital microscope.
Moticam 1-10, DC5, DC4, M10D, Swiftcam2/4/5, D-ELDB, M2252DGL, M3600DGL show up as:
Motic USB2.0
M10L, DC-128, Moticam 352, DS-2, DM52 show up as:
USB Camera or HP Starter Cam
DC3-163 and DC3-420 show up as: TVBOX
Moticam 580 shows up as: WebCam

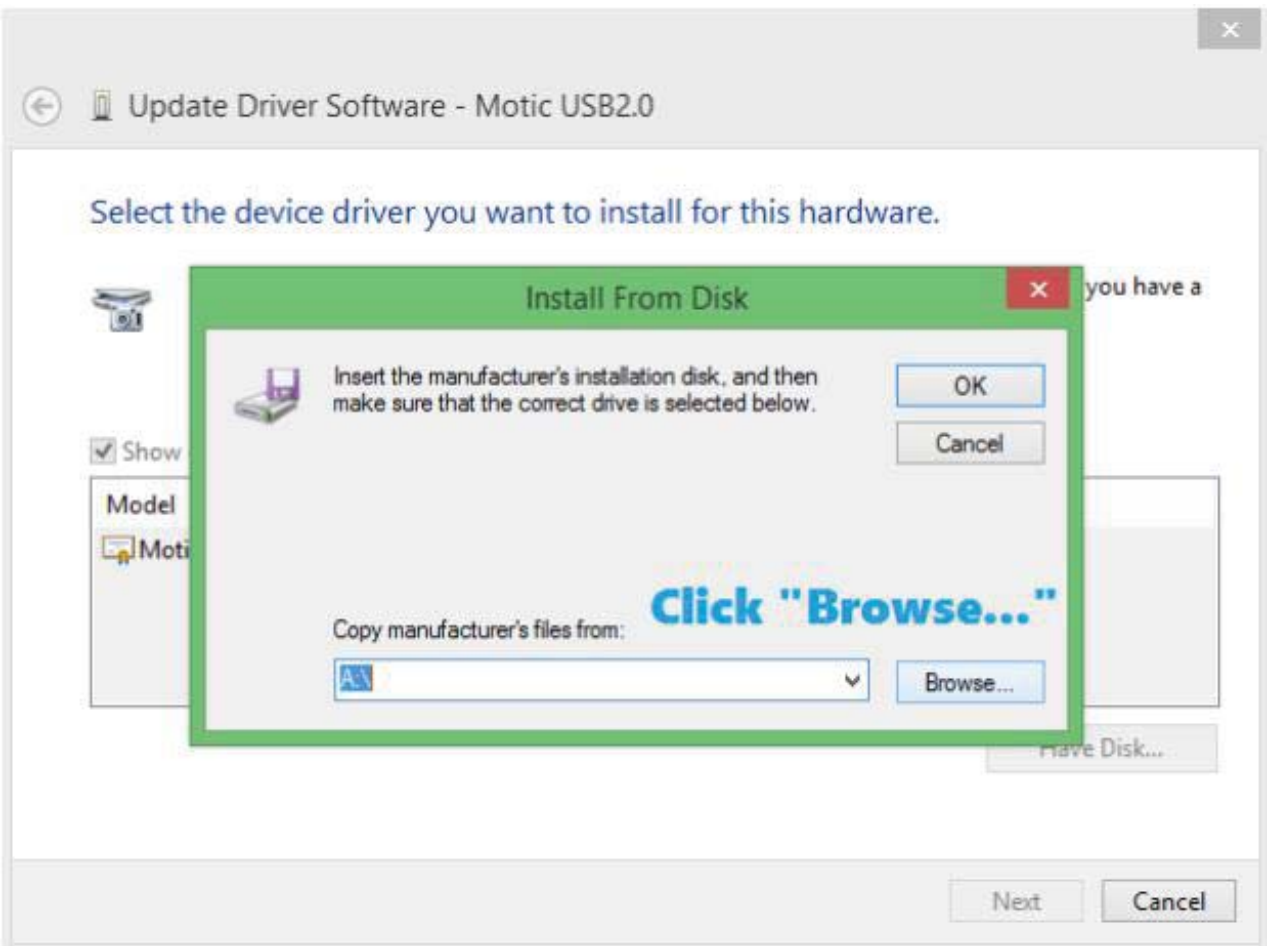

Device Manager
File Action View Help
Audio inputs and outputs
Computer
Disk drives
Display adapters
DVD/CD-ROM drives
Human Interface Devices
IDE ATA/ATAPI controllers
Keyboards
Mice and other pointing devices
Monitors
Network adapters
Other devices
Motic USB2.0
Ports (COM &
Print queues
Processors
Software devic
Sound, video a
Storage contro
System device
Universal Serial Bus controllers
Update Driver Software...
Disable
Uninstall
Scan for hardware changes
Properties
Right click on your device, then click "Update Driver Software..."
Launches the Update Driver Software Wizard for the selected device.

