

Guide to Keeping an Oyster Research Tank

How to set up, maintain, and conduct research with an oyster tank

PROGRAM GOAL:

The goal of the oyster research tank is to bring oysters and monitoring work into your classroom! Your oyster tank will be an excellent platform for regular study, data collection, and student-driven inquiry and research.



(Note: some of this information comes from "<u>Estuarine Aquarium Keeping for Beginners</u>," a guide written by the Chesapeake Bay National Estuarine Research Reserve in Virginia, a protected estuary area that is jointly managed by the National Oceanic and Atmospheric Administration (NOAA) and the College of William and Mary Virginia Institute of Marine Science.)

Table of Contents

SUPPLY LIST	3
TANK SETUP	4
Water Preparation (before adding oysters)	4
Directions for DIY Habitat for Nitrifying Bacteria	5
Cycling Your Tank	6
TANK MAINTENANCE & HELPFUL HINTS	6
Oysters Out of Water	6
Tank Size	6
Aerator	6
Bacteria	7
Different Ways to Influence Water Quality in Your Tank	7
Water Chemistry Testing	7
Water Changes	9
Oyster Feeding	9
What to Watch for When Monitoring Your Tank	9
WE WANT YOUR DATA!	10
SUGGESTIONS FOR EXPERIMENTAL TANKS	11
IF YOU HAVE AN OYSTER RESEARCH STATION	12
Directions for Adding Animals (after oysters)	12
OYSTER ANATOMY DIAGRAMS	13
MEASURING OYSTERS DIAGRAM	14
NITROGEN CYCLE DIAGRAMS	15

SUPPLY LIST

These are all the supplies you received at your ORT training.

Find prices for all supplies here.

Oyster Tank Supplies	Purpose	Quantity
Plastic Tank (5.9 gallon "XL Kritter Keeper")	home for oysters	2
Aerator w/ accessories	dissolved oxygen	2
Instant Ocean Aquarium Salt (3 lb box)	making artificial seawater	1
Bioculture (Microbacter) 125 mL bottle	adding bacteria	1
Aquarium gravel (5 lb bag)	DIY filter	1
SD Aquarist (6 oz. algae)	feeding oysters	1
Oysters for teachers within NYC	reef	2 bags
Oyster Measurement Supplies	Purpose	Quantity
Calipers	measuring oyster growth	5
Water Quality Supplies	Purpose	Quantity
Bucket 1-gallon	changing water	1
Thermometer	water temperature	1
Hydrometer	salinity	1
	pH, ammonia, nitrates,	
Water chemistry test kit	nitrites	1
Dissolved oxygen test tablets	dissolved oxygen	1

PLEASE NOTE: Billion Oyster Project is not permitted to provide live oysters to educators outside of New York City.

TANK SETUP

Water Preparation (before adding oysters)

1. Add water to the tank. There are three options for adding water to your tank. All 3 options will work for your oysters. Decide what option is best for you based on how readily you can access the Harbor. Options for adding water to your tank include using:

Water source	Please note		
Harbor water	Pro : A population of nitrifying bacteria already exists in the water and you don't need to add microbacter to your tank.		
	Con : Transporting 6-12 gallons of water from the Harbor to your school can be challenging.		
Tap water	To create salt water, you'll need to add Instant Ocean aquarium salt to the tap water in the tank. Follow the product guidelines to obtain your desired salinity. One suggestion is to make the salinity 15ppt (approximately halfway between fresh water at 0 parts per thousand (ppt) and ocean water at 32 ppt		
	Note: ½ cup of Instant Ocean in 1 gallon of water = 1.023 sg (specific gravity) = 30-35 ppt		
	Pro : Tap water is easily accessible.		
	Con : 24-hour lead time to dechlorinate water. You must dechlorinate your tap water by letting it sit for 24 hours (because the chlorine in the tap water could kill the helpful bacteria in your harbor water)		
Mixture of Harbor and tap water	Mix dechlorinated tap water and harbor water.		
	Add salt if necessary to obtain your desired salinity. (Consider making your final mixture of water have the same salinity as the harbor water you collected.)		
	Pro : A population of nitrifying bacteria already exists in the water and you don't need to add microbacter to your tank. You only have to transport ½ the water from the harbor.		
	Con : You have to access the harbor and carry water from it. 24-hour lead time to dechlorinate water. You must dechlorinate your tap water by letting it sit for 24 hours (because the chlorine in the tap water could kill the helpful bacteria in your harbor water)		

