This document is Chapter 19 of the Volunteer Estuary Monitoring Manual, A Methods Manual, Second Edition, EPA-842-B-06-003. The full document can be downloaded from: http://www.epa.gov/owow/estuaries/monitor/

Voluntary Estuary Monitoring Manual

Chapter 19: Other Living Organisms Macroinvertebrates, Phytoplankon and Non-indigenous Species

March 2006

Chapter 19

Other Living Organisms

While bacteria and submerged aquatic vegetation are popular biological parameters measured by volunteers, there are other living organisms that deserve—and receive—attention. Some of these organisms are monitored or collected to screen for potential problems in the estuary. Others serve to complement chemical, biological, and physical monitoring activities. For the most part, the monitoring of particular living organisms represents localized rather than nationwide efforts; that is, for many reasons, volunteer groups have the desire, equipment, support, and environmental need to work with these organisms in their particular estuary.

Photos (l to r): E. Ely, E. Ely, T. Monk, R. Ohrel

Overview

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For the most part, the monitoring of particular living organisms represents localized rather than nationwide efforts; that is, for many reasons, volunteer groups have the desire, equipment, support, and environmental need to work with these organisms in their particular estuary.

Clearly, there is a multitude of living organisms that a volunteer group may wish to monitor to help assess an estuary's health. This chapter discusses several of those biological parameters—macroinvertebrates, phytoplankton, and non-indigenous species—and describes their use as environmental health indicators, identifies sampling considerations, and provides steps for collecting and analyzing the organisms in the field.

Why Monitor Other Living Organisms?

The presence, absence, and abundance of many living organisms can serve as useful indicators of estuarine health. Some organisms require relatively clean water to survive, grow, and reproduce. Their presence suggests that water quality is good in that portion of the estuary. Other species are unfazed or even thrive under poor water quality conditions. If the number of pollution-tolerant organisms suddenly increases while pollution-sensitive species disappear or become difficult to find, the estuary may be under stress.

Volunteer monitoring of estuarine organisms, then, can serve as an early warning device. When biological monitoring suggests that a water quality problem may exist, the information can be used to alert government authorities, who in turn can intensify their own monitoring efforts to identify the problem's cause and solution. The identification and tracking of non-indigenous species can be used to further alert us to human disturbance of estuarine ecosystems. Early detection networks can help eradicate a non-indigenous species invasion before it becomes established.

Monitoring can also be used to complement chemical, biological, and physical measurements. Nutrient data, for example, provide useful information about the types and quantities of nutrients in the estuary, but may not tell us enough about potential impacts. The presence of phytoplankton blooms provides evidence that nutrient concentrations may have reached high levels.

As another example, turbidity and sediment deposition can affect the survival of many bottom-dwelling organisms. By monitoring these animals along with other parameters, we can gain a better sense of the estuary's overall health.

Finally, the collection and analysis of shellfish for pathogens and toxic materials complement monitoring for those pollutants in the water column. ■

MACROINVERTEBRATES

Macroinvertebrates are organisms that are large *(macro*) enough to be seen with the naked eye and lack a backbone *(invertebrate*) (USEPA, 1997). Aquatic macroinvertebrates are commonly used in freshwater stream monitoring as indicators of water quality. According to the USEPA (1997), macroinvertebrates make good indicators of stream quality because:

- They are affected by the physical, chemical, and biological conditions in the stream.
- They may show the effects of short- and long-term pollution.
- They may show the cumulative impacts of pollution.
- They may show the impacts from habitat loss not detected by traditional water quality assessments.
- They are a critical part of the food web.
- Some are very intolerant of and cannot escape pollution.

The Role of Macroinvertebrates in the Estuarine Ecosystem

Macroinvertebrates serve many of the same functions in estuarine systems as they do in streams. They are critical to the food web. Some impact water clarity through their feeding process, filtering with their gills or other body parts tiny plants, animals, and

other materials found in the water. Others, such as oysters and corals, grow together in groups, providing valuable habitat for a number of organisms. Burrowing macroinvertebrates help aerate bottom sediments.

Unfortunately, using macroinvertebrates as indicators of estuarine health is more problematic than for streams (Green, 1998; Ely, 1991).

- Estuaries support different macroinvertebrates than freshwater systems, with few key freshwater indicator species living in estuarine environments.
- Tidal fluctuations and muddy bottoms make collecting estuarine macroinvertebrates more difficult than in streams.
- In contrast with stream systems, there are as yet no identification keys and water quality indices, suitable for volunteers, that link estuarine macroinvertebrates with estuarine health.

As a result of these limitations, volunteer organizations currently find it difficult to use macroinvertebrates as indicators of estuarine water quality. However, that is not to say that volunteer programs should avoid monitoring macroinvertebrates altogether. Volunteer monitors are frequently recruited to monitor specific macroinvertebrate species, such as corals (see case study, below). Shellfish are other good examples of estuarine macroinvertebrates that volunteer groups monitor and sample.

Shellfish and Estuarine Health

Shellfish often reflect some of the most important measures of water quality. One way that volunteers can utilize shellfish is to take an inventory of their distribution throughout the estuary (see case study, page 19-4). Another way is to work with laboratories that analyze the hazardous compounds in the animals' tissues.

Case Study: Coral Monitoring

With support from the U.S. Environmental Protection Agency (EPA), The Ocean Conservancy manages the Reef Condition (RECON) Monitoring Program. RECON is an entry-level rapid-assessment protocol for volunteer recreational divers with an interest in reef conservation issues. The goals of RECON are to broaden the scope of available information about the benthic (bottom-dwelling) organisms on coral reefs, to alert local researchers and managers of changing reef conditions (e.g., mass bleaching events, outbreaks of disease, nuisance algal blooms), and to increase public understanding of the threats to coral reef ecosystems.

RECON divers take a short course from a certified RECON instructor, followed by two practice dives and a qualifying examination. Divers are trained to collect information about the reef environment, the health of stony corals, and the presence of key reef organisms and obvious human-induced impacts. Results of the cumulative data collection are posted on The Ocean Conservancy Web site for public access and archived for use by the scientific and research community.

For More Information:

The Ocean Conservancy
Office of Pollution Prevention and Monitoring
1432 N. Great Neck Road, Suite 103
Virginia Beach, VA 23454
Phone: 757-496-0920, Fax: 757-496-3207
Email: RECON@oceanconservancyva.org
http://www.oceanconservancy.org

A volunteer diver collects data at a coral reef in the Caribbean (photo by T. Monk).

Case Study: Shellfish Inventories in Florida

While some shellfish monitoring programs collect specimens for tissue analysis at a laboratory, others require only that volunteers count each organism they find in the field.

Tampa BayWatch and the Tampa Bay Estuary Program developed a volunteer activity known as the Great Bay Scallop Search. During this annual one-day event, volunteer snorkelers patrol seagrass beds and count scallops along transect lines (see Appendix A for the Scallop Search data sheet).