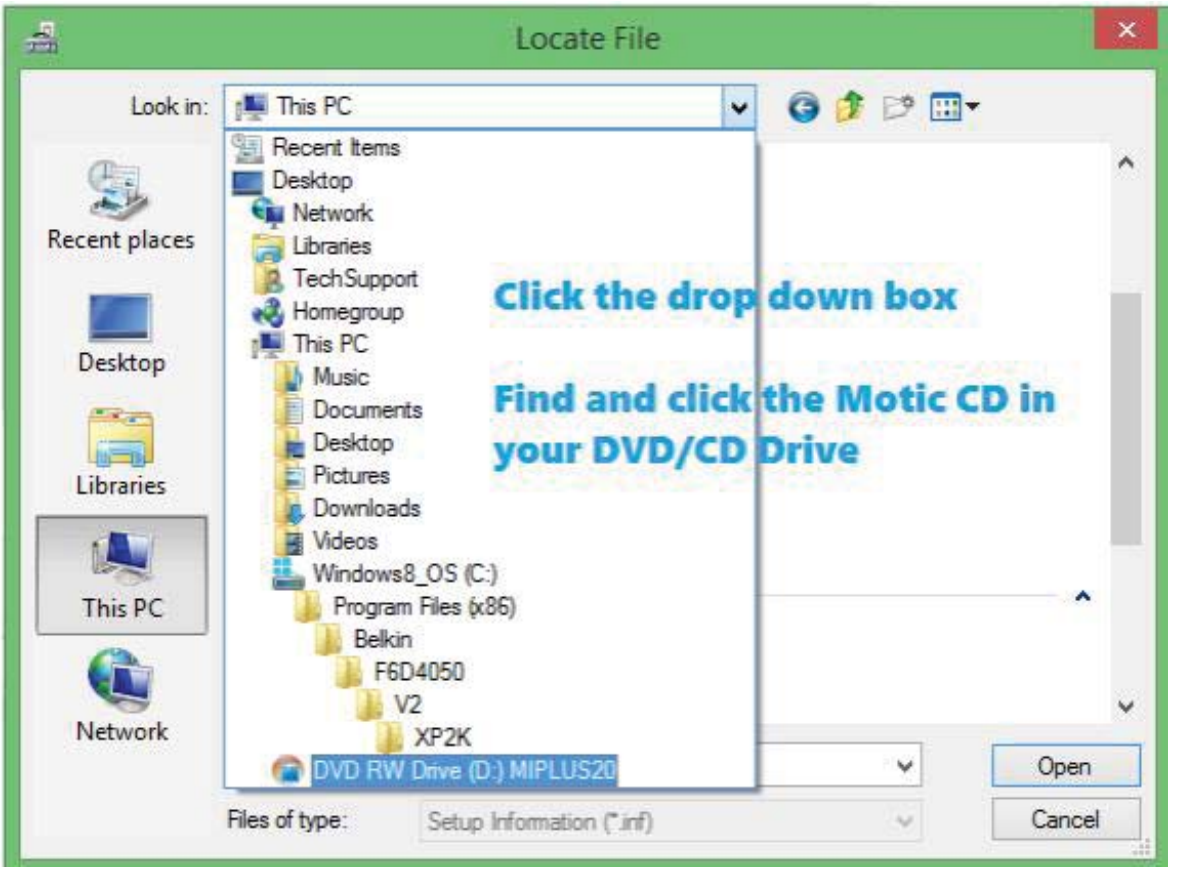

Update Driver Software - Motic USB2.0
How do you want to search for driver software?
Search automatically for updated driver software
Windows will search your computer and the Internet for the latest driver software for your device, unless you've disabled this feature in your device installation settings.
Browse my computer for driver software
Locate and install driver software manually.
Click "Browse my computer for driver software"
Cancel

