Aquaculture in Action

TOOLS FOR TEACHING SCIENCE

Sea Grant MARYLAND

NOAA National Oceanic and Atmospheric Administration U.S. Department of Commerce

A Manual for RECIRCULATING AQUACULTURE in the Classroom

CONTENTS

Flight, C., Frederick, A., Gallagher, J., Goetz, A., and Kenney, A. 2026. *Aquaculture in Action: Tools for Teaching Science, A Manual for Recirculating Aquaculture in the Classroom*. Maryland Sea Grant Publication UM-SG-TS-2026-03.

CONTENTS

Flight, C., Frederick, A., Gallagher, J., Goetz, A., and Kenney, A. 2026. *Aquaculture in Action: Tools for Teaching Science, A Manual for Recirculating Aquaculture in the Classroom*. Maryland Sea Grant Publication UM-SG-TS-2026-03.

UNIT I:

The Aquaculture in Action Model

Introduction

Aquaculture is a powerful education tool that benefits students and teachers by motivating and engaging students across all learning abilities through project-based learning. By integrating different areas of study, it allows students to apply a wide range of skills in a lab-based setting. Students are encouraged to work as teams to design and complete projects, gaining a feel for the dynamics of the modern workplace. Aquaculture projects get students involved in hands-on biology, chemistry, math, and physics skills, which can be related directly to classroom lesson plans and educational goals.

Aquaculture learning can extend beyond the classroom and promote better environmental literacy—a major goal in science education. Students form lasting relationships with local species of the Chesapeake Bay watershed, connecting aquaculture research with classroom learning, while inspiring real-world action.

Aquaculture projects require students to get involved in the process of experimental design and data collection throughout a school year, modeling the scientific process. Long-term data collection allows students to identify trends and make connections between physical and biological factors within a controlled setting. As a result, students gain greater awareness of applied skills, scientific knowledge, and environmental literacy, as well as an appreciation for the effort required to apply concepts in the field.

If you are an educator interested in starting an aquaculture system in your classroom, check out Planning for an Aquaculture System in the Classroom, our guide designed to help educators take their first steps in selecting and setting up an aquaculture system.

Goal 1: Environmental Analysis

Conduct water quality and habitat analyses of local streams in the Chesapeake Bay watershed to better understand the challenges of designing an artificial system for raising fish.

Developing an understanding of the natural environment, either near the school or during a field experience, helps develop a greater understanding of the challenges faced in aquaculture. Prior to developing an aquaculture program, teachers and students need field experiences to increase their background knowledge and enhance their problem-solving skills related to water quality issues that impact biological activity and vice versa.

Objectives

1. Research local streams to select a fish species students may support through restocking, in cooperation with the Maryland Department of Natural Resources (DNR).
2. Research the physical, chemical, and biological parameters of the water.
3. Perform various water quality analyses of local streams.
4. Apply biotic indices to streams to determine their habitability for the reintroduced species.
5. Develop datasets of the stream analyses performed.

Goal 2: Species Selection

School location, stocking opportunities, fingerling availability, and teacher experience are deciding factors in species selection. Possible species include rainbow trout, brook trout, striped bass, yellow perch, largemouth bass, shad, redeared sunfish, bluegill, tilapia, and catfish.* For more information on species selection, see Planning for an Aquaculture System in the Classroom.

**Trout species require the addition of a cooling system. All other species can be raised without a cooling system.*

Goal 3: System Design and Construction

Successfully construct a 210-gallon recirculating system at the Aquaculture in Action workshop, and design other systems based upon this model.

A prototype 210-gallon recirculating system designed by the Aquaculture in Action program (AinA) is the standard system used in the classroom (see Planning for an Aquaculture System in the Classroom). The system contains a 210-gallon central tank with a two-inch bottom drain that flows into a 30-gallon sump that collects solid waste. Water passes through the sump and is transported by a pump into an upflow biological filter containing biofilm carrier media. Water flows up through the suspended biofilter medium and is returned to the tank by gravity. An air compressor is used to supply air to the tank and biological filter.

Objectives

1. Use References and Resources to learn about the design, engineering, and maintenance of the recirculating system.
2. Construct and inoculate the biological filter and monitor the physical and biological parameters of the system prior to fish introduction.
3. Using test kits or computer probes and software, collect and graph water quality data over an extended period of time to examine normal fluctuations prior to fish introduction.
4. Continue to monitor water quality after the introduction of fish and enter data into the Aquaculture in Action online database.

Goal 4: Monitoring Growth

Monitor and record the growth of the fish before their release at the end of the school year and enter class data in the Aquaculture in Action (AinA) database.

Fish growth will be carefully monitored in relation to density and food conversion. Results will be recorded in the AinA online database so projects can be shared between schools. Fish anesthesia procedures have been developed to support data collection with minimal disturbance to the fish.

Objectives

1. Design and carry out experimental and monitoring procedures with reproducible results.
2. Use water quality kits and/or computer probes to collect and graph data on pH, dissolved oxygen, temperature, salinity, ammonia, nitrites, and nitrates.

3. Examine the relationship of the physical parameters, room temperature, and composition/amount of food to fish density.
4. Transport and release fish into local streams, lakes, and reservoirs.

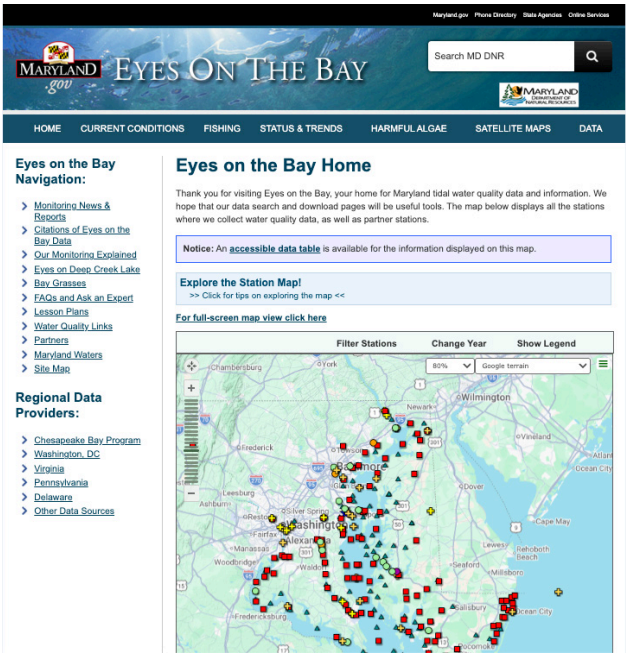
Goal 5: Comparing Artificial Systems to Nature

Make comparisons between an artificial aquaculture environment and a natural environment.

In a restocking program, it is important to compare the recirculating system environment to the natural environment. Identifying specific improvements needed within the natural aquatic system is key to the success of the reintroduction of fish to that system. It is essential to consider the possible positive and negative effects of reintroduction, not only on the local aquatic system but also to the general health of the Chesapeake Bay.

Objectives

1. Compare the fish tank environment to the natural environment of the selected species by examining chemical, physical, and biotic parameters.
2. Identify specific projects to enhance or protect the environment of the natural aquatic system.
    a. Examine the positive and negative effects of clearing a stream of debris.
    b. Describe specific structures that could be constructed in the stream (e.g., dams, wings, channel constrictors) to enhance the environment.
3. Reduce the impact of people moving through a stream (reducing turbidity) by building bridges and walkways.
4. Reduce turbidity by constructing stream bank stabilization devices.
5. Reduce turbidity and nutrient runoff by planting stream buffers.
6. Monitor local construction activities to ensure they comply with local and state regulations for controlling site runoff.
7. Compare water quality data collected in open portions of the Chesapeake Bay to data collected at the selected site. Eyes on the Bay is a source of water quality data that can be used for this comparison.
8. Monitor the effectiveness of the restocking program by electrofishing on a yearly basis to estimate the population of various fish species.



UNIT 2:

Recirculating Systems and Parameters

Photo, Don Adams

2.1 Background

One goal of aquaculture is to raise a large number of fish to as great a size as possible in a short period of time. This is accomplished by feeding the fish an amount of food equal to a specific percentage of their body weight (usually from 1-3%) every day. The specific amount depends on factors such as fish species, fish size, and water temperature.