The purpose of the project is to document scallop population recovery. Poor water quality caused scallops to disappear from Tampa Bay during the 1960s. Thanks in part to regulatory action, the scallops are slowly returning. Stocking efforts are underway to help boost scallop recovery and establish viable breeding colonies in the bay.

For More Information:

Tampa BayWatch
Phone: 727-896-5320
http://www.tampabaywatch.org

Tampa Bay Estuary Program
Phone: 727-893-2765
http://www.tbep.org/

Chemical Uptake

Even water that appears clear and untainted may still contain harmful levels of chemical pollutants. Shellfish living in the water may assimilate and accumulate these chemicals through the intake of polluted water and sediment or by eating other contaminated organisms.

Several types of chemical contaminants can accumulate in shellfish. These include:

- heavy metals such as mercury and cadmium;
- petroleum hydrocarbons such as polyaromatic hydrocarbons (PAHs);
- pesticides such as endrin, dieldrin, endosulfan, mirex, and malathion; and
- industrial pollutants such as polychlorinated biphenyls (PCBs).

Bivalve shellfish, such as clams, mussels, and oysters, are filter-feeders and strain large quantities of estuarine water through their systems to extract small particles of food. Because they filter such large quantities of water, however, even relatively low concentrations of a waterborne contaminant may eventually translate to high tissue concentrations.

Non-bivalve shellfish, such as crabs, lobsters, and shrimp, are mobile scavengers which consume plants, small animals, and detritus from the estuary's waters and bottom. Contaminated prey or sediments can produce high contaminant levels in the tissues of these shellfish.

Biological Uptake

Studies or surveys often use shellfish as indicators of biological contamination as well. The non-mobile bivalves are particularly helpful as they pinpoint specific areas of contamination.

Shellfish collect fecal bacteria in their gut, making them good indicators of recent exposure to sewage waste. Since fecal coliforms can indicate the presence of human or animal pathogens, tainted shellfish serve as a warning and signal that an area may not be suitable for recreation or fishing. Unlike water sampling for bacterial contamination (see Chapter 17), shellfish tissue analysis acts as a market test; that is, it determines whether the shellfish are fit for human consumption.

Officials can set predetermined levels of fecal coliform in shellfish as a management standard. Areas where levels exceed this standard should be closed to commercial and recreational shellfish collection until the problem is resolved.

In addition to accumulating bacteria, shellfish can also consume phytoplankton, some of which produce toxins. When shellfish ingest the phytoplankton, the toxins accumulate in their tissues. The toxins can be transferred to humans who consume the shellfish. ■

Shellfish contaminated with pathogens or other pollution indicate that surrounding waters may be unsafe for fishing or swimming. It may be unsafe for humans to eat the shellfish (photo by R. Ohrel).

Shellfish Sampling Considerations

Shellfish are easier to collect than finfish because most tend to move more slowly or not at all. Moreover, they often congregate—an oyster bed or a boulder studded with mussels are two examples—and are fairly easy to reach.

Some shellfish are more susceptible to certain contaminants than others. While a species may easily tolerate high concentrations of one chemical, low concentrations of another can be lethal.

The life stage of an individual—larva, juvenile, or adult—will also greatly affect its response to a toxic substance. In general, larvae and juveniles are more vulnerable to injury or death from exposure to these substances. Studies of the effects of toxic compounds must consider both the age and species of the specimens to fully assess the chemical's toxicity. ■

Helpful Hint

Before collecting any organism, check with the appropriate government agency to determine whether you will need a permit.

How to Collect Shellfish

The tests for shellfish contaminants require sophisticated analyses, expensive equipment, and rigorous quality assurance procedures. Trained scientists must perform these tests to ensure accurate, scientifically valid results. Volunteers can assist the scientists, however, by collecting shellfish for analysis in designated study areas.

Scientists may need data to:

- identify areas of concern for a particular toxic substance;
- aid policy makers in setting regulatory limits on the recreational or commercial collection of shellfish species;
- identify the contaminant sources;
- examine the effects of particular contaminants on a species; and
- determine whether shellfish are safe for consumption.

The training of volunteers should include a session on the identification of the species required for testing. Most of the popular field guides for the coastal regions include sections on shellfish identification.

Before proceeding to the monitoring site and collecting samples, volunteers should review the topics addressed in Chapter 7. It is critical to confirm the monitoring site, date, and time; have the necessary monitoring equipment and personal gear; and understand all safety considerations. Once at the monitoring site, volunteers should record general site observations, as discussed in Chapter 7.

STEP 1: *Check equipment.*

In addition to the standard sampling equipment and apparel listed in Chapter 7, the volunteer should bring the following items to the site for each sampling session:

- field guide for species identification;
- waterproof copy of any required collection permits;
- collection bucket;
- tools necessary to dig shellfish, dislodge clustered shellfish, or otherwise collect targeted shellfish species;
- sample containers; and
- sample preservative, if necessary.

STEP 2: *Collect the shellfish sample.*

Once at the sample site, volunteers should capture animals using the method designated by the program manager.

The volunteers should carefully label the sample container in which the animal will be transported with the date, site name, shellfish type, and the name of the collector. An indelible marker is best for ensuring that the labeling is permanent. Make sure that the sample container is not wet before using the marker. If required by the program manager, volunteers should add a preservative to the sample bottle.

STEP 3: *Clean up and send off sample and data.*

Volunteers should transport the live specimens at chilled temperatures appropriate for the species. The program manager should designate pickup locations for volunteers to deliver the specimens and supporting data sheets to program personnel. As with all data sheets, the volunteer should make a duplicate in case the original becomes lost.

Make sure to thoroughly clean all equipment. ■

Case Study: Shellfish Collection in Washington and Alaska

Very few programs currently use volunteers to collect shellfish for laboratory analysis. The Department of Health in Washington State, however, has successfully worked with different volunteer groups to gather shellfish at commercial and recreational beaches.

Youth Area Watch, managed by the Chugach School District, in Alaska, is also involved with shellfish collection. Students in the organization collect mussels for scientists who monitor planktonic activity and production capacity in Prince William Sound.

For More Information:

Washington State Department of Health
Food Safety and Shellfish Programs
Phone: 360-236-3330

Adopt A Beach
4649 Sunnyside Ave. N. #305
Seattle, WA 98103-6900
Phone: 888-57-BEACH or 206-632-1390
Email: aab@halcyon.com

Youth Area Watch
Web site: http://www.micronet.net/users/~yaw/

PHYTOPLANKTON

Organisms lacking the means to counteract transport by water currents are referred to as **plankton**, a name derived from the Greek word planktos for "wandering." Included in this group are bacterioplankton (bacteria), phytoplankton (plants), and zooplankton (animals). In general all plankton are very small and, in many cases, microscopic. However, relatively large animals, such as the jellyfish, are also included in the definition of plankton (Table 19-1; Figure 19-1).

Phytoplankton are microscopic plants that are common components of our natural waters. These plants are algae and contain an assortment of pigments in their photosynthetic cells. They are represented by single cell or colonial forms that are the primary food and oxygen producers within freshwater, estuarine, and marine

(a)

(b)

Volunteer phytoplankton monitors at work in Maine. (a) Samples are collected using a 1-meter plankton net. (b) Subsamples are examined immediately at 100X magnification (photos by E. Ely).