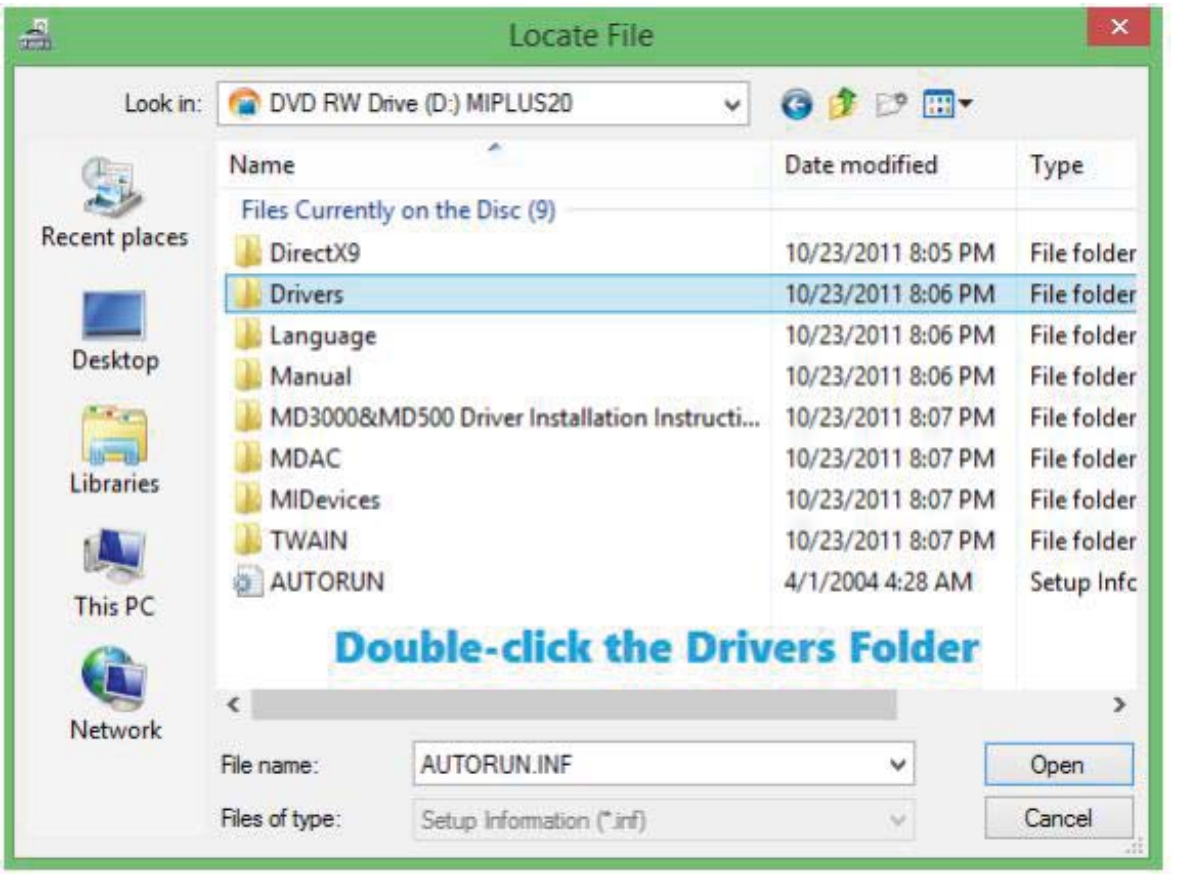

Update Driver Software - Motic USB2.0
Browse for driver software on your computer
Search for driver software in this location:
Browse...
Include subfolders
Click "Let me pick..."
Let me pick from a list of device drivers on my computer
This list will show installed driver software compatible with the device, and all driver software in the same category as the device.
Next
Cancel

