

# Water Sampling Guide for Shellfish Hatcheries and Growers

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Shellfish hatcheries are the base of an emerging and profitable aquaculture industry. During a hatchery season, it is common for unknown water quality issues to impair production for short periods of time and, in extreme cases, for a significant portion of the production season. One useful approach is to collect discrete samples during production failure and/or to archive water samples throughout the hatchery season for later analysis if a production problem is experienced

after the fact. Proper protocols for sampling and storage prior to analysis are required to obtain accurate and meaningful results.

This guide is meant to inform shellfish hatcheries of proper techniques for water sampling and for storing water samples for analysis of harmful algal blooms, metals, pesticides, herbicides, and bacteria. The collection and preservation of these samples is summarized in table 1.

**Table 1. Quick guide for sampling water.**

Determination	Container <sup>1</sup>	Minimum sample size (mL)	Sample type <sup>2</sup>	Preservation	Maximum storage recommended	Regulatory <sup>3</sup>
Harmful algal blooms	P(C)	100	g, c	Live sample: Do not filter. Store at room temperature. Filter and store at -20 C.	18-24 hours; deliver as soon as possible.	18-24 hours
Metals	P(A), G(A)	1,000	g, c	For dissolved metals, filter immediately; add nitric acid (HNO <sub>3</sub> ) to pH<2.	6 months	6 months
Pesticides	G(S), PTFE <sup>4</sup> -lined cap	1,000	g, c	Refrigerate <sup>5</sup> ; add 1,000 mg ascorbic acid/L if residual chlorine present.	7 days	7 days until extraction; 40 days after extraction
Herbicides	G(S), PTFE <sup>4</sup> -lined cap	1,000	g, c	Refrigerate <sup>5</sup>	7 days	7 days until extraction; 40 days after extraction

<sup>1</sup>Type of container: P(C) = plastic coliform; P(A) or G(A) = rinsed with 1 part water + 1 part HNO<sub>3</sub>; G(S) = glass rinsed with organic solvents or baked.

<sup>2</sup>Sample types: g = grab; c = composite.

<sup>3</sup>Additional storage recommendations, if any.

<sup>4</sup>PTFE = polytetrafluoroethylene (i.e., Teflon).

<sup>5</sup>Refrigerate = storage at 4 C ± 2 C.

## Harmful Algal Blooms

The first step in the harmful algal bloom water collection protocol is to get two water samples (replicate samples) from each sampling location. One sample will be used for microscopic identification; the other sample will be filtered for genetic analysis.

Collect replicated samples in 120-mL disposable plastic coliform water bottles. Coliform water bottles are sterile until opened and are generally sealed. Fill the bottles to at least the 100-mL mark.

One of these collected samples will be used for microscopic identification and for determination of the number of cell types. Next, wrap the sample in a wet paper towel, deliver it promptly to the testing facility. Wrapping in a towel will keep the sample cooler than the air temperature. This is considered a live sample. If the sample is not available for analysis within 18-24 hours, add 400  $\mu$ l of aqueous iodine (e.g., Lugol's solution) to the sample for future evaluation. Lugol's solution contains 10 g iodine crystals, 20 g potassium iodide, 200 mL distilled water, and 20 mL glacial acetic acid. Once mixed, store it at room temperature. Ready-made Lugol's solution can also be purchased from scientific supply companies.

Filter the second sample collected for harmful algal bloom sampling as soon as possible to reduce the possibility of a change in the composition of the sample. The filtration process begins with attaching a funnel adapter and stopper to a test filter funnel that contains a 3-mm Isopore membrane filter (Millipore Corp., Darmstadt, Germany). Then place the funnel adapter into the neck of the flask with a filter funnel placed on the rim. Filter a total of 100 mL of the sample unless it is not possible due to turbidity; record the volume if a smaller volume is filtered. Using disposable forceps, remove the filter, fold it in half, and place it in a new plastic storage bag. Label the bag and store it in a freezer at minus 20 degrees Celsius. Dispose of the forceps, test filter funnel, and bottle after completing the filtration process to avoid potential contamination of future samples. Label the bottles for water samples and the storage bag for the filter with the location, date, and time of collection. If the temperature and salinity are available, note the data in both the lab/data book and on the bag.