Table 19-1. Common types of phyto- and zooplankton *(see Levinton, 1982).*

	Taxonomic Grouping		Taxonomic Grouping	
Phytoplankton		diatoms	*Biddulphia* *Nitzschia* *Thalassiosira*	*Chaetoceros* *Skeletomena* *Melosira*
		dinoflagellates	*Dinophysis* *Gyrodinium*	*Ceratium* *Prorocentrum*
		cryptomonads	*Cryptomonas*	
		coccolithophorids	*Coccolithus*	*Phaeocystis*
		green algae	*Chlorella* *Cladophora*	*Codium*
		blue-green algae	*Oscillatoria/Trichodesmium*	*Lyngbya*
		red algae	*Porphyridium*	*Porphyra*
		brown algae	*Ectocarpus*	
Zooplankton	Crustaceans	copepods	*Calanus* *Temora*	*Acartia*
		euphausiids	*Euphausia*	
		cladocerans	*Podon*	
		amphipods	*Euthemisto*	*Hyperia*
	Protistans	radiolarians	*Globigerina*	
		foraminiferans		
	Ctenophores	comb jellies	*Pleurobrachia*	*Mnemiopsis*
	Chaetognaths	arrow worms		
	Coelentertates	true jellyfish	*Aurelia*	*Physalia*
	Pteropods			
	Tunicates	larvacea salps	*Pyrosoma*	

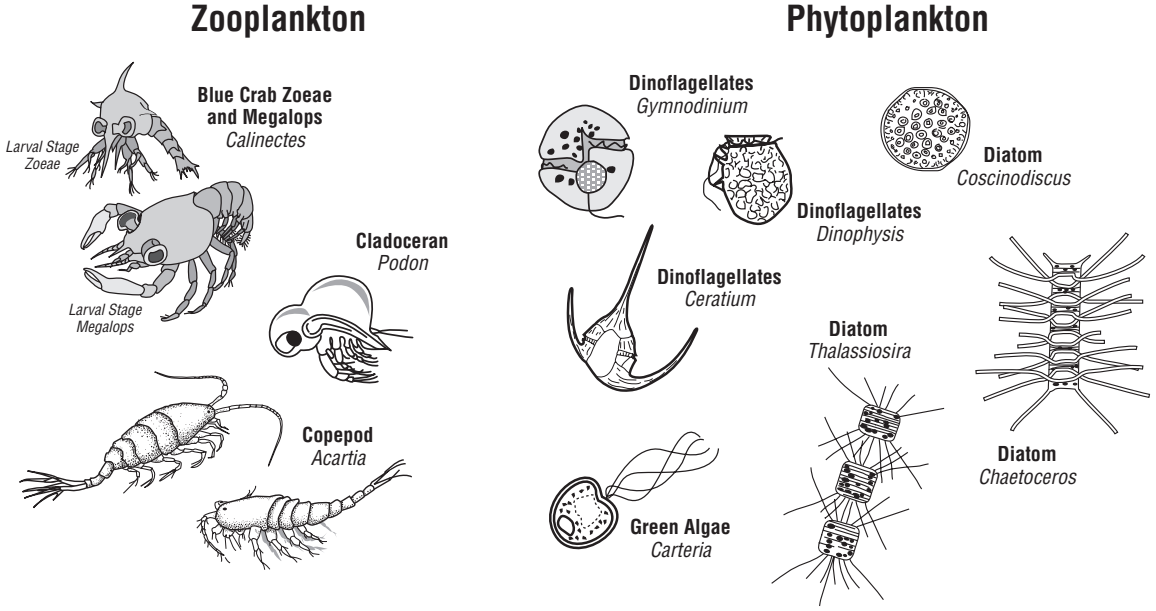

Figure 19-1. Examples of various planktonic forms found in coastal and estuarine waters.

habitats. **Zooplankton** are animal plankton that range in size and complexity. They include the larval forms of large adult organisms (e.g., crabs, fish) and small animals that never get larger than several millimeters (e.g. copepods).

The abundance of planktonic organisms in the water column follows predictable, geographically based seasonal patterns related to nutrients, light intensity, temperature, and grazing (predation) pressures. Monitoring the types and relative abundances of plankton populations in conjunction with nutrient and other environmental parameters can provide significant insight into the health of an aquatic ecosystem.

The Role of Phytoplankton in the Estuarine Ecosystem

Without phytoplankton, the intricate web of estuarine plants and animals would collapse. Phytoplankton are primary producers and form the base of the estuary's food pyramid. As photosynthesizers, phytoplankton transfer the sun's energy into plant matter and provide nourishment for the next level of organisms.

Phytoplankton are consumed primarily by zooplankton, which in turn are fed upon by other larger organisms. If the phytoplankton community is altered in composition or abundance, these changes may have serious ramifications throughout the food web and upset what may be considered a more favorable balance of life in these waters.

Water quality conditions will influence the composition and abundance of phytoplankton. Since phytoplankton respond rather rapidly to changes in nutrient concentrations, they are good indicators of nutrient-rich conditions. Waters having relatively low nutrient levels are dominated by diatoms, which are a highly desirable source of food. In water with higher nutrient concentrations, cyanobacteria and dinoflagellates become more abundant. These phytoplankton species are less desirable as a food source to animals.

As nutrients—and consequently phytoplankton—increase, various water quality variables are affected. Waters with low nutrient levels are generally clearer than water containing high concentrations of nutrients. As nutrient levels increase and the phytoplankton concentrations become more dense, the water often takes on the color of the algal pigments (e.g., reddish brown, green, brown) and odors become noticeable. In estuaries, the cells frequently collect along tidal fronts, where their presence is more evident.

Phytoplankton also influence oxygen concen-

trations in the estuary. As photosynthesizers, they produce oxygen, which is critical to all but a few estuarine organisms. When sunlight is unavailable (e.g., at night), phytoplankton respire, removing oxygen from the water. Oxygen is also consumed when bacteria work to decompose phytoplankton. A common aftermath of excessive phytoplankton growth in confined waterways is that oxygen is depleted, thus producing hypoxic conditions that may result in the deaths of many organisms.

Levels of Phytoplankton

At certain times, conditions are very good for phytoplankton growth. When there are adequate nutrients, increased light intensity, warmer water, and minimal predation pressures from zooplankton, phytoplankton population explosions, or **algal blooms**, may occur. The phytoplankton will continue to bloom until one or more of the key factors promoting phytoplankton growth is no longer available.

For example, during the spring months in temperate regions, diatom blooms usually coincide with increases in nutrient levels, water temperature, and light intensity. This increase in phytoplankton biomass is typically followed by an increase in zooplankton (often copepods) into the summer months. During the summer, the dominant phytoplankton assemblage shifts to include dinoflagellates and the grazing pressures of the zooplankton subsides. Another, but smaller, bloom of diatoms occurs in the fall, leading to a successive repeat in the nutrient/bloom cycle. Figure 19-2 illustrates seasonal bloom fluctuations in different geographic locales.