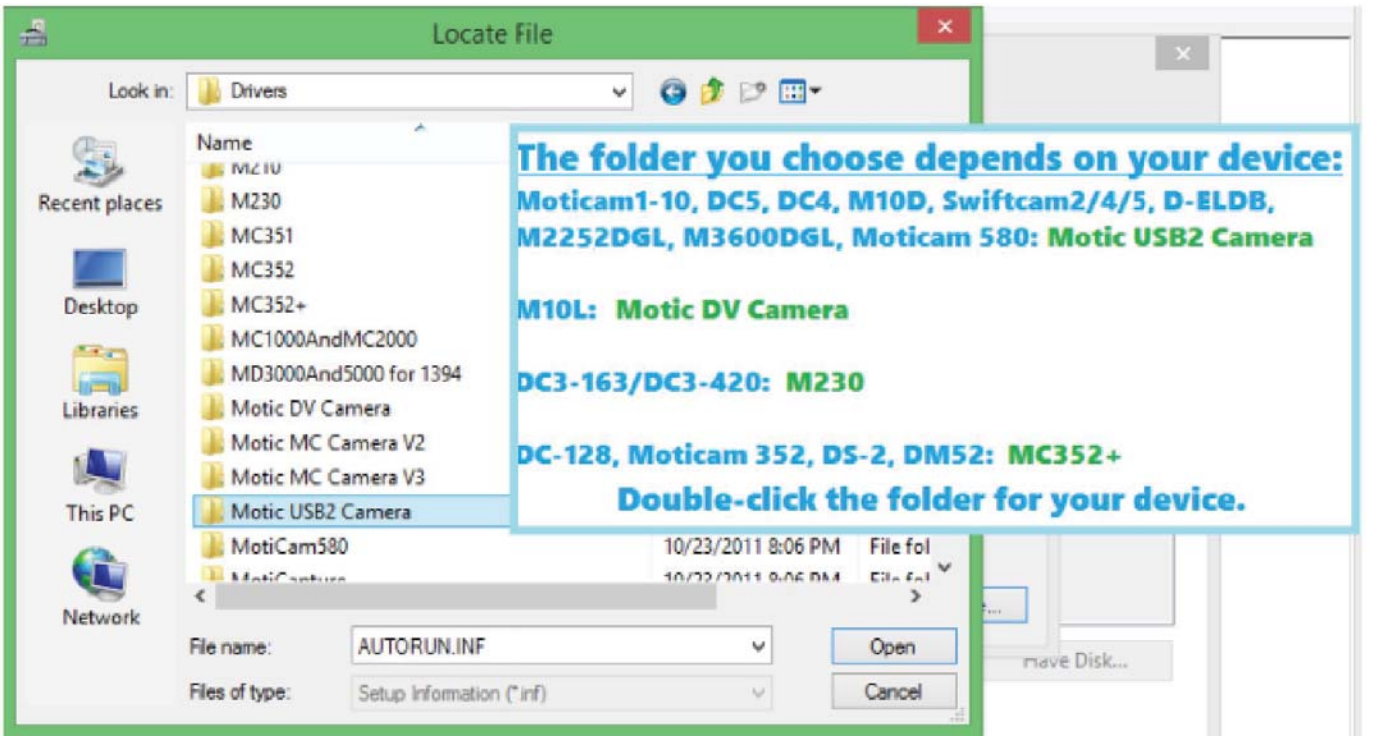

Update Driver Software - Motic USB2.0
Select your device's type from the list below.
Common hardware types:
IDE ATA/ATAPI controllers
IEEE 1284.4 compatible printers
IEEE 1284.4 devices
IEEE 1394 and SCSI printers
IEEE 1394 host controllers
IEEE 1667 silo and control devices
Imaging devices
Infrared devices
Keyboards
Media Center Extender
Medium Changer devices
Memory devices
Select Imaging Devices, then click Next
Next
Cancel