## Metals

Samples that are to be analyzed for total metal contaminants are not filtered. The following procedure is appropriate for nonvolatile metals like copper, zinc, and lead.

The most basic approach is to collect an unfiltered water sample for total metals, which includes both phases (solid and aqueous) and reduces the amount of required handling. Samples should be approximately 250-1,000 mL. Clear or translucent plastic containers usually have the lowest levels of common contaminant metals; low-density polyethylene, high-density polyethylene, and polypropylene are all acceptable plastics.

Soak the container in deionized water and 10 percent hydrochloric acid — known as an acid bath — for a minimum of one week to clean the container and then rinse it with deionized water. The bottles can also be left soaking indefinitely until needed. After rinsing, bottles can be capped and stored in clean, reclosable plastic storage bags to avoid exterior contamination by dust. A convenient acid bath can be made in a new, clean, 5-gallon bucket (preferably a white-colored bucket because some pigments contain metal contaminants). Wear gloves, protective eyewear, a lab coat, and suitable closed-toed shoes whenever working around acid. (Note: Use of acids other than hydrochloric acid can deteriorate plastic bottles, making them brittle and unfit for sample collection.)

If acid washing is not available, use new bottles.

Before sample collection, rinse both new and acid-washed bottles five times with the water being sampled. Be careful to include the cap and bottle threads during rinsing and to ensure that nothing touches the water sample or bottle interior except for the sample. When sampling for metals, take care to avoid contamination from hair, dust, and other particles. Bottles should not be filled completely; leave approximately 5 percent headspace to allow for acid addition and mixing.

Once the sample has been collected, place the bottles in reclosable plastic storage bags. To determine the amount of contamination from the sample container, set aside at least one empty container for testing. Samples that are ready to be analyzed within about five days do not need to be acidified. Samples that cannot be analyzed within a few days require acidification.

After collection, acidify samples for metals analysis to  $\text{pH} < 2$  (usually approximately 2 mL concentrated hydrogen chloride per liter sample) with high-purity metal-free acid (TraceMetal or Optima grade). If this is not possible, document that samples are non-acidified; acids can be added at a later time before analysis. Documentation should include the date, time, salinity measurement, and whether the sample is acidified or non-acidified and filtered or unfiltered.

Samples that are acidified and tightly capped can be stored (in bags) at room temperature for extended periods. The maximum recommended storage time is approximately six months. Commercial analytical labs usually analyze these samples using mass spectrometry to determine the metal contaminants.

## Pesticides

The maximum holding time when testing for pesticides is seven days, and the minimum sample size is 1,000 mL. Use a glass container rinsed with organic solvents such as benzene when collecting a sample. Another option is rinsing the bottle in diluted hydrochloric acid and distilled water, but then it has to be heat-treated overnight at 300 C (Goerlitz and Brown 1984). If the sample can't be analyzed right away, store it away from light and refrigerated at 4 C or below. To preserve a sample being tested for pesticides, add 1,000 mg of ascorbic acid to 1 liter of the sample if residual chlorine is present (APHA, AWWA, and WEF 1998). Whether the sample can be used right away or it has to be preserved, it should always be refrigerated.

## Herbicides

Samples to be analyzed for herbicides must be refrigerated at 4 C within four to five hours of collection (Goerlitz and Brown 1984). Acidify samples with concentrated sulfuric acid at a rate of 2 mL per liter. A sample that is not acidified must reach the laboratory within 24 hours.

Herbicides, like pesticides, can be analyzed by a few different detectors, some of which are more sensitive than others; this is why it is imperative to use proper handling to prevent contamination. For example, electron-capture detectors, which can be used in gas chromatographic analysis, are very sensitive to interfering substances.

Approximately three 1,000-mL samples need to be collected in polytetrafluoroethylene (PTFE; i.e., Teflon)-lined glass jar that has been prerinsed with deionized water. When collecting a sample, there should be little to no air space in the top of the jar. Preserve the sample immediately by putting it on ice and placing it in the dark (TCEQ 2012). Samples can be preserved at a temperature below 4 C for up to seven days.