In recent years, there has been an increasing presence of bloom-forming algae in estuaries worldwide. This has been attributed to increased levels of nutrients entering these waters.

Harmful Algal Blooms

Some phytoplankton—mostly dinoflagellates and some diatoms—produce toxins, which have been known to cause illness or death in many aquatic organisms, including fish, shellfish, and marine mammals, by causing lesions, clogging gills, and depleting oxygen in the water. The toxins can also have serious impacts on humans (Table 19-2). The toxins can be transferred to humans through the consumption of shellfish (e.g., clams, oysters, mussels, and scallops). These organisms feed by filtering water through their gills to extract various forms of plankton. The plankton may not be toxic to the shellfish, but they could be toxic to humans who consume them. Therefore, state or local authorities routinely close areas to shellfish harvesting if excessive amounts of toxins are detected in the water.

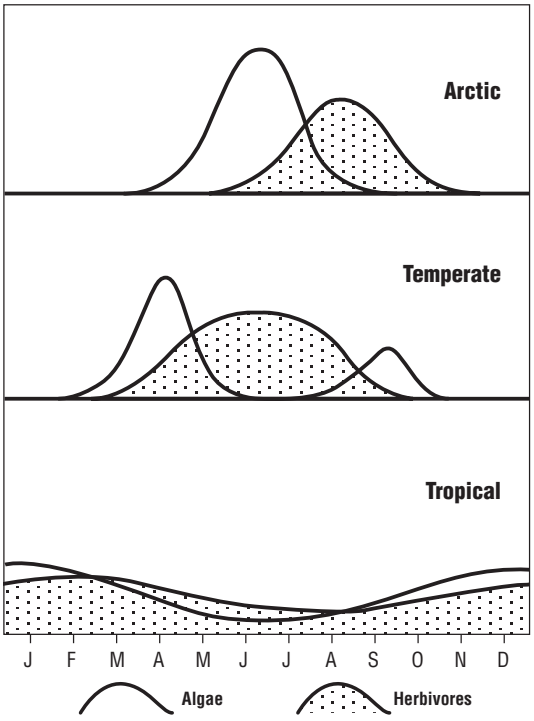

Figure 19-2. Typical seasonal cycles for plankton in arctic, temperate, and tropical regions.

One example of a harmful algal bloom is a "red tide." It is so named because the bloom is intense enough to change the color of the water. Some phytoplankton will produce a reddish, brown, or green color. In some cases, however, no color is produced at all—thus dispelling the myth that there is a definite connection between abnormally colored water and toxicity. Although there are many species that can bloom enough to change the color of the water, only a few species are toxic.

An increase in the frequency of harmful algal blooms in the U.S. and worldwide has led to increased efforts to develop effective monitoring and detection methods. By detecting blooms early, we can better ensure the safety of seafood products. The states of Maine and California have instituted coast-wide volunteer monitoring programs aimed at early detection of harmful blooms (Ely, 1998). Other states have a combination of formal and informal mechanisms to detect the blooms. The cost of toxic plankton monitoring is relatively high, so volunteer monitoring is an important way to protect public health in a cost-effective manner (Griffin, 1998). ■

Table 19-2. Some toxic phytoplankton important in the United States and their human impacts *(excerpted and adapted from Ely, 1998).*

Phytoplankton	Illness Caused	U.S. Outbreaks	Symptoms
Alexandrium spp.	PSP (paralytic shellfish poisoning)	New England, West Coast (including Alaska)	Numbness of lips and fingers; lack of coordination. Respiratory failure in severe cases. Can be fatal.
Pseudonitzschia spp.	ASP (amnesic shellfish poisoning)	No human illness reported in U.S. Human illness reported in Canada (east coast) and marine mammal illness on U.S. West Coast.	Abdominal cramps, disorientation. Permanent memory loss in severe cases. Can be fatal.
Gymnodinium breve	NSP (neurotoxic shellfish poisoning)	Mid-Atlantic and Southeast Coast, Gulf of Mexico	Gastroenteritis, painful amplification of sensation. No deaths.
Dinophysis spp.	DSP (diarrhetic shellfish poisoning)	No human illness reported in U.S.	Gastroenteritis. Nonfatal.

Sampling Considerations

When and Where to Sample

As stated in the discussion on algal blooms, dominant plankton populations change throughout the year. This should be considered when planning a sampling program. For long-term monitoring, a consistent time period for sampling is needed for comparability.

What to Sample

In temperate regions, the cyclic pattern of increased nutrient availability, sunlight, and algal blooms provides a baseline for comparison to other spikes in the phyto-plankton populations. If an increase in the numbers of a given species or genus is conspicuously absent when one normally

Helpful Hint

Adversely affected by strong light, zooplankton groups descend to great depths during the day and ascend during the night to feed on phytoplankton. Staying in deeper, colder waters during the day requires less energy for respiration and aids in avoiding predation by fish and seabirds.

If sampling zooplankton populations, considerations must be made to collect samples from depths what will yield representative samples of the plankton assemblages being monitored. This determination should be made as part of the overall planning process and establishment of a monitoring protocol.

expects an increase, or vice versa, this may serve as a warning that some environmental parameter has changed. In this case, the data set from phytoplankton monitoring serves as a general indicator of estuarine conditions.

Choosing a Sampling Method

Several different methods of obtaining phytoplankton data are available. The monitoring coordinator should choose a method based on the precision of the data required, the reason for collecting phytoplankton data, and the money available for this portion of the monitoring effort.

Visual Assessment

This method is the easiest and least expensive way to monitor phytoplankton. In this approach, volunteers estimate phytoplankton abundance based on field observations. This gross assessment of the waterbody is very limited and should be used as an indicator to follow-up with a more rigorous assessment procedure.

Plankton Net Tow

This method uses a cone-shaped mesh net, towed by boat or by hand along a dock or bridge through the water, to collect a variety of plankton species. This approach provides a decent estimate of phytoplankton density, but smaller species are likely to be excluded from the sample because they are able to pass through the net. If a more comprehensive quantitative assessment is required for the taxonomic identification of phytoplankton species, an alternative method of sampling, such as water samplers, should be used.

Water Samplers

If plankton population density measures are needed (number of cells/liter), then a monitoring technique to collect a specific amount of water must be used. A device that can be lowered into the water to capture a precise amount of water at a set depth is needed.

Traditional water samplers, such as Kemmerer or Van Dorn (see Chapter 7), are useful for collecting samples at different depths, thus ensuring better representation of the entire plankton community. In addition, small plankton are unable to escape the samplers, which is not the case for nets. ■

Reminder!

To ensure consistently high quality data, appropriate quality assurance and quality control measures are necessary. See Chapter 5 for details.