- Set up your <u>DIY Habitat for Nitrifying Bacteria</u> (i.e. biological filter) with the aerator and air stone. Add microbacter following the directions on the bottle and using the directions below under "<u>Directions for DIY Habitat for Nitrifying Bacteria</u>".
- 3. Let the tank sit for 24 hours.
- 4. Check salinity to make sure it's stabilized at desired level.
- 5. If you're using Harbor water, your tank is now ready for oysters. If you are only using tap water, follow the Directions for <u>Cycling Your Tank</u> on page 6 to make sure your tank is ready for oysters.

Directions for DIY Habitat for Nitrifying Bacteria

First you will need to create the habitat for the nitrifying bacteria. This can be done with a plastic bottle, an aerator, and gravel.

- 1. Poke or drill 6 holes around the plastic bottle about ½ inch up from the bottom. This can be difficult depending on your tool and the type of plastic bottle. Make sure the holes are about the same diameter as the aerator tube.
- 2. Feed the aerator tube (without the airstone) into the plastic bottle through one of the holes you just made.
- 3. Feed the aerator tube out of the top of the bottle.
- 4. Put the air stone on the aerator tube. Pull the aerator tube (with air stone) back down through the top of the bottle, so the air stone rests *near* the bottom, inside the plastic bottle.
- 5. Fill the plastic bottle with gravel.
- 6. Place the bottle in the tank.



Cycling Your Tank

"**Cycling**" your tank means that you are establishing a population of nitrifying bacteria in your biological filter (i.e. DIY Habitat for Nitrifying Bacteria) to remove the toxins that the oysters' metabolism (i.e. feces) creates. You only need to do this if you are using tap water. If you are using Harbor water, you don't need to cycle your tank.

- 1. To cycle your tank, first follow the "Water Preparation" directions above.
- 2. Then, monitor and record ammonia, nitrites, nitrates and pH every day for at least two weeks before adding oysters.
- 3. When ammonia and nitrites are at 0, and nitrates are present, and pH stays relatively stable (a little basic, between 7 and 8), your tank is cycled and you are ready to add your oysters.

TANK MAINTENANCE & HELPFUL HINTS

Oysters Out of Water

- Spat that are 1 cm or less can survive out of water for ~4 hours (no guarantees though)
- Spat that are 1 cm to 5 cm can survive out of water for ~12 hours
- Spat larger than 5 cm can survive out of water for ~24 hours
- In direct sun, the time out of water is cut to ¼ of the above suggested time. For example, spat that are 1cm or less can survive out of water for ~1 hour in direct sun. If it is necessary to work with oysters in direct sunlight, frequently dunk/spray/wet the oysters to prevent death from desiccation.
- Oysters that are out of the water for more than the recommended time above should be kept in the refrigerator (not freezer). Your oysters should be fine in the fridge for 24-48 hours.

Tank Size

A larger tank will make for happier oysters. You do not want to overcrowd your tank. The volume of water in the tank and the surface area of the water/air interface are important components to keep in mind when thinking about creating the best habitat for your oysters. For instance, it is better to purchase a 20-gallon long aquarium instead of a 20-gallon tall model. The long model has more bottom surface area than the 20-gallon tall model.

Aerator

Tanks must be kept aerated at all times to ensure adequate levels of oxygen and regulate bacteria. Never turn off your aerator or allow the hose to become blocked.