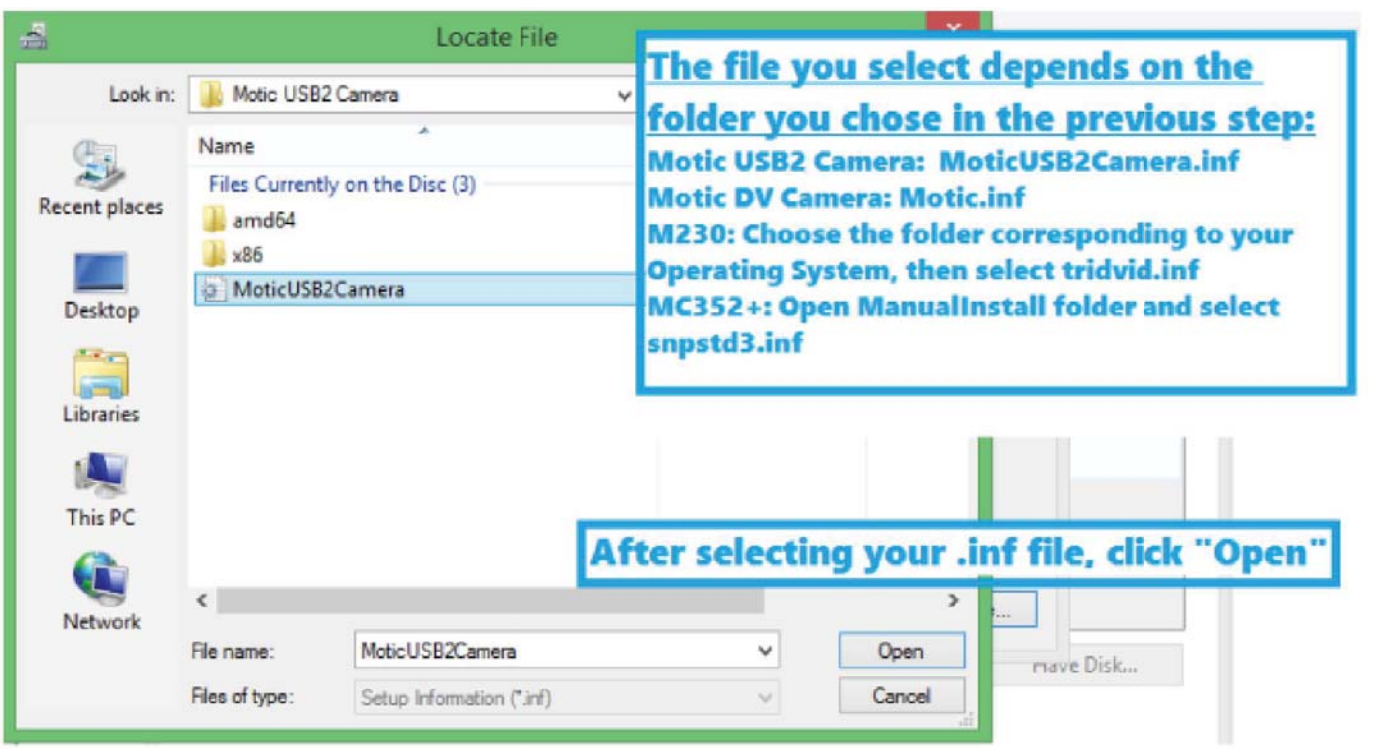

Update Driver Software - Motic USB2.0
Select the device driver you want to install for this hardware.
Select the manufacturer and model of your hardware device and then click Next. If you have a disk that contains the driver you want to install, click Have Disk.
Show compatible hardware
Model
Click "Have Disk..."
This driver has an Authenticode(tm) signature.
Tell me why driver signing is important
Have Disk...
Next
Cancel