How to Monitor Phytoplankton

General procedures for collecting and analyzing phytoplankton samples are presented in this section for guidance only; they do not apply to all sampling methods. **Monitors should consult with the instructions that come with their sampling and analyzing instruments. Those who are interested in submitting data to government agencies should also consult with the agencies to determine acceptable equipment, methods, quality control measures, and data quality objectives (see Chapter 5).**

Before proceeding to the monitoring site and collecting samples, volunteers should review the topics addressed in Chapter 7. It is critical to confirm the monitoring site, date, and time; have the necessary monitoring equipment and personal gear; and understand all safety considerations. Once at the monitoring site, volunteers should record general site observations, as discussed in Chapter 7.

Besides the general considerations in Chapter 7, each phytoplankton sampling method involves a specific set of steps. Regardless of the method used, the program manager should designate how the data will be provided to program personnel. If the data is used as an early warning indicator of harmful algal blooms, volunteers may need to submit data sheets immediately (e.g., via fax) to program staff. As with all data sheets, volunteers should make a duplicate in case the original becomes lost.

When finished sampling, volunteers should also make sure to thoroughly clean all equipment with fresh water and store dry. Nets should be kept out of sunlight whenever possible.

Specific instructions for measuring phytoplankton using the different methods are presented here.

VISUAL ASSESSMENT

STEP 1: Check equipment.

In addition to the standard sampling equipment and apparel listed in Chapter 7, the volunteer should bring a Secchi disk to measure transparency (see Chapter 15).

STEP 2: Record phytoplankton assessment.

Estimate the presence of phytoplankton blooms based on water color, transparency (using a Secchi disk), and odor. In general, a waterbody with a significant phytoplankton bloom will display a green, brown, or red color, although—as discussed earlier—this is not always the case. The transparency level will be considerably reduced when compared with prior measurements. There may also be an odor (usually a sulfur or "rotten egg" smell) due to the decomposition of phytoplankton cells at the end of the bloom period.

PLANKTON NET TOW

STEP 1: Check equipment.

In addition to the standard sampling equipment and apparel listed in Chapter 7, the volunteer should bring the following items to the site for each sampling session:

- properly-sized tow net, sampling container, and towing apparatus;
- dropper;
- field microscope with slides (e.g., depression slides) and slide covers;
- guide for plankton identification; and
- sample preservative (iodine- or formalin-based solution for phytoplankton and zooplankton, respectively) if species identification will not be made soon after collection.

STEP 2: *Collect the sample.*

The deployment method of the plankton net is important, as many phytoplankton assemblages tend to form clumps within the water column. Numerous horizontal tows, done in a diagonal sweep through the water column, are useful to account for zooplankton migration patterns and phytoplankton clumps. A diagonal sweep conducted during a horizontal tow requires that the plankton net be deployed to a depth that is 0.5 m off the bottom. During the tow, the net is pulled diagonally toward the surface, thus transecting the water column.

In the horizontal tow method, volunteers should allow the net to be pulled through the water for a predetermined distance at a set speed. Slow speeds are recommended—less than 3 feet per second if towing manually; 1-3 miles per hour if towing by boat. A record should be maintained as to the duration of the tow and distance so that the quantity of water filtered can be computed and plankton density derived.

STEP 3: *Calculate density.*

Figure 19-3 shows how to calculate plankton density. The plankton captured by the net represent the number of organisms in the pre-measured volume of water. A slide sample of 0.05 ml (a drop from an eyedropper) should be prepared and plankton cells counted using a microscope. Multiplying the number of cells by 1,000 reveals the number of plankton per liter. This density figure can then be converted to the number of individuals per cubic meter, again multiplying by 1,000. A sample equation for extrapolating plankton density is given

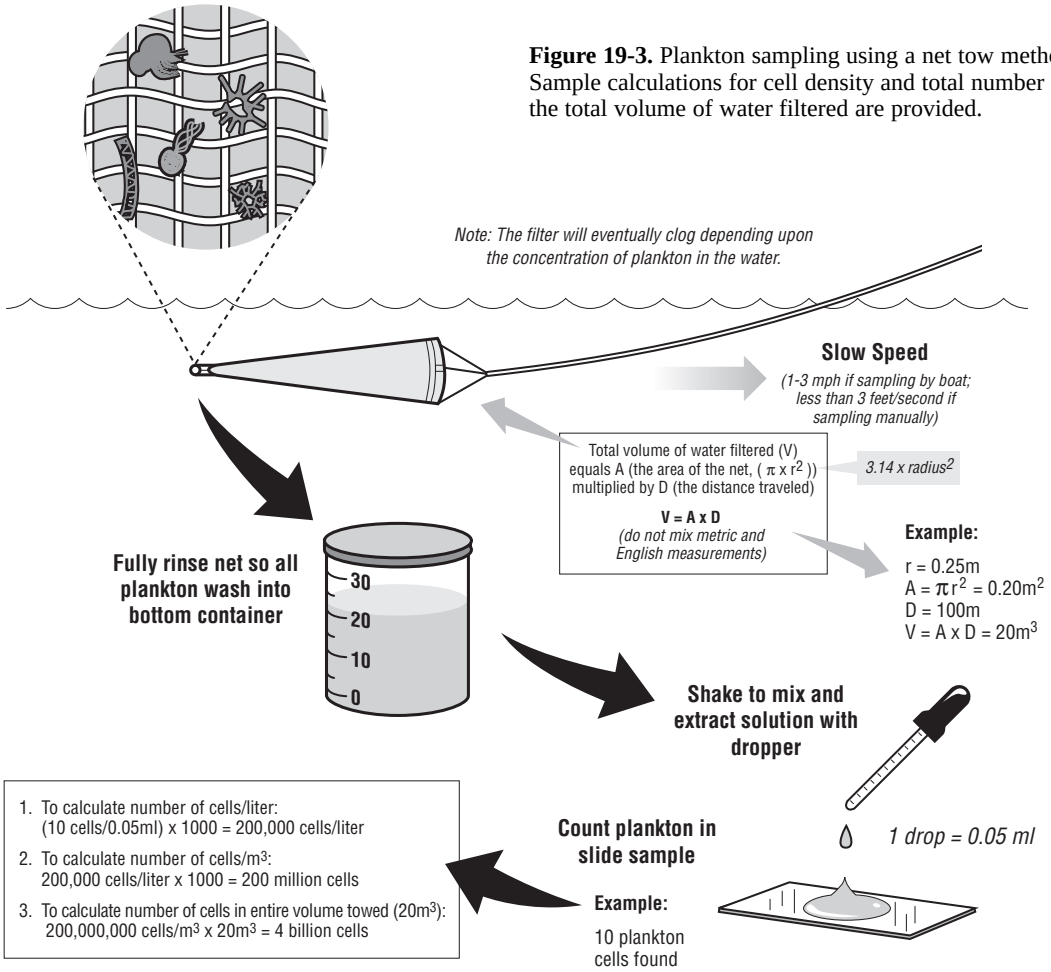

Figure 19-3. Plankton sampling using a net tow methodology. Sample calculations for cell density and total number of cells in the total volume of water filtered are provided.

below using a slide sample of 0.05 ml containing 10 plankton cells:

(a) (10 cells/0.05 ml) x 1,000 = 200,000 cells/liter

(b) 200,000 cells/liter x 1,000 = 200,000,000 cells/m^3

Calculating further to determine the total number of cells in the tow (total volume = $20m^3$ in our example; see Figure 19-3):

(c) 200,000,000 cells/m^3 x $20m^3$ = 4,000,000,000 cells

The density calculation is only approximate due to the "net factor"—the effect of the net as it is towed—forcing some water off the side rather than through its opening.