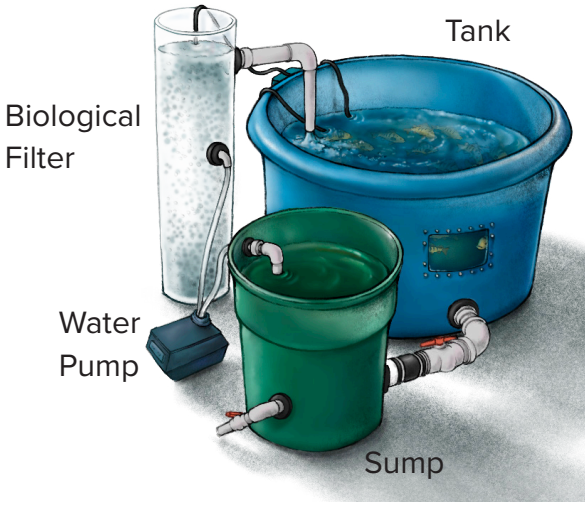
As a result of feeding, fish produce ammonia. If left unchecked, ammonia can slow growth and eventually kill the fish. In recirculating systems, it is a primary concern to treat the water before it is used again. This treatment is usually accomplished in three steps. The first two steps involve clarification, in which suspended solids settle out of the water and are physically removed by a filter. In a typical classroom system, these two steps will take place in the first filter or sump. The last step is ammonia conversion, in which ammonia is converted first to nitrite and eventually to nitrate. Biological filtration (bacteria) is used for this step. The total amount of surface area of the filter medium in your system will determine the nitrification rate. This, in turn, will determine the maximum number of fish and the feeding rate for each system.

2.2 Objectives

1. Calculate the volume and surface area of the tank and each of its filters.
2. Calculate the flow rate of a tank system.
3. Calculate ammonia production rate and the amount of filter surface area required for nitrification for the fish that will be raised.

2.3 Classroom Resources

2.3.1 Recirculating System Components



There are four basic components of a recirculating system:

1. Tank
2. Sump
3. Water pump
4. Biological filter

There are many variations within these four components, but their basic functions remain the same. Depending on its size, the design and construction of a basic recirculating system can be simple and affordable. There are also more expensive designs that use automated monitoring, advanced filtration techniques, and automated feeding. Here is a breakdown of the four basic components of a recirculating system and their functions.

Tank

Most aquaculture tanks used for raising fish are round and opaque. This allows fish to naturally school in one direction or another. The opaque tank has a calming effect on the fish and reduces the stress associated with glass or acrylic tanks often found in the classroom. Most tanks are constructed from a type of monoform plastic or fiberglass that is durable and unlikely to leak (until holes are drilled to connect the tank to other parts of the system). The number of fish the system can accommodate is determined, in part, by the size of the tank.

Sump

The sump is a container connected to the tank via a pipe. It serves as an area to collect sediment and particulate matter. Water travels from the tank to the sump via a bulkhead located on the lower side of the tank. This helps sediment and particulates collect in the bottom of the sump so they do not pass back into the tank. Collecting and disposing this material is important for maintaining water quality in the system. The sump has an outlet at its base that can be easily opened to drain collected sediment and particulate matter.

Water Pump

Pumps used for aquaculture systems differ from pumps used in standard aquariums. Aquaculture system pumps are located outside the tank and do not need to be submerged. They provide the link between the sump and the biological filter. There are a variety of pumps on the market; the tank size and desired rate of flow will determine the size of the pump needed. Before purchasing a pump, consider whether it will need to lift water vertically over a specific distance. This is referred to as "head height" on the pump rating. For example, if your pump sits on the floor and needs to raise water 30" to reach the level of the tank, a head height rating of at least 3 feet would be appropriate.

Biological Filter

Whether designed or purchased, the function of the biological filter remains the same: provide an area for the growth of denitrifying bacteria to break down the toxic waste (ammonia) produced by aquatic organisms. When acted upon by specific bacteria, ammonia becomes nitrite, a very toxic compound. Nitrite is then acted upon by another species of beneficial bacteria and becomes nitrate—a harmless nitrogen-based compound. The key is to provide the correct environment and substrate for these beneficial bacteria to grow and to design a biological filter that meets the demand of the size of the system and the number of fish. Unit 3 of this manual covers the details of biological filtration. Reference it before setting up a system and after the system has been stocked with fish.

2.3.2 Handy Conversions and Formulas

Conversions

1 m^3 = 1,000 L

1 L = 0.264 gal

1 gal = 3.79 L

1 gram = 0.00221 lb

1 oz = 0.0625 lb

Volume and Surface Area Formulas

Tank Shape	Tank Volume	Surface Area of Water Exposed
Cube	s^3	s^2
Rectangular	$l \times w \times h$	$l \times w$
Trapezoidal	$0.5 \times h \times (b_1 + b_2)$	b_1
Cylinder	$\pi \times r^2 \times h$	$\pi \times r^2$
Cone	$\frac{1}{3} \times \pi \times r^2 \times h$	$\pi \times r^2$
Oval	$h \times ((\pi \times w^2) \div 4) + (l \times w) - w^2$	$((\pi \times w^2) \div 4) + (l \times w) - w^2$

s = side
x = multiply
r = radius

w = width
l = length
h = height

π = 3.14
b_1 = area of top (l x w)
b_2 = area of bottom (l x w)

2.4 Classroom Activities

2.4.1 Volumes and Surface Area

Measure all tank dimensions; record them below and complete calculations using the formulas provided.

	Shape	Height	Weight	Length	Radius	Volume*	Area Exposed to Air*
Tank							
Filter 1							
Filter 2							
Filter 3							
Filter 4							

**Show all work below*

Work for volume calculations:

Work for area exposed to air (surface area) calculations:

2.4.2 Calculating Flow Rate

Obtain a beaker, bucket, or other container that measures either liters or gallons. For each location where water returns to the tank, measure the time it takes to collect one liter or one gallon of water. Repeat three more times (for a total of four times) for each return location. Record all data below. Complete all calculations to determine the flow rate of each filter returning water and the overall flow rate of the system. Then, calculate the "turnover" time of the system—the time it theoretically takes to circulate all the water in the tank.

Time in seconds to fill one liter or gallon

Trial	Time in seconds						
	Return 1	Return 2	Return 3	Return 4	Return 5	Return 6	
1							
2							
3							
4							
5							
Total							
Average							Total for whole system _____ (use below)

For liters: (_____ liters/sec (from chart)) x (60 sec/min) = _____ liters/min

(_____ liters/min) x (0.264 gal/liter) = _____ gallons/min

(_____ gallons/min) x (60 min/hour) = _____ gallons/hour

For gallons: (_____ gallons/sec (from chart)) x (60 sec/min) = _____ gallons/min

(_____ gallons/min) x (60 min/hour) = _____ gallons/hour

Turnover time: (_____ gallons in system) x (gal/min) = _____ min for turnover

2.4.3 Ammonia Production

The following formulas are for 100 lb of fish. TheYieldGrid is a resource with an interactive calculator for estimating biofilter volume and nitrification capacity.

Formula 1: Ammonia = $0.0157 + (0.0455 \times O_c)$

Where O_c = lb of oxygen consumed per 100 lb of fish per day,

$O_c = K \times T^n \times W^m$

Where K = species dependent constant* (3.05×10^{-4} for trout),

T = degrees in Fahrenheit,

n = constant* (1.855 for trout),

m = constant* (0.138 for trout),

W = weight of individual fish, average or mean.

**These constants are specifically for trout and won't work for other fish.*

Formula 2: $N_a = 0.0289 \times F$

Where F = feeding rate,

0.0289 is a constant for 100 lb of fish.

Ammonia Production Rates

Use either Formula 1 and your average fish weight (trout only) or Formula 2 and your intended feeding rate to calculate daily ammonia production. (TAN = Total Ammonia Nitrogen).

Formula 1: TAN = 0.0157 + 0.0455 x K x Tn x Wm

ammonia production rate for 100 lb of trout =_____lb ammonia/day

100 lb trout/_____lb your trout = _____ (no unit)

lb ammonia/day (your number) = _____ lb ammonia/day your trout

OR

Formula 2: TAN = 0.0289 F

TAN = 0.0289 X _____ % feeding rate (Note: for 2% enter 2, not .02)

TAN = _____ lb ammonia produced/100 lb fish/day

2.4.4 Filter Surface Area

Formula 3: $F_a = N_p / N_o$

Where F_a = surface area required in square feet,

N_p = lb ammonia produced per unit biomass per day,

N_o = ammonia oxidized per square foot per day = 1.56×10^{-4}.