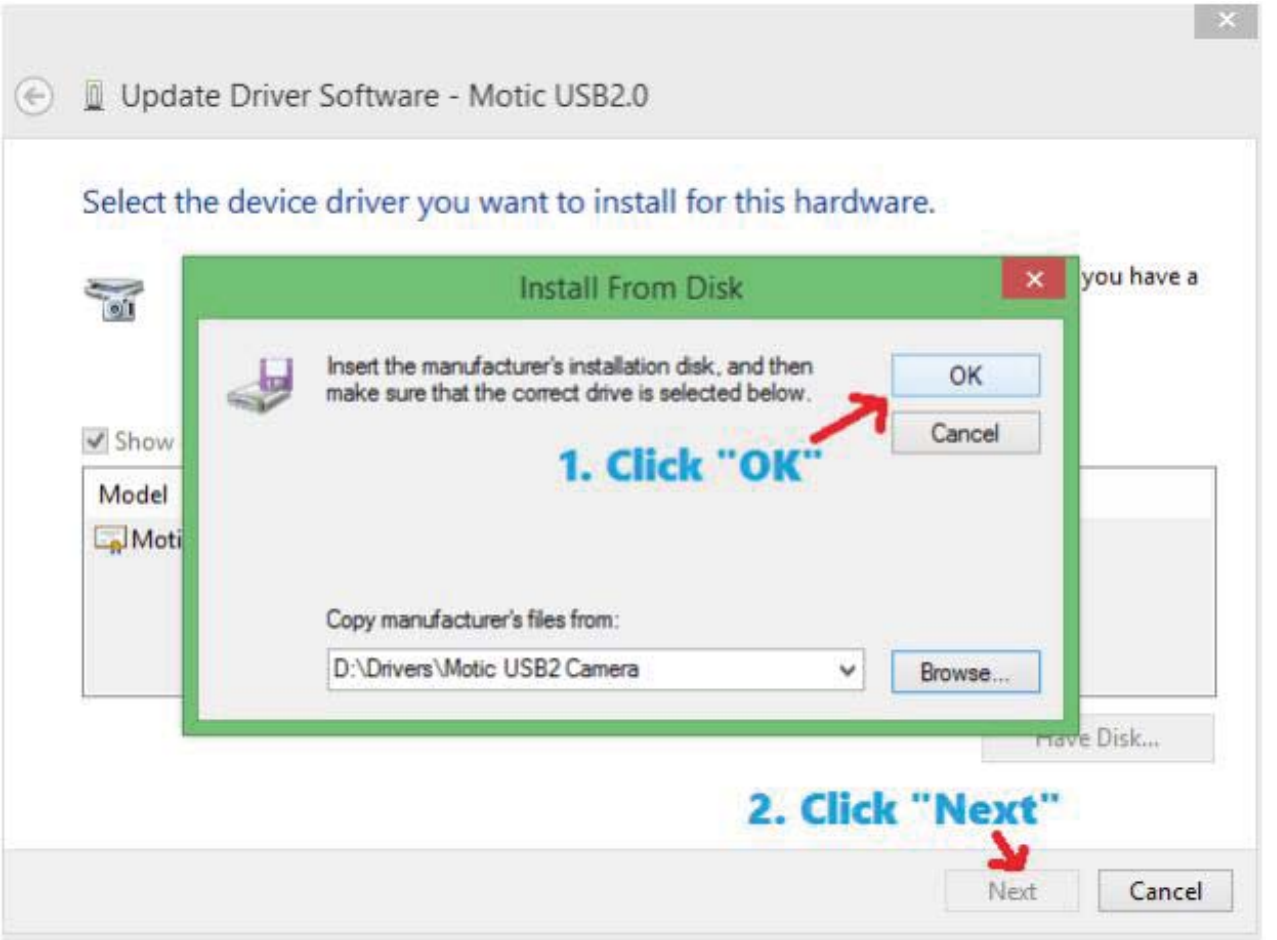

Update Driver Software - Motic USB2.0
Select the device driver you want to install for this hardware.
you have a
Install From Disk
Insert the manufacturer's installation disk, and then make sure that the correct drive is selected below.
OK
Cancel
Model
Copy manufacturer's files from:
Click "Browse..."
A:\
Browse...
Next
Cancel