Bacteria

You need nitrifying bacteria in the tank to deal with the ammonia that builds up from keeping animals in the enclosed space. This bacteria can take weeks to develop, and if you let the ammonia build up, that is more than enough time to kill all your oysters. Using harbor water to start adds a wide variety of bacteria. You can also add the bacteria with a product called "Microbacter" which is included in your tank kit. Be prepared to do water changes very frequently for the first few weeks.

Different Ways to Influence Water Quality in Your Tank

We are not providing a typical aquarium filter for your tank because it would be catching necessary phytoplankton and nutrients for oysters, making it harder for them to feed. Instead, your tank will use other types of filtration to keep the water clean enough for oyster survival.

There are three types of aquarium filtration:

- 1. Mechanical Filtration -- something that works more or less like a sieve, collecting particles above a certain size all in one place, so they don't float in the water and make it turbid.
 - Oysters can accomplish some of this on their own, as long as the particles are not too big for them. But instead of mechanically sieving out the particles, oysters coat the particles in mucus and release them as pseudofeces, or digesting them and excreting them as feces. That mucus makes the particles sink to the bottom of the tank, and that's why the water in oyster tanks usually looks pretty clear.
- 2. Chemical "Filtration" -- a material that can remove dissolved substances from the water, by transforming them into other substances and/or capturing them so they're no longer dissolved in the water.
 - Oysters cannot do this on their own, but it is not necessary for your classroom tank.
- 3. Biological Metabolism of Animal Waste -- this refers to changes that are carried out inside the cells of living bacteria. These changes are chemical changes -- most importantly oxidizing ammonia and nitrites -- but because they only happen as part of the metabolism of specific bacteria, people call this process "biological filtration."
 - Oysters cannot do this on their own, which is why you definitely need some biological filtration happening in your tank. See the "<u>Directions for DIY Habitat for Nitrifying</u> <u>Bacteria</u>" section above on page 5.

Water Chemistry Testing

Water chemistry testing includes all of the parameters listed on the next page. Test the water chemistry in your tank once a week once it's stable, except when cycling your tank - then you need to test ammonia, nitrates, and nitrites every day. If issues with the tank arise otherwise (e.g. a high ammonia reading) you should test the tank 2-3 times a week until it's stable again.

Parameter	Please note	ldeal Range
Temperature	Classrooms are notoriously warm, and this can be a major factor in your oysters' survival. Water temp in your tank can easily climb into the 80°F's if there's no air conditioning. The oysters will be much easier to care for if you have cooler water.	Below 70°F Cooler is better.
	Ideally, keep your tank in an air conditioned room, or at least out of direct sunlight. Oysters tolerate cold water well. As the water temperature in your aquarium decreases, the oysters will simply slow down and may stop eating entirely. It should not be a problem if your water temps decrease to 50°F or below.	
	Tank chillers are effective, but very expensive. A low-cost solution is to freeze six bottles of water. Float three bottles on the top of your tank to cool the water. Each day swap out the three used bottles for the three frozen bottles. To make the frozen bottles even more effective, put a splitter on your aerator and put a second hose from your aerators next to the frozen bottles to move even more water over them.	
Salinity	Set up the desired tank salinity BEFORE adding oysters. See the instructions for setting up tank water on page 4 under " <u>Water</u> <u>Preparation</u> ."	15ppt is ideal, or 10-25ppt
Dissolved Oxygen	Your aerator should do a good enough job of keeping the dissolved oxygen levels consistent in your tank but it is still good practice to test DO with your students, as that is especially important for organism survival in the Harbor.	Above 6ppm
рН	The pH of Harbor water is generally a little basic (7.5-8), and that will be fine for your tank as well. pH is unlikely to change unless the ammonia levels get too high, in which case you will see a spike in pH as ammonia is fairly basic.	7-8
Ammonia	Any ammonia above 0.5ppm should be considered too high. Check ammonia daily at first. Do a water change any time the ammonia readings are too high. Change no more than 50% of the water as this will keep more of the helpful bacteria in the tank. See more info about water changes in the section on page 9 titled " <u>Water Changes</u> ."	0-0.5ppm
Nitrate/Nitrit e	You might consider using the Nitrogen Cycle diagrams for yourself or your students starting on page 12 of this guide. Nitrites should be under .05ppm and Nitrates should be present in order to be a good habitat for your oysters.	Nitrites: <0.05ppm Nitrates: >0ppm