Calculations

N_p = _____ lb ammonia (from above)

N_o = 1.56 x 10-4 ft^2

F_a = _____ / 1.56×10^{-4} ft^2 = _____ ft^2 media required/100 lb of fish

Formula 4*: F_{au} = Unit biomass (your tank) / (100lb x F_a)

Calculations

Unit biomass (your tank) = _____lb fish

F_a = _____ ft^2 media required/100 lb of fish (from Formula 3)

F_{au} = _____ / (100lb x _____) = _____ ft^2 media for your tank

**Use either the total weight obtained for your fish or that you intend to have at maximum capacity.*

2.4.5 Air Lift Technology

Objectives

1. Explain the principles and benefits of using air to move water in an aquatic system by conducting several experiments.
2. Describe the construction of a lift tube and its possible uses in an aquatic system.
3. Calculate the volume and surface area of a bubble.
4. Compare the bubble size produced by different diameter lift pipes to that produced by medium pore diffusers.
5. Correlate bubble size to the resulting volume and height of water lifted.

Background

Airlifts can be used to lift water into biological filters, aquaponic tubes, and satellite tanks connected to larger systems. Lift systems are inexpensive if you have plenty of air.

Activity 1: Research

1. Have students conduct an online search and answer the following prompts.

   Please preview any resources and ensure the content is appropriate and accessible for your students.

   a. Draw a diagram of the airlift pipes.

   b. Why are airlifts more efficient for moving rather than lifting water?

   c. What causes the water to move up a pipe?

   d. Why are large bubbles better for lifting water?

   e. What is the relationship between air volume and lift capacity?

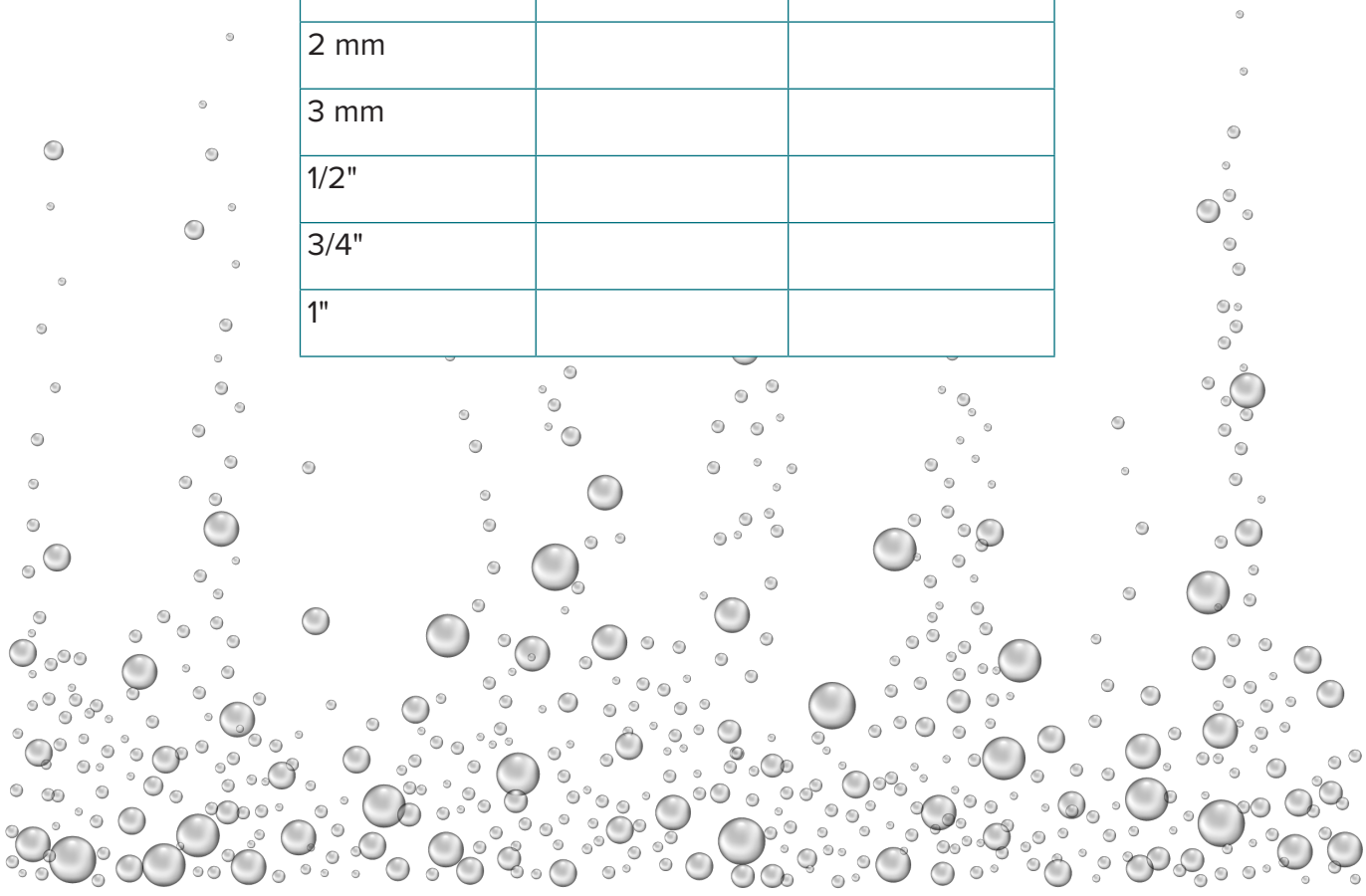
   f. List the three scientific principles describing the function of an air lift tube.

2. Have students research water quality parameters and then:

   a. Give the formulas for volume and surface area of a sphere.

   b. Calculate the volume and surface area for air bubbles that would be produced by a standard medium pore diffuser (1-3 mm bubbles) and the largest bubbles that could hypothetically be carried in 1/2", 3/4", and 1" air lift tubes. Record answers in the table below.

Bubble Size	Volume	Surface Area
1 mm		
2 mm		
3 mm		
1/2"		
3/4"		
1"		



Activity 2: Laboratory – Airlift Designs

Problem: Design an airlift system and determine the parameters of height and volume for moving water.

Hypotheses: Assuming that air bubbles achieve the size of the airlift tube, predict which lift tube will move the greatest volume of water.

Materials

- Sections of 1/2", 3/4", and 1" PVC pipe
- PVC elbows, tees, and couplings for 1/2", 3/4", and 1" pipe
- Airline, 2 air valves, air source, 2 air diffusers
- Meter stick and container to measure volume
- Stopwatch, zip ties

Note: It is not necessary to glue the PVC pipe when designing the lift tube. The lengths of the PVC sections should vary. You can also use two air lines, in the same or different locations and with two air line valves (optional). Zip ties can be used to attach air lines.

Procedure

Experiment with various lift tubes to come up with a design that will give:

a. Maximum height

b. Maximum volume

c. Maximum height and volume

Data and Observations

Draw and show measurements of the three designs. Be sure to include diameter of pipe, joints, lengths of all sections of pipe, location of airline(s), air diffusers, valves, depth of main tank, amount of pipe above and below water, as well as volume of water per minute and height of lift.

Design 1: Maximum lift drawing with measurements

Height of lift = _____cm

Volume = _____liters/minute

Depth of tank = _____cm

Design 2: Maximum volume drawing with measurements

Height of lift = _____cm

Volume = _____liters/minute

Depth of tank = _____cm

Design 3: Maximum volume and lift drawing with measurements

Height of lift = _____cm

Volume = _____liters/minute

Depth of tank = _____cm

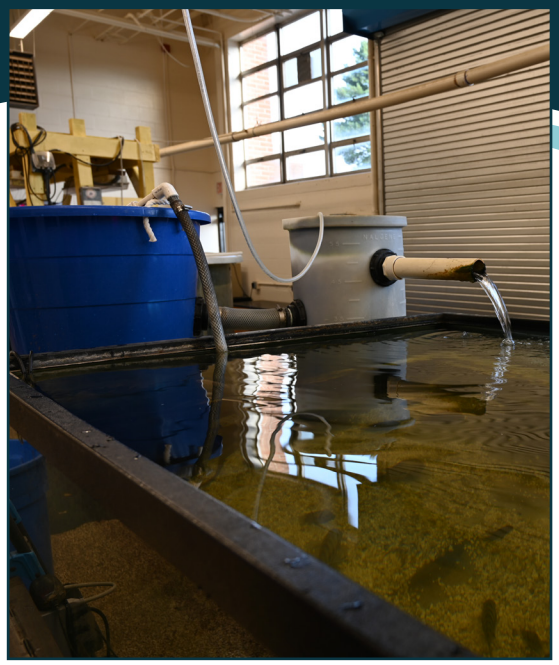
Conclusions

Answer the following questions.