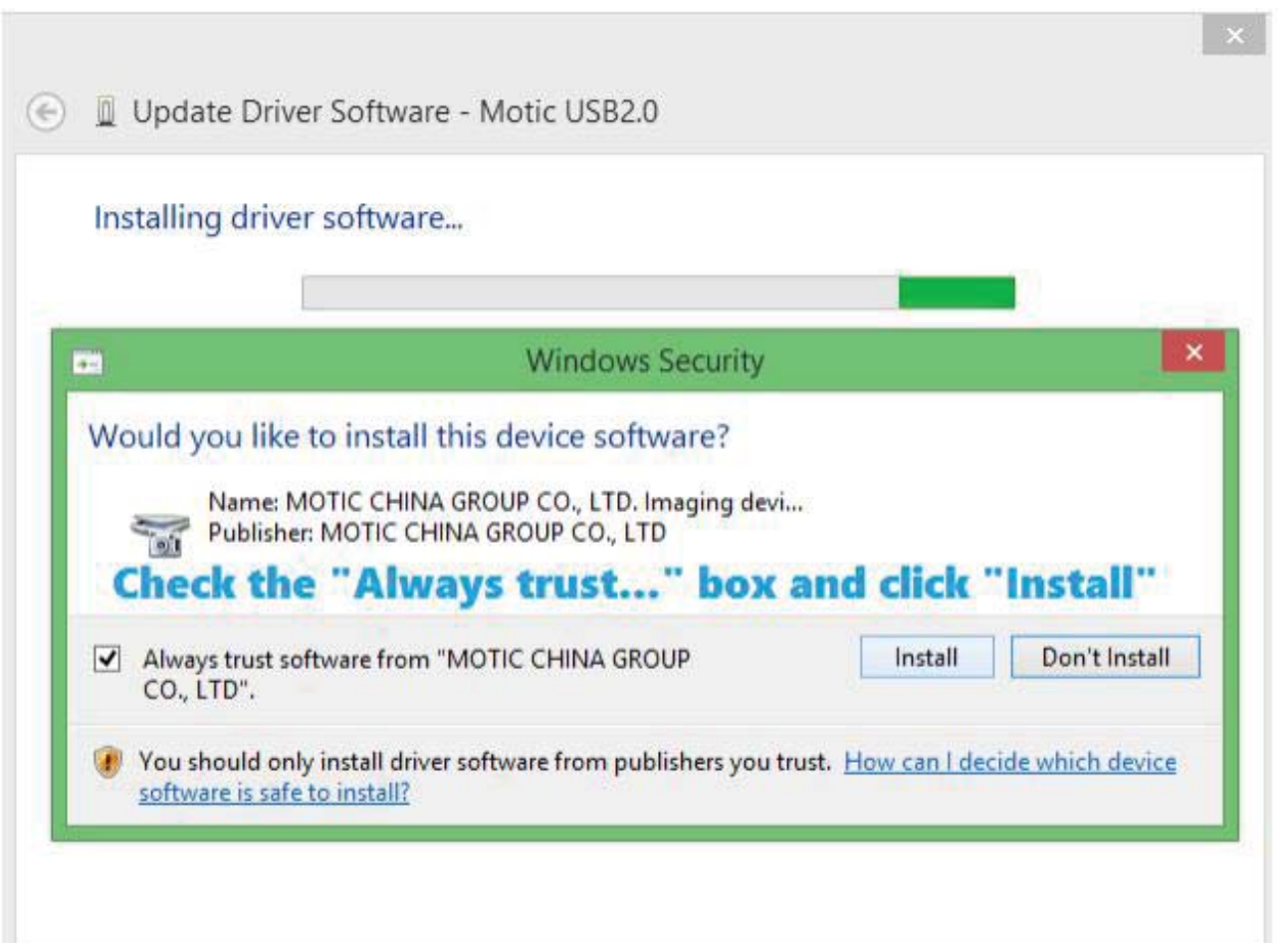

Locate File
Look in:
This PC
Recent Items
Desktop
Network
Libraries
TechSupport
Homegroup
This PC
Music
Documents
Desktop
Pictures
Downloads
Videos
Windows8_OS (C:)
Program Files (x86)
Belkin
F6D4050
V2
XP2K
DVD RW Drive (D:) MIPLUS20
Recent places
Desktop
Libraries
This PC
Network
Click the drop down box
Find and click the Motic CD in your DVD/CD Drive
Open
Cancel
Files of type:
Setup Information (*.inf)