## Bacteria

Bacteria are more difficult to test for due to the many different types of bacteria, the natural levels of bacteria in the water, rainfall, and contamination. Bacteria concentrations are strongly impacted by the amount of rainfall; therefore, depending on the season, the data collected could result in an inaccurate outcome. Additionally, analytical methods used for testing bacteria can also introduce inaccuracies, which is why preventing contamination is important.

Sample containers can range from glassware to plastic. If glassware or plastic containers are used or reused, they must be rinsed and sterilized at 121 C for 15 minutes using an autoclave. Preferably, sampling is done with a presterilized disposable Whirl-Pak bag because no additional preparation is necessary. Duplicate samples — where another sample is collected at the same place and time as the first sample — should also be collected; this allows a determination of the laboratory's precision to be made. Once the samples are collected, take them to a lab for immediate testing because the maximum holding period is only six hours. Store these samples at 4 C.

When transporting samples, place them on fresh ice in a cooler to maintain a temperature below 4 C at all times.

## Sample Analysis

Some tests are easy to complete and analyze in the hatchery; however, others need to be done in a commercial lab or at a university. For example, harmful algal bloom samples should be sent to the Virginia Institute of Marine Science, Marine Aquaculture and Molecular Genetics Laboratory (Kim Reece, 804-684-7407). Samples collected for analysis of bacteria, pesticides, or herbicides need to be tested in a commercial analytical laboratory. Table 2 contains a list of some laboratories and their contact information. Each laboratory has different analytical capabilities that can be found on its website or by calling its office.

**Table 2. List of commercial analytical laboratories.**

Laboratory name	Contact info	Website
Air Water & Soil Laboratories Inc.	804-358-8295	www.awslabs.com/
A & L Eastern Laboratories Inc.	804-743-9401	http://al-labs-eastern.com/
Analytics Corporation	804-365-3000	http://analyticscorp.com/
Babcock & Wilcox Lynchburg Technology Center	434-522-5130	www.babcock.com/Pages/default.aspx
B & B Consultants Inc.	434-372-3393	http://bandbcons.com/
EMS Inc.	276-228-6464	—
EnviroCompliance Laboratories Inc. – Ashland	804-550-3971	www.envirocompliance.com/
Environmental Hazards Services LLC	804-275-4788	www.leadlab.com/
Environmental Monitoring Inc.	276-679-6544	www.emilab.com/
Environmental Systems Service LTD – Culpeper	540-825-6660	www.ess-services.com/
Greenway Engineering Inc.	540-662-4185	http://greenwayeng.com/
Hampton Roads Sanitation District, Central Environmental Laboratory/Wet Laboratory	757-460-4217	www.hrsd.state.va.us/
Inboden Environmental Services Inc.	540-477-3300	www.4ies.com/
James R. Reed & Associates	757-873-4703	http://jrreed.com/
Jennings Laboratories Inc.	757-425-1498	www.jenningslab.com/
Metropolitan Solutions	757-853-4000	http://metrosolutionsusa.com/
Newport News Shipbuilding, A Division of Huntington Ingalls Industries	757-380-3244	www.huntingtoningalls.com/
Schneider Laboratories Global Inc.	804-353-6778	https://www.slabinc.com/content/
Universal Laboratories – Hampton	757-865-0880	www.universallaboratories.net/

## References

APHA, AWWA, and WEF (American Public Health Association, American Water Works Association, and Water Environment Federation). 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th ed. Edited by L. S. Clesceri, A. E. Greenberg, and A. D. Eaton. Washington, DC: APHA.

Goerlitz, D. F., and E. Brown. 1984. “Methods for Analysis of Organic Substances in Water.” *In Techniques of Water-Resources Investigations of the United States Geological Survey*. Washington, DC: U.S. Government Printing Office. <https://pubs.usgs.gov/twri/05a03/report.pdf>.

TCEQ (Texas Commission on Environmental Quality). 2012. “Collecting Water Samples.” *In Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods*.” Austin, Texas: TCEQ.