1. What is the relationship between pipe size and lift height? Explain.
2. What is the relationship between pipe size and lift volume? Explain.
3. What happens to the volume of water as the height is increased for the same diameter pipe? Explain.
4. What is the advantage or disadvantage to using air diffusers. Explain using bubble theory.
5. What is the best lift tube diameter and delivery method (diffuser, air line, etc)?
6. Explain the benefit of lift tubes for dissolved oxygen levels.
7. What are the advantages and disadvantages of lift tubes compared to pumps?
8. Based on your results:
    a. What was the flaw in your original hypothesis?
    b. Estimate the size of the bubbles produced that gave maximum lift/volume. Defend your estimate.

UNIT 3:

Filtration



3.1 Background

The filtration system is the heart of a closed aquatic system. The filtration system is not only responsible for removing uneaten food and fish waste, but more importantly, provides an environment for the conversion of highly toxic nitrogen waste to a non-toxic form. Filters can be purchased (or designed and built) to:

- Trap or settle out particulate matter from a system
- Aid the breakdown of nitrogenous waste products
- Remove protein wastes from the water
- Improve overall water quality

In this unit, students will compare different types of filtration systems. They will examine how the communities of bacteria that form on filter media help drive the nitrification process. Students will calculate the correct size of a biological filter, construct the filter, and seed the filter as part of the start-up procedure for the aquaculture tank. Students may develop individual research projects to test how effectively different biological filtration systems break down waste.

3.2 Objectives

1. Compare a variety of aquaculture filtration techniques, including mechanical, chemical, and biological.
2. Build a filter for an aquaculture system that incorporates biological, chemical, and mechanical methods.
3. Explain the fundamental principles of the role of microorganisms in filtration.

4. Demonstrate the proper techniques for maintaining a healthy filtration system.
5. Develop a research project that compares different commercially available filter media to determine which performs best in an aquaculture system.

3.3 Classroom Resources

3.3.1 Filters, Filters Everywhere: The basics and options

Aquaculture filtration methods can be broken down into three groups: mechanical, biological, and chemical. In most cases, these three types of filtration can be incorporated into a single design. For large systems, it may be necessary to separate the mechanical filtration from the biological and chemical filtration. It is fairly easy to design a filter. You can minimize costs by using common materials instead of prefabricated materials found in pet stores and aquaculture supply catalogs.

Mechanical Filtration

Mechanical filtration is accomplished by trapping or settling out sediment, particulate matter, and waste from a recirculating system. This is the simplest type of filtration and is often used in wastewater treatment plants. As large materials enter a wastewater treatment plant, they are screened out and mechanically removed by conveyer belts. Smaller particulates are trapped, settled out, or scraped off the surface of the water. Particulates need to be removed so the rest of the treatment process can function properly. A recirculating aquaculture system is very similar; particulate matter should be removed before, or in conjunction with, biological and chemical filtration. Particulate matter that is not removed from a recirculating system can cause water quality problems, impacting fish health and growth.

The simplest way remove particulates from a system is to use a settling tank or incorporate an area where particulates can "settle out" without interfering with other filtration processes. Water can be fed into a settling tank from the bottom, side, or top of a system, as long as the water moving out of the settling tank does not lift the settled particulates off the bottom. The settled material can be easily drained or siphoned out, making this a common and effective filtration method.

Filter materials that trap particulates—including air condition filter pads, filter floss, and nylon stockings—can aid mechanical filtration. However, they may not be cost effective. Filter materials must be routinely removed and rinsed or replaced. Otherwise, material buildup and decay can lead to ammonification and excess nutrients. This is especially important in larger systems where the goal is to rapidly grow fish.

Biological Filtration

Biological filters go by many names, including wet/dry filters (commonly used in saltwater aquariums), trickle filters, fluidized beds, and sponge filters. Regardless of the name, biological filtration is the life support system for an aquarium or aquaculture setup. Its purpose is to maximize contact among ammonia-rich water from fish waste, beneficial bacteria (nitrosomonas and nitrospira), and oxygen.

Nitrosomonas and nitrospira are naturally occurring soil bacteria that can grow on a variety of filter media if given sufficient food (ammonia and nitrite), oxygen, and time. As these bacteria colonize surfaces, they form a biofilm. Increasing the surface area available for biofilm growth improves the efficiency of a biological filter, which in turn supports fish health and growth. Biological filtration can also be partially achieved through aquaponics, where plants help remove nutrients from the water (see Unit 6: Aquaponics in the Classroom for more details).

Biological filters are inexpensive to build and can hold a larger volume of biofilm media than many prefabricated models at the same cost. The biological filter should receive water that has already passed through a settling tank, or it should include a prefilter area that allows particulates to settle out or become trapped.

Water can enter the biological filter from the bottom (upflow) or from the top (downflow). In an upflow filter, biofilm media remains submerged in water. Because water contains much less oxygen than air, an air pump is needed to supply the bacteria with sufficient oxygen. In a downflow filter, biofilm media is exposed to air and water, which increases oxygen and enhances the bacteria's ability to convert ammonia to nitrate. Recirculating systems may use one or a combination of these filter types.

Both upflow and downflow filters rely on oxygen and are therefore aerobic processes. Another process, anaerobic ammonium oxidation (anammox) has also been used in wastewater treatment plants and recirculating aquaculture systems to convert ammonia and nitrite into dinitrogen gas (van Kessel et al., 2011). This process is naturally carried out by planctomycetales bacteria in oxygen-free (anaerobic) sludge but can also be cultivated on biofilm media (Tal et al., 2006). Additional information on biological filtration and the anammox process can be found in Unit 4.3.2.

Biofilm media can include a range of materials, such as hair curlers, bird netting, PVC pieces, gravel, oyster shell, lava or tufa rock, bioballs, ceramics, glass beads, and sand. Commercially manufactured biofilm media is often more expensive and may not offer significant advantages over more conventional, homemade options. Any material selected should meet the following criteria:

- High surface area for biofilm growth
- Allows free movement of water in and around itself
- Will volatilize or degrade into the system
- Does not trap particulates and sediment

Adding a large amount of biofilm media to the biological filter will improve the efficiency of waste conversion and support the health of your fish.

Chemical Filtration

Chemical filtration uses specialized media that scavenge or bind toxic compounds, nutrients, and odors to improve water clarity. The most common form is activated carbon, which is typically housed in a mesh bag or filter canister.

Chemical filtration offers many advantages, especially when using a water source with poor water quality. Specialized media (a complex of chemical compounds) can help control or remove a range of contaminants found in water supplies, from pesticides to chlorine. However, chemical filtration can be expensive in large aquaculture systems. One way to reduce costs is to prefilter the water before it enters the system. This can be done by connecting a large canister filled with activated carbon to the water supply. These canisters are usually rated for a specific flow rate and a maximum number of gallons they can treat before the carbon must be replaced.

3.3.2 Biofilm Biology: The link between microbes and filter media

Biofilms are found throughout the human body and in many parts of the environment, including on teeth, in the intestines, on medical devices and contact lenses, inside drainage pipes, and on the bottoms of ships. Dental plaque is a familiar example of a biofilm that forms on teeth and can cause tooth decay. On ship hulls, biofilms can attract and accumulate small organisms, creating enough drag to slow a ship down! Biofilms have even been found aboard space stations, where they can clog machinery or make astronauts sick. In 2019, a study conducted on the International Space Station examined how biofilm formation differs in space.