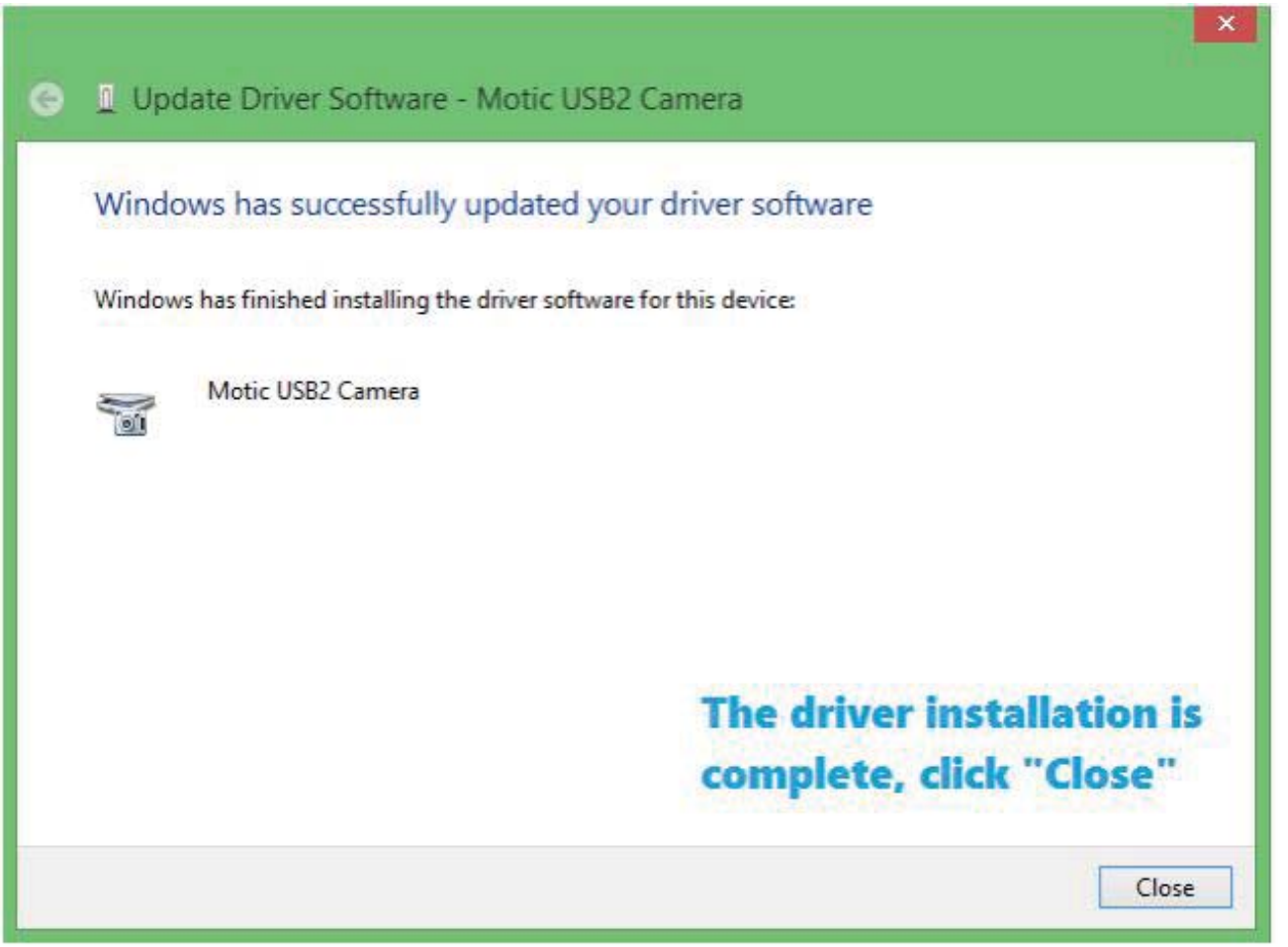

Locate File
Look in:
DVD RW Drive (D:) MIPLUS20
Name
Date modified
Type
Files Currently on the Disc (9)
DirectX9 10/23/2011 8:05 PM File folder
Drivers 10/23/2011 8:06 PM File folder
Language 10/23/2011 8:06 PM File folder
Manual 10/23/2011 8:06 PM File folder
MD3000&MD500 Driver Installation Instructi... 10/23/2011 8:07 PM File folder
MDAC 10/23/2011 8:07 PM File folder
MIDevices 10/23/2011 8:07 PM File folder
TWAIN 10/23/2011 8:07 PM File folder
AUTORUN 4/1/2004 4:28 AM Setup Infc
Recent places
Desktop
Libraries
This PC
Network
Double-click the Drivers Folder
File name:
AUTORUN.INF
Files of type:
Setup Information (*.inf)
Open
Cancel


Locate File
Look in: Drivers
Recent places
Desktop
Libraries
This PC
Network
Name
M230
MC351
MC352
MC352+
MC1000AndMC2000
MD3000And5000 for 1394
Motic DV Camera
Motic MC Camera V2
Motic MC Camera V3
Motic USB2 Camera
MotiCam580
10/23/2011 8:06 PM
File fol
File name: AUTORUN.INF
Files of type: Setup Information (*.inf)
Open
Cancel
The folder you choose depends on your device:
Moticam1-10, DC5, DC4, M10D, Swiftcam2/4/5, D-ELDB, M2252DGL, M3600DGL, Moticam 580: Motic USB2 Camera
M10L: Motic DV Camera
DC3-163/DC3-420: M230
DC-128, Moticam 352, DS-2, DM52: MC352+
Double-click the folder for your device.


Locate File
Look in: Motic USB2 Camera
Recent places
Desktop
Libraries
This PC
Network
Name
Files Currently on the Disc (3)
amd64
x86
MoticUSB2Camera
File name: MoticUSB2Camera
Files of type: Setup Information (*.inf)
Open
Cancel
The file you select depends on the folder you chose in the previous step:
Motic USB2 Camera: MoticUSB2Camera.inf
Motic DV Camera: Motic.inf
M230: Choose the folder corresponding to your Operating System, then select tridvid.inf
MC352+: Open ManualInstall folder and select snpstd3.inf
After selecting your .inf file, click "Open"


Update Driver Software - Motic USB2.0
Select the device driver you want to install for this hardware.
you have a
Install From Disk
Insert the manufacturer's installation disk, and then make sure that the correct drive is selected below.
OK
Cancel
1. Click "OK"
Show
Model
Moti
Copy manufacturer's files from:
D:\Drivers\Motic USB2 Camera
Browse...
Have Disk...
2. Click "Next"
Next
Cancel


Update Driver Software - Motic USB2.0
Installing driver software...
Windows Security
Would you like to install this device software?
Name: MOTIC CHINA GROUP CO., LTD. Imaging devi...
Publisher: MOTIC CHINA GROUP CO., LTD
Check the "Always trust..." box and click "Install"
Always trust software from "MOTIC CHINA GROUP CO., LTD".
Install
Don't Install
You should only install driver software from publishers you trust. How can I decide which device software is safe to install?


Update Driver Software - Motic USB2 Camera
Windows has successfully updated your driver software
Windows has finished installing the driver software for this device:
Motic USB2 Camera
The driver installation is complete, click "Close"
Close