Water Changes

When doing a water change, change about half the water at a time because you do NOT want to get rid of all the wonderful nitrifying bacteria that has grown in your tank! Go back to page 4 of your guide to prepare your water, making sure to wait the necessary 24 hours before adding it to your tank if using tap water. When ready to do a water change, use your small bucket to take out up to half the water in your tank, and replace it with the water you have already prepared. Make sure to pour the water in slowly so as to not upset the living organisms too much.

We recommend changing the water between once per week and once per month. The exact frequency is up to you and some experimental designs may call for changing water more or less frequently than the recommended range. Generally, the more water changes the better. Refer to the "<u>Watch Watch</u> <u>Watch</u>" section on page 10 for more information about when you might need to do a water change. Outside of an actual water change, always keep the salinity at a constant level by adding more brackish water to account for evaporation.

Oyster Feeding

Oysters must be fed a steady diet of phytoplankton (single- and multi-celled algae). In nature phytoplankton is abundant; however you must add a concentrated solution to a tank.

- 1. Start by mixing 15 drops of the SD Aquarist phytoplankton with a little water (about 3mL) to thin it out (approximately four parts water to one part algae).
- 2. Generally feed 3-5 times a week depending on how quickly your tanks clears.
- 3. Keep a very light yellowish/brownish/greenish tint to the water.
- 4. Feed the oysters at the beginning or end of the work/school day.
- 5. If the water is perfectly clear 6-12 hours later, feed the oysters again and try increasing the feeding by 5-10 drops.
- 6. When increasing food amounts, it is a good idea to check ammonia more often to help maintain good water quality.
- 7. Don't forget to ask for more algae before you run out!

What to Watch for When Monitoring Your Tank

A close eye on your oyster tank, during feeding and at different times during the day, will give you invaluable information as to how everything is working. Keep an eye on:

- The tint of the water. You want to keep a very light yellowish/brownish/greenish tint to the water to ensure your oysters have enough phytoplankton available for food. If your tank is too clear, refer to our <u>Oyster Feeding Guide</u> on page 9 to add phytoplankton. If your tank is too cloudy, refer to our <u>Water Change Guide</u> on page 9.
- Make sure the aerator is running at all times. Dissolved oxygen is extremely important for your oysters and other organisms. If your DO is lower than 6ppm in the tank, consider adding an additional aerator to the tank, and watch out for dead organisms and other conditions that can reduce DO levels.

- If there is a lot of buildup of sediment at the bottom of the tank, try to take some out when doing water changes. You can do this by simply stirring up the water when taking it out of the tank – it will capture a lot of that sediment.
- If you have other organisms in the tank, make sure they're alive. Dead organisms can change the water chemistry of the tank very easily. If you have determined that one of your organisms is dead, remove it from the tank to avoid water quality issues, and then do a partial water change.
- If you notice that your ammonia/nitrite counts become high in the tank after initial cycling, perform a partial water change, making sure to add tap water conditioner if needed. If counts continue to stay high, make sure to check your tank for dead organisms and sediment buildup.

WE WANT YOUR DATA!

Please submit measurements from your oyster tank at least twice a year to this Google Form.

You can jog your memory on how to take the measurements with this <u>Tank Measurement Protocol</u>, and use <u>this data sheet</u> to record your data points. More images are below on page 14.