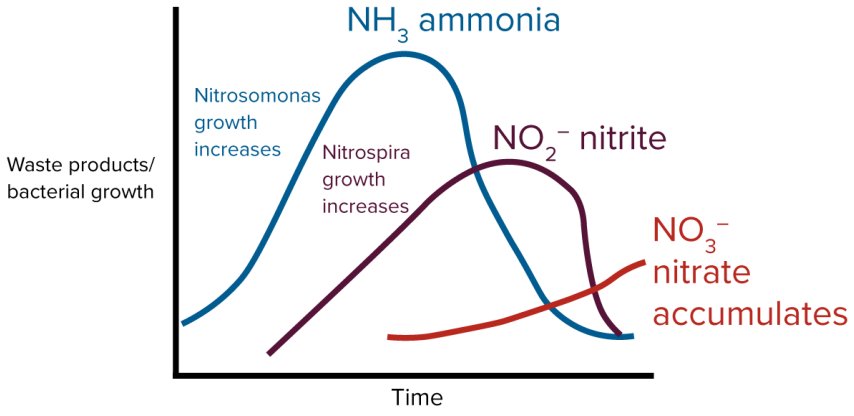
All biofilms share a common feature: a primary layer of bacteria that attaches to a surface and provides an attractive environment for other bacteria and larger organisms. These slimy layers can be resistant to antibiotics, so scientists are studying how the bacterial colonies form, adhere to surfaces, and how they can be either prevented or encouraged to grow. To further explore biofilms in the classroom, use Maryland Sea Grant's interactive Biofilms and Biodiversity lessons (mdsg.umd.edu/topics/k-12-lesson-plans/biofilms-and-biodiversity).

Biofilms with nitrosomonas and nitrospira are especially important for the health of aquaculture systems. Biofilm formation on filter media in an aquaculture system is not an overnight process. A well-established biofilm capable of converting ammonia may take a few weeks to form and depends on environmental factors, including:

- Water temperature
- Amount of food (ammonia) available
- Amount of oxygen available
- pH of the water

Given the right conditions, bacteria will use different mechanisms to attach to all available surfaces in the system. Some bacteria produce a sticky polysaccharide material. Others form threads that grab onto surfaces, much like kelp holdfasts, the climbing tendrils of ivy, or the hooks and loops of Velcro. Attachment allows the bacteria to establish residence and rapidly reproduce within a very small area until their population reaches a maximum. In natural environments, this slimy, sometimes encrusting layer provides a habitat for other microscopic and macroscopic organisms, forming a living community.

Biofilms of nitrosomonas and nitrospira grow in stages. After nitrosomonas begins to convert ammonia to nitrite, nitrospira start to increase. If there is a delay or interruption to this process, the levels of nitrite could rise and kill the fish. This is why stocking a new system with fish too quickly is risky. Water tests can show the rise and decline in ammonia levels as nitrosomonas establishes itself, as well as the rise and decline of nitrite levels as nitrospira grows. This is why water testing, especially at the start of a new system, is critical for fish health. The diagram at below illustrates the relationship between bacterial and waste production.

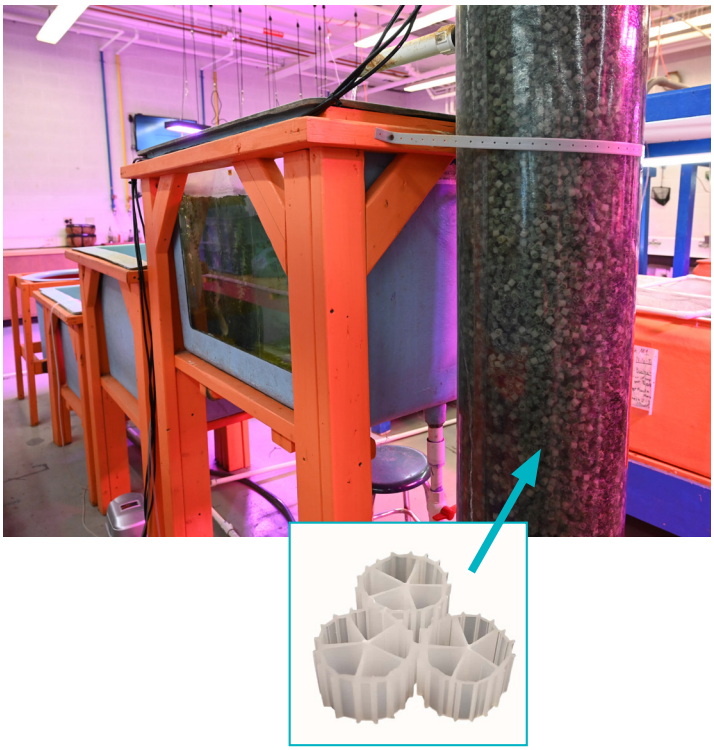


There are ways to speed up biofilm formation in an aquaculture system. One option is to add commercially prepared nitrosomonas and nitrospira. These products, available in liquid or powder form, add large amounts of beneficial bacteria to the system. However, the bacteria need a food source to become established and grow. For that reason, gradually stock the system with fish at the start. Used properly, these supplements can shorten the time it takes a biofilm to become established. Using proper filter media, with the criteria described above, can also speed up biofilm formation.

Some commercially available bioballs claim greater efficiency, and researchers have studied how bioball material, size, and shape affect biofilm growth. One popular type is Kaldnes media—a small, white plastic medium with outer "fins." In an upflow filter mixed with air, this media forms a fluidized bed. It becomes neutrally buoyant, maximizing contact between the biofilm, the surrounding water, and dissolved oxygen. The media is engineered in a wheel shape and is slightly positively buoyant, allowing a small amount of water flow (created by adding air to the process) to circulate the media throughout the vessel.

Oxygen and food (ammonia and nitrite) give the bacteria the means to grow, while the Kaldnes media provides maximum surface area for the bacteria to colonize and produce biofilm. This process removes harmful ammonia and nitrite from the water. As the Kaldnes media chaotically circulates within the biotank, dead bacteria and biofilm are removed, making space for new bacteria and biofilms to colonize.

Kaldnes Media Biofilter Guidelines



Each 100 L will handle up to 1.5 kg of food per day.

To calculate liters of media required for a filter chamber:

L of media recommended = V x 0.0164 x 0.5

Where V is the volume of X (the filter vessel) in cubic inches.

To calculate the volume in a non-cone vessel:

$$V = l \times w \times d$$

Where l is the length of X in inches,

w is the width of X in inches,

d is the depth of X in inches.

To calculate the volume for a straight side vortex chamber/cone shaped:

$$V = r^2 \times \pi \times h$$

Where r is the radius in inches,

π is 3.14,

h is the height of the top of cone to water level

Benefits of Kaldnes Moving Bed™ process:

- Creates an oxygen rich environment for your fish
- Promotes even water distribution throughout the media, preventing the dead zones commonly associated with static filtration systems
- Old bacteria is constantly removed, promoting new highly active bacteria
- Provides significantly more surface area than other types of filter media
- Self-cleaning and requires little to no maintenance
- Creates an oxygen-rich environment that improves oxygen transfer in moving bed systems
- Biological filter performance can be increased by adding more Kaldnes media
- 50 L of Kaldnes media can handle up to 0.25 kg of food per day

3.4 Classroom Activities

Activity 3.4.1 Seeding and Activating a Biological Filter

"Seeding" a filter ensures an aquaculture system is ready to handle the biological load (waste products) of fish without depleting dissolved oxygen or developing toxic levels of ammonia or nitrite. There are many ways to "seed" a biological filter, including using "garden water" or bacterial additives.

- **Bacterial Additives**: Using commercially prepared bacteria, in the form of liquid or powder additives, to add large amounts of beneficial bacteria to the system.
- **Garden Water**: Introducing beneficial bacteria to the system using garden soil. Several handfuls of soil are added to a mesh bag or stocking, which is tied off and placed in a tub of water. Biofilter media and an aerator are then added to the tub. The media sits in this solution for several days (up to a week) while the soil bacteria colonize the media. The media is then removed from the water tub and added into the system's biofilter.

Seeding the biological filter jump-starts the denitrification cycle and the conversion of ammonia and nitrite, creating a smoother introduction for the fish.

Formulas for biological activation

Chemicals are in solid form. Select a formula based on what you have available.