STEP 4: *Identify the species.*

Plankton collected in the cod-end jar at the base of the net can also be analyzed microscopically for species identification. Trained volunteers can conduct a gross field analysis of species found in the water column. A more rigorous laboratory analysis may be needed to fully quantify species composition and density. This method could provide a means to establish a joint research project with a local university.

Species identification must be conducted soon after collection (4-8 hours), unless a recommended preservative is used. Many phytoplankton samples can be preserved in an iodine-based preservative (e.g., Lugol's solution),

Case Study: Searching for Toxic Phytoplankton in Maine

In 1996, the United States Food and Drug Administration, the Maine Department of Marine Resources, and the University of Maine Cooperative Extension developed a marine phytoplankton monitoring program for the state. This volunteer-based monitoring effort has proven to be integral to harvesting safe shellfish.

In this program, community members and students use plankton nets and field microscopes to monitor for toxic algal species. Guidelines are established for volunteers to quantify their observations on the various species of algae. The volunteers collect data at least once a week, providing valuable information that otherwise would not be available to scientists. The data:

- help agencies identify toxic condition trends and where more thorough sampling is needed;
- serve as an early warning system for harmful algal blooms, which can lead to shellfish bed closures; and
- help officials understand the correlations between toxins in the water column and toxins in shellfish.

Volunteers receive training on phytoplankton identification and receive preserved samples of toxic species to take home as references. Agency staff periodically visit volunteers in the field to help with species identification and to answer questions. Many volunteers simultaneously test their sites for other water quality parameters to give a more complete summary of estuary health.

For More Information:

Maine Shore Stewards
University of Maine Cooperative Extension
235 Jefferson Street
P.O. Box 309
Waldoboro, ME 04572
Phone: 207-832-0343
Fax: 207-832-0377
http://www.ume.maine.edu

which "fixes" the sample and stains the cellulose walls of the phytoplankton cells to aid in identification. A zooplankton sample should be preserved using a formalin-based solution.

WATER SAMPLE

STEP 1: *Check equipment.*

In addition to the standard sampling equipment and apparel listed in Chapter 7, the volunteer should bring the following items to the site for each sampling session:

Gross Density Sample Technique

- sampler (e.g., Kemmerer, Van Dorn, or plankton net);
- dropper;
- field microscope with slides (e.g., depression slides) and slide covers;
- guide for plankton identification; and
- sample preservative (iodine- or formalin-based solution for phytoplankton and zooplankton, respectively) if species identification will not be made soon after collection.

Composite Cell Count Technique

- sampler (e.g., Kemmerer or Van Dorn sampler);
- 500 ml labeled bottles;
- guide for plankton identification; and
- sample preservative (iodine- or formalin-based solution for phytoplankton and zooplankton, respectively).

STEP 2: *Collect the sample.*

Using a Kemmerer or Van Dorn sampler, collect plankton samples from various depths. In many cases, phytoplankton clumps within the water column can cause problems for analysis. These problems can be compensated for in multiple and/or composite samples.

STEP 3: *Analyze the sample.*

Samples can be analyzed using the gross density sample technique or the composite cell count technique.

Gross Density Sample Technique

The collected water can be poured through the mesh of a plankton net where the plankton are strained out of the water sample. Calculate density as explained in the plankton net tow method and Figure 19-3.

It should be noted that a density figure calculated from a mesh-strained plankton sample will likely not contain the smaller phytoplankton forms as they will pass through the mesh of most plankton nets. To obtain a more accurate density measure, an alternative method of analysis (e.g., composite cell count) should be used to better assess the types and numbers of phytoplankton cells obtained in each sample.

Composite Cell Count Technique

Using water sampling devices such as a Kemmerer or Van Dorn, collect a series of 1-liter volumes of water from predetermined areas representing vertical and horizontal distributions. Combine these samples into a larger, composite sample. This method of creating composite samples provides the mechanism to reduce the effects of distribution patterns of phytoplankton assemblages and also ensures that a better sampling is obtained of smaller phytoplankton forms for analysis as the sample is not filtered through a mesh.

While in the field, mix the composite sample thoroughly and pour subsamples into 500 ml labeled bottles containing a preservative solution. At a laboratory, these samples will be processed into concentrates for more rigorous microscopic analysis. Volunteer monitors can be trained to scan slide samples of these composite samples to determine species types and density levels. This method could provide a means to establish a joint research project with a local university. ■

Special Topic: Chlorophyll Collection for Lab Analysis

Another procedure for determining the abundance of phytoplankton is to measure the amount of chlorophyll that is present. Chlorophyll is a pigment common to all photosynthetic algae, and its amount in the water is in relation to the algal concentration. This analysis is conducted in a laboratory where the density of chlorophyll may be determined with appropriate instrumentation.

Three replicate water samples, often ranging from 250-500 ml, are usually taken for this analysis. The samples should be collected in opaque bottles, which are placed on ice, kept in the dark, and transported to a laboratory.

In the lab, the water sample is processed through a glass fiber filter, which is then placed in acetone and ground up. Technicians measure the amount of chlorophyll in the processed sample using a fluorometer, an instrument that measures the fluorescence of a substance. If the laboratory cannot process the sample immediately, it may be frozen and filtered at a later date. ■

NON-INDIGENOUS SPECIES

Recently, attention has been given to the great numbers of organisms that have been introduced to ecosystems outside their normal range of occurrence. These "alien invaders" are known by many names, including non-native species, non-indigenous species, nuisance species, invasive species, and exotic species. Regardless of the name, some of these organisms can wreak havoc on any ecosystem—including estuaries—once they become established.

Non-indigenous species (NIS) enter estuarine systems by a variety of pathways. These include:

- Boats and Ships

  Vessels often take on ballast water and sediment to keep them stable at sea. Often, the ballast and associated sediment contain small aquatic plants, animals, and pathogens which can be introduced to the estuary when the ballast is discharged near ports. Fouling organisms (e.g., barnacles) on the vessel's hull can also be transported to different regions. Plant fragments get caught on boat propellers and fishing gear, creating another introduction pathway.

- Seafood Imports and Processing

  Packing materials for live seafood (e.g., seaweed and seawater) can contain living organisms. When materials are improperly discarded, species introductions are possible. Organisms living in or on seafood can also find a way into the estuary.

- Aquaculture and Fishery Enhancement

  This includes introductions of non-indigenous fish and shellfish that are intentionally released to the estuary or escape from captivity. Intentional introduction of one species can inadvertently bring other associated species, such as parasites.