The number of oysters in your tank can range from a handful to tens of oysters. If you have a smaller number, you might want to print out one data sheet and use it to collect data from multiple dates of oyster measurements. Then, you can hang or keep this datasheet near the tank to enable quick comparisons. If you have a large number of oysters in your tank, this strategy might be less useful because it can be hard to compare large amounts of data visually without calculations.

SUGGESTIONS FOR EXPERIMENTAL TANKS

- 1. The only things that must be placed in this second, experimental tank are :
 - Water (with some amount of salt)
 - Oysters
 - The aerator
 - Some amount of food
- 2. Other than that, what is put in the second tank is based on the experimental question.
- 3. Possible variables include:
 - Cycling vs not cycling
 - Amount of salinity
 - Kind of water (Instant Ocean or NY Harbor)
 - Substrate (sand, oyster shells, concrete, etc.)
 - Number of oysters
 - Volume of water
 - Conditioning drops
 - Other organisms
 - Amount of food: How much are they eating in correlation with the temperature?

IF YOU HAVE AN OYSTER RESEARCH STATION

If you already have an Oyster Research Station (ORS), you have the opportunity to add invertebrates other than oysters to your classroom tank that are taken from your ORS. See instructions below.

If you do not already have an ORS, look for ORS training opportunities on <u>Eventbrite</u>. The ORS not only provides opportunities for your students to practice the skills they need in the field to monitor their oysters, but also provides a point of comparison to the ORT.

Directions for Adding Animals (after oysters)

Once you have had your oysters in your tank for a couple of weeks, and the water quality in your tank is stable, you can add other organisms that you gather from your Oyster Research Station (ORS).

- 1. Bring **invertebrates** back from your ORS in a bucket of harbor water with a portable aerator.
- 2. Put the bucket with organisms next to your tank.
- 3. Test the water chemistry (temp, salinity, DO, pH, ammonia, nitrate, phosphate) of both your tank and the bucket.
- 4. In order to not shock the invertebrates, you need to make the water between the tank and the bucket more similar. Take a little bit of water out of your tank using a cup or other tool, and discard. Then take that amount of water from the bucket and pour it into the tank.
- 5. Give your tank a few minutes to adjust. Test the water chemistry again.
- 6. If the water is still uneven between the tank and bucket, repeat steps 4 and 5 until the water chemistry is about equal between them.
- 7. Once water quality measurements are nearly identical, add the ORS organisms to your tank.
- 8. Note: If you're wondering what organisms to add, shrimp do a good job at scavenging uneaten food, and even the feces/pseudofeces of our beloved oyster. Some organisms in the Harbor are predators of oysters (like blue crabs and oyster drill snails). You may want to avoid putting those organisms in your tank, unless doing a particular experiment involving predators.

OYSTER ANATOMY DIAGRAMS



From Billion Oyster Project





Diagrams from Billion Oyster Project

MEASURING OYSTERS DIAGRAM



From Billion Oyster Project

NITROGEN CYCLE DIAGRAMS



Source #1 https://matrixaquatics.com/fishless-cycle/



Source #2 https://diamondaquatics.com/blog/understanding-the-nitrogen-cycle/



Source #3 https://aqualabaquaria.com/blogs/aquarium-basics/



Source #4 http://www.fishlore.com/NitrogenCycle.htm

Figure 1. The nitrification pathway in fish tanks. (1) Decomposition of organic matter (e.g., fish excrement and uneaten fish food) leads to an increase in ammonium levels in fish tanks. In addition, fish excrete ammonium directly into the water. (2) Nitrifying bacteria (*Nitrosomonas, Nitrosospira*) oxidize ammonium to nitrite. (3) Nitrifying bacteria (*Nitrobacter, Nitrospira*) oxidize nitrite to nitrate. The latter is utilized as a plant fertilizer.



Source #5: https://www.mdpi.com/2079-6382/9/9/564



Source #6 https://www.theaquariumwiki.com/wiki/File:Cycling_graph.png