Ammonium Chloride

Number of gallons of water x 0.0113 = grams to add to system (to raise the ammonia level to 1 ppm in the system)

Example: 250 gal x .0113 = 2.83 g (add 2.83 g to raise the ammonia level to 1 ppm in the system)

Ammonium Hydroxide

Number of gallons of water x 0.0135 = grams to add to system (to raise the ammonia level to 1 ppm in the system)

Ammonium Nitrate

Number of gallons of water x 0.0169 = grams to add to system (to raise the ammonia level to 1 ppm in the system)

Activity 3.4.2 Calculating the Size of a Biological Filter

Adapted from *Applications of Biotechnology in Aquaculture*, National Council for Agricultural Education, 1996.

Before you construct a biological filter, let's clarify some details about recirculating systems. Biological filters must be able to handle the waste from the fish that are going to be raised in the system. The filter size needs to be constructed with fish growth in mind; your fish will not remain 2 inches in size for very long! These calculations will help you estimate the appropriate size for the biological filter you will need.

1. Determine pounds (lb) of ammonia (NH_3) produced by your fish per day:

   _____lb of fish food fed each day x 0.03 lb NH_3 = _____ lb NH_3 per day

2. Convert this to kilograms (kg) per day:

   _____lb of NH_3 x 0.4536 kg = _____ kg NH_3

3. What is the water temperature of your system?

   _____°C

4. Calculate the nitrification rate of your system:

   Nitrification rate (kT) = (_____ °C X 0.11) - 0.2 = _____

5. Calculate the surface area of filter needed:

   _____ Kg NH_3 per day (step 2)

   Surface area (S) = _____ square meters

   _____ Nitrification rate, (kT) (step 4)

6. Using the formula above, calculate the surface area of the biofilm media you are using in your filter and the amount needed to provide the square meters of surface area from step 5.

Activity 3.4.3 Building a Biological Filter

Now that you have calculated the size of your biological filter, it is time to build one.

Trash Can Filter Supplies

- 3-gallon trash can
- 1 ft of ¾" PVC pipe
- ¾" PVC elbow
- ¾" male PVC adapter
- ¾" female PVC adapter
- 1 ft of ½" PVC pipe
- ½" PVC end cap
- ½" – ⅜" PVC adapter
- small hose clamp
- 3 ft of ⅝" plastic tubing
- Aquarium pump (requires at least 2 ft of head, a pump strong enough to push the water up 2 feet from the tank)
- Air conditioning filter pad
- Bag of bird netting or lots of plastic hair curlers
- Hole saw or very sharp knife
- Two ½" rubber gaskets

Preparing the Trash Can Filter

1. Clean all parts with water to remove dust and dirt.
2. You will need a hole saw or a very sharp knife to make the holes in the trash can. **Get help from an adult when using tools and follow safety precautions to avoid injury.**
3. On each narrow side of the trash can, about 2" down from the lip, make a ½" hole. The holes should be neat and directly opposite from one another. A hole saw works best for this. The 1 ft (more or less, depending upon the size of the can) of ½" PVC pipe should fit snugly through the holes. If the pipe fits well, remove it and proceed to step four.
4. Drill small holes along one side of the ½" PVC pipe. This will allow water to spray down onto the filter media inside the trash can.
5. Make a ½" hole on the wide side of the trashcan (only on one side!). This hole should allow the ¾" male and female PVC adapters to be connected on either side of the trashcan. Place a rubber gasket on the male and female adapters and thread them together through the hole. The rubber gaskets should make a nice seal on either side of the trashcan. Do not over-tighten the male and female adapters, as this could lead to stress on the rubber gaskets over time.
6. At this point, fill the trash can with water to make sure there are no leaks. If you have a leak, aquarium sealant can help. Place the sealant between the male and female adapters at the point of contact with the trash can.
7. Take the 1 ft of ¾" PVC pipe and cut it into 3 pieces: two 2" pieces and one 8" piece.
8. Connect one 2" piece of the PVC pipe to the end of the female adapter outside the trash can. This will serve as the pipe for the outflow of the water. Connect the other 2" piece to the male adapter inside the trashcan. This will serve as the connector to the elbow inside the trashcan.
9. Take the ¾" PVC elbow and cut off the point of the elbow so there is a hole in the end. This is where the water will flow out from the inside the trash can. Connect the elbow to the 2" piece of ¾" PVC pipe you placed inside the trashcan. The elbow should point upward inside the trashcan.
10. Connect the 8" piece of ¾" PVC pipe to the elbow inside the trash can. This will serve as the overflow pipe in case the hole in the elbow gets clogged.
11. Take the ½" PVC pipe and slip it through the holes in the top of the trash can. Make sure the small holes are face down so water does not spray out of the can. Place the end cap on one end. To make the seal watertight, use aquarium sealant on the inside of the end cap.
12. Connect the ½" - ⅜" PVC adapter to the other end of the ½" PVC pipe.

Filter Pads and Other Media

In the bottom third of the container, place filter media that allows for a lot of air space and provides large amounts of surface area for waste-busting bacteria to grow. You can use a variety of materials from the local pet store or hardware store as filter media. Materials like bird netting and hair curlers are good choices, because they are cheap and you do not need to replace them. Remember, the more oxygen the bacteria get, the better they will break down waste (as long as the environment is wet and the water is free of harmful substances).

On top of the filter media, place something that will trap waste, dirt, and sediment from the tank. Air conditioning filter pads and artificial sponges are good choices, as long as they allow water to easily flow through to the lower part of the trash can. Place these materials below the holes in the ½" PVC pipe. After a few days, these pads will become dirty. It is important to clean them with water or replace them to ensure the health of the fish in the tank.

Testing the Filter

Take the plastic tubing and connect it to the adapter on the end of the ½" PVC pipe. Connect the other end of the plastic tubing to the submerged pump in the tank. Make sure the tubing fits snugly. If it seems loose, use the hose clamp to tighten the fit on the adapter.

Plug in the pump and watch it go to work! After your filter is up and running, think about modifications you could make or different designs you could use. The possibilities are endless. Have fun!

Activity 3.4.4 Research Projects

1. **Designing an external filter.** Design a filter that can use different types of media for a specific tank. Establish the water quality by conducting chemical tests on the first group of fish. These fish are the control group. Change the biological media and document the change in water quality to evaluate the filter media. Students will observe dramatic swings in water quality with the addition of each new media until the nitrification bacteria are established. This time can be reduced from two weeks to 24 hours by adding Aqua Gold (freeze-dried bacteria) each time a new media is introduced. Examples of media include sand, cell-pore, lava rocks, bioballs, bio-clam, bioglass, scrub pads, and biofilm carrier elements. Students can also calculate the effectiveness of each media based on surface area. Over time, ammonia production will rise as fish grow. The same biomass of the tank can be maintained by adjusting the size of the fish population to compensate for their growth.

2. **Testing biofilter media efficiency.** This project has a similar objective but uses a different approach. Place a variety of biofilter media in biofilter media bags or similar containers. Then, submerge them in an established recirculating system (or its filter), to establish bacteria colonies. After several weeks, these media should have bacteria growing on them in numbers relative to their respective surface areas. Next, add ground-up fish food to the water to make a nitrogen-rich solution. This solution can be tested for ammonia or nitrite, and changes can be made (i.e., dilutions) to obtain a desired starting concentration. Expose the various biofilter media to this nitrogenous solution for varying lengths of time, from 24-72 hours, and retest the solutions. The research and design of the filters is up to the students. Regardless of experimental design, have students set up a control in which the solution is circulated over no biofilter media.

3. **Do bacterial additives really work?** Many kinds of bacteria participate in the biological filtration of pond water. Many bacterial additives are commercially available to reduce fish waste. However, there is some controversy regarding the effectiveness of bacterial starter cultures. Have students research several additives and design and carry out an experiment to test the effectiveness of one or more of the additives. Some examples of additives include:

    - Accelobac (American Biosystems)
    - Aqua Gold (National Fish Pharmaceuticals)*

- Microbe-Lift Nite-Out II (Ecological Labs)
- Bio-Spira (Instant Ocean)
- Quick Start (API)

Research the ingredients and characteristics of each additive. Determine their effectiveness by adding them to a newly established aquaculture system and monitoring ammonia, nitrite, and nitrate levels. Remember to include a control system without any bacterial additives. Alternatively, samples of tank water can be inoculated and tested daily or weekly.