- Biological Control

  Some organisms are introduced to control the growth and spread of other species.

- Artificial Waterways

  Channels, canals, and locks are artificial connections between waterways. They facilitate movement of various organisms to new locales via vessels or natural spread.

- Live Bait

  Bait species and their packing material can be intentionally and accidentally released to the estuary.

- Research and Education

  Laboratories, schools, and aquariums use NIS for testing, teaching, and research. Poor management can allow organisms to escape or be intentionally released.

- Aquarium and Nursery Trades

  These industries transport and sell NIS. Intentional releases and escapes of organisms can lead to NIS invasions.

- Natural Spread

  Some organisms are transported to new locations by natural means, including migration and transport (e.g., by birds, insects, natural floating debris, etc.).

NIS have been introduced into environments for hundreds of years, but the rate of these introductions is increasing, thanks in part to greater and faster international shipping traffic and international trade. The problem of NIS is worldwide and involves nearly all taxonomic groups (Heimowitz, 1999).

The Role of NIS in the Estuarine Ecosystem

Some NIS have impacts on estuarine ecosystems that are being felt in many ways. The following sections describe these various impacts:

Ecological Impacts

Many NIS are relatively innocuous to their new environments; others, however, are notorious for causing major problems to estuarine ecology. In fact, NIS are the second most significant threat to threatened and endangered species, behind only habitat loss (Wilcove et al., 1998).

Through predation and competition, NIS have disrupted many native populations—some to the point of total disappearance from the estuarine system. Because many NIS have few, if any, predators or competitors in their new homes, they are able to reproduce essentially unchecked. As a result, they dominate their habitats and cause a reduction in biodiversity.

NIS can also affect habitats. Atlantic smooth cordgrass, for example, is native to the eastern United States but has been introduced to the Pacific Northwest. It is now causing havoc along Pacific Northwest estuaries, where it traps sediment and converts extensive mudflats to nearshore meadows. The increased elevation and root mass displaces animal communities adapted to surviving in the mud, destroys foraging habitat for fish and birds, and out-competes other plants that are included in animals' diets (*Coastlines*, 1999).

Other impacts are also being seen. Some non-indigenous herbivores (plant-eating animals), such as the nutria, have decimated wetland, marsh, and submerged aquatic vegetation. This leads to increased erosion and loss of food and habitat for native species. Some NIS crossbreed with native species, a situation which can ultimately lead to local extinction of the natives. NIS may also carry diseases or parasites with them, against which local species have no defense. Finally, some NIS can transform estuarine chemical dynamics, exposing the food web to new or increased amounts of toxins.

Human Health Impacts

Some NIS threaten human health. Ballast water discharges are suspected as causes of bacterial and viral outbreaks. Ballast water can also contain the dinoflagellates which cause red tides (see previous sections in this chapter). These occurrences can have severe health consequences for people who swim, boat, or eat fish or shellfish from contaminated waters.

Non-Indigenous Species: A Different Kind of Pollution

Non-indigenous species (NIS) are viewed as a biological pollutant requiring management under the federal Clean Water Act. NIS is markedly different from most traditional pollutants, however.

Unlike many other forms of pollution, such as nutrients, oil spills, or sewage, NIS do not eventually dissipate over time. Instead, they grow and reproduce, spreading their impacts throughout the estuary. Because of this characteristic, an NIS is very difficult to eliminate once it becomes established. Consequently, prevention and early detection are the only cost-effective options for keeping NIS out of estuaries.

Socio-Economic Impacts

Not only can NIS have significant impacts on ecosystems and human health, but they also exact a great financial cost. NIS that grow in unchecked abundance can clog water intake pipes (the zebra mussel is probably the most notorious NIS in this regard) and cause instability to levees. These problems keep municipal utilities and land managers on the lookout, and cost millions—even billions—of dollars to address. One study has estimated that environmental damages caused by NIS add up to $138 billion annually (Pimentel et al, 1999). In the marine environment alone, it costs $5 billion each year to control NIS (Valigra, 1999).

Because of the damage they cause to native populations, NIS can have a direct impact on local fisheries. The Chinese mitten crab has been blamed for shutting down fish salvage operations in the Sacramento River delta. In the Chesapeake Bay, scientists and watermen fear that the recently discovered veined rapa whelk—an Asian native with a voracious appetite for shellfish—will decimate the region's already suffering oyster and clam fisheries.

Levels of NIS Invasion

All estuaries in the United States probably have some NIS, but no one knows exactly how many. San Francisco Bay is one of the most invaded estuaries in the world, supporting approximately 230 non-indigenous organisms (Cohen and Carlton, 1998). Approximately 160 NIS are known to infest the Chesapeake Bay (SERC Web site), and the numbers are growing. ■

Sampling Considerations

Once non-indigenous species become established, they are very difficult—or even impossible—to eradicate. Therefore, early detection of NIS invasions is critical. Volunteers can serve an important function by working with experts to find NIS. In fact, one of the most firmly established and destructive species in the San Francisco Bay area—the Asian clam—was first discovered by a college biology class doing basic monitoring exercises in 1986 (Sheehan, 2000).

NIS include the full range of plants, animals, and microbes, so sampling approaches will vary depending on the species. To help find and control NIS, it is important to understand the organisms' life histories and habitats (Graves, 1999). Awareness of native natural history is important for volunteer monitors, who may not know all NIS in the region but can at least recognize what doesn't look typical. ■

Helpful Hint

It may be illegal to possess or transport certain NIS specimens. Volunteer leaders should check with the appropriate government agency about obtaining the necessary collection and transport permits. Obtain the permits before starting any sampling activities.

How to Monitor Non-Indigenous Species

Because NIS include nearly all taxonomic groups, there is no standard procedure for monitoring them. **Monitors should therefore consult with their data users to determine acceptable equipment, methods, quality control measures, and data quality objectives (see Chapter 5) for their organisms of interest.**

Regardless of the method used, volunteers should review the topics addressed in Chapter 7 before proceeding to the monitoring site and collecting samples. It is critical to confirm the monitoring site, date, and time; have the necessary monitoring equipment and personal gear; and understand all safety considerations. Once at the monitoring site, volunteers should record general site observations, as discussed in Chapter 7.

The training of volunteers should include a session on the identification of the organisms of interest.

STEP 1: *Check equipment.*

In addition to the standard sampling equipment and apparel listed in Chapter 7, the volunteer should bring the following items to the site for each sampling session:

- guide to aid species identification;
- equipment necessary to collect and transport the organism(s) of interest;
- waterproof copy of any required species collection/transport permits; and
- sample preservative, if necessary.

STEP 2: *Collect the sample.*

Volunteers should capture the organisms using the method designated by the program manager and approved by the data users.

The volunteers should carefully label the sample container in which the organism will be transported with the date, site name, organism name (if known), and the name of the collector. An indelible marker is best for ensuring that the labeling is permanent. Make sure that the sample container is not wet before using the marker.