**If you can only get one bacterial additive, we recommend using Aqua Gold. This dry additive consists of nitrifying bacteria and enzymes that boost the breakdown of ammonia and nitrite wastes. It has a much longer shelf life than liquid additives.*

UNIT 4:

Water Quality

4.1 Background

Water quality refers to the measurement of chemical and physical properties that help determine the condition of the water and its ability to support life. In fresh, estuarine, and marine ecosystems, parameters such as temperature, dissolved oxygen, pH, hardness, alkalinity, carbon dioxide, turbidity, ammonia, nitrite, and nitrate are measured to understand water quality. Salinity is also a driving force in estuarine and marine ecosystems. Carbon dioxide and acidity are concerns for many shell-producing animals. Several helpful articles explaining this process can be found in the March 2012 issue of *Chesapeake Quarterly*, "Acid Test for the Great Shellfish Bay?".

Understanding water quality can be challenging due to the interactions that occur between parameters under different environmental conditions. Water quality is also impacted by human activity and the variety of chemicals we introduce to aquatic ecosystems, both directly and indirectly.

In aquaculture, water quality is essential for raising fish to market size in a short period of time. The wide range of commercially available filtration systems reflects extensive research and development into maintaining high water quality standards in aquaculture systems. This unit gives an overview of key water quality parameters, but further research is encouraged.

4.2 Objectives

1. Demonstrate the skills needed to measure fundamental water quality parameters: temperature, dissolved oxygen (DO), pH, carbon dioxide, alkalinity, hardness, ammonia, nitrite, nitrate, hydrogen sulfide, chlorine, and chloride.
2. Apply a variety of monitoring techniques for each water quality parameter.
3. Demonstrate an understanding of the interactions of specific water quality parameters.

4. Demonstrate the appropriate nutrient cycling concepts that directly relate to maintaining good water quality.
5. Apply strategies for maintaining and correcting water quality.

4.3 Classroom Resources

4.3.1 Water Quality Parameters

Dissolved Oxygen (DO)

- **Sources**: Diffusion at the air/water interface and photosynthesis from aquatic plants and algae.
- **Removal**: Respiration or biological oxygen demand.
- **Interaction**: Decreases with increasing temperature, decreases with increasing salinity, competition with CO_2 for solubility in water.
- **Healthy Levels**: Above 5 mg/L or parts per million (ppm) is a minimum level for most fish.
- **Periodicity**: Seasonal fluctuations with temperature and salinity; daily fluctuations due to photosynthesis and respiration.
- **Measurement Options**: Dissolved oxygen (DO) can be measured with oxygen meters or probes or using chemical test kits which give results in mg/L or ppm. DO meters can cost about $600 or more. A well maintained and calibrated probe can give a quick, accurate, and safe reading. Another option, if you have a computer, is to purchase a DO probe and software from Vernier Software™, Inc. One advantage to this type of measurement is that data is collected continuously, in real-time and can be instantly graphed. Chemical test kits are still the most reliable, inexpensive way to test DO. Chemical methods vary from snap-cap ampules that give a general indication of DO range, to colorimeters that interpret the color of the final DO test and give very accurate readings in ppm. However, the chemicals involved can be dangerous, and they must be properly handled and disposed of.

Carbon Dioxide (CO_2)

- **Sources**: Respiration, minimal diffusion, hard water sources like well water.
- **Removal**: Photosynthesis, diffusion, and addition of $Ca(OH)_2$.
- **Interactions**: Competes with oxygen for solubility in water, increases in pH stabilize CO_2 levels, and increased CO_2 levels can cause decreases in pH.
- **Healthy Levels**: 20-60 ppm is a safe range for most species.
- **Periodicity**: Daily fluctuations with respiration levels and decomposition rates.
- **Measurement Options**: Chemical test kits are the most accurate means of measurement. The cost for one kit ranges from $35-$45.

pH

- **Interactions**: pH fluctuations are minimized in aquatic systems that have a good buffering capacity or alkalinity. Increased pH levels can cause the toxicity of the ionic form of ammonia (NH_4^+) to increase. Decreased pH levels can cause hydrogen sulfide (H_2S) toxicity to increase.
- **Healthy Levels**: 6.5-9.0 for most fish.

- **Periodicity**: Daily fluctuations with respiration and photosynthesis. High photosynthetic levels during the day can cause dramatic increases in pH in water with poor buffering capacity.
- **Measurement Options**: Although the cost is lower, the options are very similar to those of DO measurement.

Alkalinity (the buffering capacity of a system)

- **Sources**: Bicarbonates, carbonates, and hydroxides.
- **Interactions**: Increased alkalinity can stabilize pH fluctuations and can provide a storehouse of carbon for photosynthesis.
- **Healthy Levels**: Above 100 ppm is optimum for most species and below 25 ppm is considered a low level.
- **Measurement Option**s: Chemical test kits are the most reliable and inexpensive method.

Hardness (dissolved levels of Ca^{2+} and Mg^{2+})

- **Sources**: Calcium carbonate and magnesium carbonate.
- **Interactions**: Hardness has a direct relationship to alkalinity and pH levels concerning increases and decreases in levels.
- **Healthy Levels**: Above 100 ppm is optimum for most species.
- **Measurement Options**: Chemical test kits are the most reliable and inexpensive method.

Ammonia (NH_3)

- **Sources**: Fish waste, anaerobic respiration, decomposition of organic matter (ammonification).
- **Removal**: Biological by nitrosomonas bacteria.
- **Interactions**: Decreases in pH cause a decrease in NH_3 but increase the toxicity of NH_4^+; increased temperature causes increased respiration rates leading to higher ammonia production, and increases in an aquatic system's biomass will increase ammonia levels.
- **Healthy Levels**: 0-0.02 mg/L or ppm.
- **Periodicity**: Increased production during warm seasons.
- **Measurement Options**: Chemical test kits are the most reliable and inexpensive method.

Nitrite (NO_2^-)

- **Sources**: Decomposition of ammonia.
- **Removal**: Biological by nitrospira bacteria.
- **Interactions**: Associated with increased ammonia production, toxicity of NO_2 increases as pH levels increase, increases in Cl can lower nitrite levels.
- **Healthy Levels**: Less than 0.2 ppm.
- **Periodicity**: Increased production during warm seasons.
- **Measurement Options**: Chemical test kits are the most reliable and inexpensive method.

Nitrate (NO_3^-)

- **Sources**: Decomposition of nitrite.
- **Removal**: Water changes and plants will use nitrate directly as a nutrient.
- **Interactions**: Source of N for plants and can cause stress at extremely high levels.
- **Healthy Levels**: Most fish can tolerate levels above 100 ppm; ranges vary above this level.
- **Periodicity**: Increased production during warm seasons.
- **Measurement Options**: Chemical test kits are the most reliable and inexpensive method.

Hydrogen Sulfide (H_2S)

- **Sources**: Industry waste, anaerobic decomposition of sewage and organic matter.
- **Removal**: Increases DO levels, increases in pH will help dissociate H_2S.
- **Interactions**: Decreases in pH can lead to increased toxicity of H_2S.
- **Healthy Levels**: Less than 0.002 ppm.
- **Periodicity**: Increased production during warm seasons.
- **Measurement Options**: Chemical test kits are the most reliable and inexpensive method.

Chlorine (Cl^-)

- **Sources**: Gas used for treatment of tap water.
- **Removal**: Agitate or "age" the water for 24 hours.
- **Interactions**: Increased toxicity with increased ammonia levels.
- **Healthy Levels**: Less than 0.003 ppm for freshwater. Less than 0.01 ppm for saltwater.
- **Measurement Options**: Chemical test kits are the most reliable and inexpensive method.

Chloride (Cl_2)

- **Sources**: Salts.
- **Removal**: Water changes.
- **Interactions**: Increases in chloride can lower nitrite toxicity, increases salinity levels, decreases DO levels, increases concentration as water evaporates from system.
- **Healthy Levels**: Depends upon species adaptability.
- **Measurement Options**: Chemical test kits are the most reliable and inexpensive method.

Salinity

- **Sources**: Salts, primarily NaCl and MgCl.
- **Lowering or Removal**: Perform water changes with freshwater gradually.
- **Interactions**: Increases in chloride can lower nitrite toxicity, increases salinity levels, decreases DO levels, increases in concentration as water evaporates from system.
- **Healthy Levels**: Depends upon species adaptability.
- **Measurement Options**: Chemical test kits, a specific gravity meter or device, or a refractometer.