STEP 3: *Clean up and send off sample and data.*

Volunteers should transport live specimens at chilled temperatures appropriate for the species collected in containers supplied by the program. The program manager should designate pickup locations for volunteers to deliver the specimens and supporting data sheets to program personnel. As with all data sheets, the volunteer should make a duplicate in case the original becomes lost.

Make sure to thoroughly clean all equipment. ■

Hunting for NIS—and Bounty

In 1999, the Virginia Institute of Marine Science began offering a bounty for the veined rapa whelk. Citizens collect and donate the animals in return for money or t-shirts.

The project is used to help scientists document the whelk's distribution in the Chesapeake Bay and investigate potential impacts on the Bay's ecosystem.

For More Information:

The Virginia Institute of Marine Science
P.O. Box 1346
Gloucester Point, VA 23062-1346
Phone: 804-684-7000
http://www.vims.edu/fish/oyreef/rapven.html

Case Study: Keeping Tabs on Green Crabs in the Pacific Northwest

Spreading northward from California, the non-indigenous European green crab (*Carcinus maenas*—see Figure 19-4) first appeared on the Oregon coast in 1997. By 1999, green crabs occupied most Oregon estuaries, Washington's Willapa Bay and Grays Harbor, and sites in British Columbia. A capable predator, this invader raises concerns about impacts to native and commercial shellfish in the Pacific Northwest.

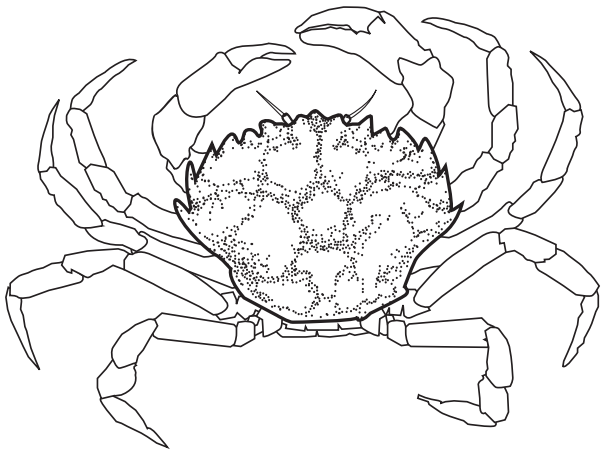

Figure 19-4. The European green crab, which can have severe environmental and economic impacts in the Pacific Northwest.

Given the invasion rate and extensive area at risk, agency monitoring alone is insufficient to detect the spread of the green crab. Volunteer programs provide many more sets of eyes to watch for this species as well as other NIS invasions.

To help the public distinguish green crabs from similar native crabs, Oregon and Washington Sea Grants have produced a color photo identification guide. In addition, Washington Sea Grant (with support from the U.S. Department of Fish and Wildlife) held a workshop for volunteer groups to provide information on green crab biology and monitoring techniques. Washington State's Department of Fish and Wildlife has contracted with Adopt-A-Beach, a nonprofit volunteer group to train and coordinate volunteers on green crab monitoring. Between July and September of 1999, 35 volunteers were trained and 32 monitoring sites, ranging from south Puget Sound to the San Juan Islands and the U.S.-Canadian border, were established. Volunteers search for the crab using baited traps in the intertidal zone.

Through the combined actions of agencies, tribes, schools, and volunteers, approximately 80 sites are monitored for green crabs in Washington. Numerous efforts to track the status of the green crab in Oregon estuaries also continue. For example, volunteers and high school students along the northern coast are monitoring for green crabs using live bait and modified minnow traps in six estuarine and marine sites.

For More Information:

Washington Department of Fish and Wildlife
1111 Washington St. SE
Olympia, WA 98501
Phone: 360-902-2200
Fax: 360-902-2230

Oregon Sea Grant,
Marine Invasive Species Team
500 Kerr Admin. Bldg., OSU
Corvallis, OR 97331-2131
Phone: 541-737-2714
Fax: 541-737-2392

Washington Sea Grant,
Marine Invasive Species Team
3716 Brooklyn Avenue NE
Seattle, WA 98105-6716
Phone: 206-543-6600
Fax: 206-685-0380

Biological Monitoring: Something for Everyone

Volunteers have many opportunities to become involved in biological monitoring activities. This chapter discusses just a few organisms monitored by volunteers. Below are references to other biological monitoring parameters. Volunteer leaders should contact their state monitoring agencies for information on other monitoring opportunities.

Birds

- "Bird Monitoring in North America"-U.S. Geological Survey Web site: http://www.mp1-pwrc.usgs.gov/birds.html.
- International Black Brant Monitoring Project: http://www.sd69.bc.ca/~brant/. (See also Alexander, G. 1998. "The International Black Brant Monitoring Project: Education That Spans a Flyway." *Coastlines* 8.2. Web site: http://www.epa.gov/owow/estuaries/coastlines/spring98/blackbrt.html.)

Salt Marshes/Wetlands

- Ferguson, W. 1999. "Fixing a Salt Marsh: Citizens, Shovels, and Sweat." *The Volunteer Monitor* 11(1). Web site: http://www.epa.gov/owow/volunteer/spring99/index.html.
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- Lipsky, A. 1996. *Narragansett Bay Method: A Manual for Salt Marsh Evaluation*. Save the Bay, Providence, RI. 22 pp. (Save the Bay, 434 Smith St., Providence, RI 02908-3770; phone: 401-272-3540).
- *The Volunteer Monitor* 10(1). 1998. Issue Topic: "Monitoring Wetlands." Web site: http://www.epa.gov/volunteer/spring98/index.html.

Replanting/Restoration Projects

- The Volunteer Monitor 11(1). 1999. Issue Topic: "Restoration." Web site: http://www.epa.gov/owow/volunteer/spring99/index.html.

References and Further Reading

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Web sites:

Shellfish

U.S. Food and Drug Administration

1. Center for Food Safety and Applied Nutrition

   Fish and Fishery Products Hazards and Controls Guide: http://vm.cfsan.fda.gov/~dms/haccp-2.html

   Foodborne Pathogenic Microorganisms and Natural Toxins Handbook: http://vm.cfsan.fda.gov/~mow/chap37.html

   Seafood Information and Resources: http://vm.cfsan.fda.gov/seafood1.html

2. Office of Seafood: http://vm.cfsan.fda. gov/~mow/sea-ill.html

Harmful Algal Blooms

Washington Sea Grant: http://www.wsg.washington.edu/outreach/mas/aquaculture/algalfacts.html

Non-Indigenous Species

Washington Sea Grant: http://www.wsg.washington.edu/outreach/mas/nis/nis.html

San Francisco Estuary Institute: http://www.sfei.org/invasions.html

Smithsonian Environmental Research Center (SERC): http://www.invasions.si.edu

U.S. Fish and Wildlife Service Invasive Species Program: http://invasives.fws.gov/

Sea Grant Non-Indigenous Species Site: http://www.sgnis.org/

Phytoplankton

Melbourne Parks and Waterways, Biological Surveys: http://140.211.62.101/streamwatch/swm13.html