4.3.2 The Nitrogen Cycle

Nitrogen (N) occurs in several forms in aquatic systems, most commonly ammonia (NH_3), nitrite (NO_2-), and nitrate (NO_3-). Other important forms include amine (NH_2-), ammonium (NH_4+), and elemental diatomic nitrogen (N_2). The cycling of nitrogen between these forms is an important aspect of the health of the system. The biogeochemical nitrogen cycle has four essential parts: nitrification, denitrification, ammonification, and nitrogen fixation. Each of these processes, represented in the diagram below, is mediated by bacteria.

Nitrogen Cycle Chemistry

Ammonification

Ammonification involves the degradation of proteins from dead plant and animal tissue and the release of ammonia into the environment. The process starts with the release of proteolytic enzymes from bacteria that hydrolyze proteins of plant and animal origin into amino acids. The amino acids are subsequently deaminated, accompanied by the release of ammonia.

Guidelines for Reducing Ammonification

Immediately remove dead plants and animals from the system. Over-feeding is another common pitfall. Uneaten food can get trapped in filter media, a sump, a settling tank, or on the bottom of the system. Uneaten food begins to go through ammonification within a few hours, depending upon the temperature of the system. If uneaten food accumulates, especially in smaller systems, it will begin to cause water quality problems. Rinsing or replacing filter media and performing 20% water changes reduces the impact of ammonification.

Nitrification

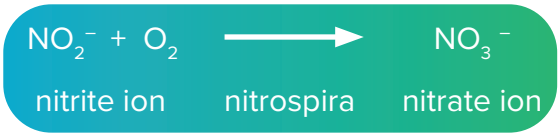
In aquaculture systems, the nitrification cycle begins when fish are introduced into a tank and begin feeding. Fish food is normally high in proteins; a family of compounds composed of very large molecules containing hundreds of thousands of atoms. These molecules are digested by the fish into amino acid molecules, which contain nitrogen atoms. The fish then metabolize the amino acids to release energy. In the metabolic process, amine (NH_2-) is removed from the amino acid molecules. The amine is then converted to ammonia (NH_3) and released into the water through the fish's gills.

Accumulation of ammonia in the water can be disastrous to fish. For example, ammonia levels between 0.2 and 0.5 mg/L (milligrams of ammonia per liter of water) are normally lethal to koi. Therefore, something must be done to remove this waste from the water.

The first step is the conversion from ammonia (NH_3) to nitrite (NO_2-). This is accomplished by a common soil bacterium, nitrosomonas.

Nitrite, while less toxic to fish, is still a problem. Nitrite disrupts the hemoglobin's ability to carry oxygen in the blood. If nitrite is allowed to accumulate in water, it can cause listlessness and oxygen starvation in fish. For instance, a nitrite concentration of 0.15 mg/L can kill small koi (under 15 cm long). If fish are listless, will not eat, and stay at the bottom of the tank, there may be a nitrite problem. The remedy is to replace 20% of the tank water with fresh water. Usually, the second stage of the nitrogen cycle takes care of excess nitrite.

Nitrospira, another common bacterium, metabolizes nitrite (NO_2-) to nitrate (NO_3-).



Fortunately, all fish can tolerate nitrate concentrations up to 500 mg/L. Water plants included in the tank, or diverting water to a separate aquaponic system, can help use up nitrate. Plants uptake nitrate as fertilizer and use it in the synthesis of proteins, completing the cycle.

If ammonia, nitrite, and nitrate levels are monitored over time as a new tank is established, the concentration of each will increase, then decrease until a balance is reached. This balance is achieved when the bacteria population grows to a point where it can metabolize all the fish waste products. This cycle offers an opportunity for data collection, analysis, and inquiry about the metabolism of fish and biological filtration in an aquaculture system.

Guidelines for Reducing Ammonia

Increases in fish biomass, either due to fish growth or fish added to a tank, may create a condition called "new tank syndrome," where fish become lethargic and less responsive. The syndrome results from the buildup of excess ammonia and nitrite. Until the bacterial population grows to meet the new needs of the tank, the system will be out of balance. If ammonia and nitrite continue to build up and fish health does not improve, the fish load may be too great for the tank size. To remedy this situation, remove the newly added fish, harvest fish, add another biological filter, or perform a 20% water change.

Denitrification

When dissolved oxygen levels fall to 0.6-0.7 mg/L, nitrification is inhibited and anaerobic conditions may develop. Pseudomonas, an anaerobic bacterium, may begin to grow. Pseudomonas is a denitrifier; it converts nitrate to nitrogen gas.

This may seem good, since the product is nitrogen gas, which will eventually escape into the atmosphere. But other anaerobic processes may occur. This causes organic materials to break down into hydrogen sulfide (H_2S), which can be lethal in concentrations less than 1 ppm. The gas, which smells like rotten eggs, prevents the hemoglobin in the fish's blood from carrying oxygen.

Anaerobic Ammonium Oxidation (Anammox)

Another possible path within the nitrogen cycle is anaerobic ammonium oxidation, also called anammox. This process occurs under anaerobic conditions. Ammonium (NH_4^+) and nitrogen dioxide (NO_2) are directly converted to water and nitrogen gas by bacteria belonging to the phylum Planctomycetes.

$$NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O$$

Discovered in the early 1990s, anammox is used in wastewater treatment plants to remove ammonia from wastewater sludge. In fact, this process reduces the energy associated with treating wastewater across the globe. Researchers estimate wastewater treatment in the European Union uses 1% of the total amount of energy consumed. In the United States, the percentage is higher (4%) with roughly 75 billion kilowatt hours going to wastewater treatment. China's wastewater treatment systems use over 100 billion kWh (Gurung et al., 2018). In the US, this energy usage costs roughly $7.5 billion. Much of that cost is associated with adding oxygen and aerating wastewater. The anammox process is one of several emerging technologies that can transform wastewater treatment plants from energy consumers to energy producers. This process is already underway in Finnish wastewater treatment plants (Gurung et al., 2018).

Annamox is useful in recirculating aquaculture systems because it reduces the amount of excess sludge produced, removes nitrate, and allows the system to run with minimal water changes and no aeration. In anammox processes, small amounts of water are diverted from the system and slowly passed over sludge containing anaerobic bacteria. The water flow must be very slow, with minimal aeration. After passing through the anaerobic bacteria, the treated water returns to the main system with reduced levels of ammonia.

4.4 Classroom Activities

4.4.1 The Nitrogen Cycle Assignment

Objectives

1. Describe the role of the nitrogen cycle in aquaculture.
2. Explain the role of bacteria in the nitrogen cycle.
3. Explain the interactions of water quality parameters in the N cycle.

Questions

1. List the forms of nitrogen found in an aquatic ecosystem.
2. What is the waste product of protein metabolism in fish, and at what levels is it lethal to koi?
3. What are the effects of changes in pH on ammonia?
4. What is the effect of nitrite on fish, and what symptoms does it produce?
5. If nitrite approaches lethal levels, what should be done?
6. Describe the role of the following bacteria in terms of their effect on nitrogen compounds:

   A. Nitrosomonas

   B. Nitrospira
7. What are indicators that excess nitrate exists? How are excessive amounts of nitrate removed from an aquaculture system?
8. Discuss the need for biological filtration in recirculating aquaculture systems.
9. Describe "new tank syndrome" and why it occurs in a system.
10. What problems arise if anaerobic conditions develop in a system, and how can these conditions be prevented?

4.4.2 Research Topics

1. Monitor a local stream or pond to observe changes in water quality during different seasons.
2. Aerate cold and warm water to explore oxygen solubility at different temperatures.
3. Titrate samples of water to test the alkalinity or buffering capacity of the water against changes in pH.
4. Use Eyes on the Bay data to graph changes in Chesapeake Bay water quality.
5. Compare data collected with different monitoring devices, such as chemical test kits versus probes, to compare for accuracy.
6. Build a colorimeter to measure turbidity of water samples.

4.4.3 Understanding Alkalinity

Search the Southern Regional Aquaculture Center's research publications database for article number 464, *Interaction of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds*, by Wurts and Duborrow, or find the article in References and Resources.

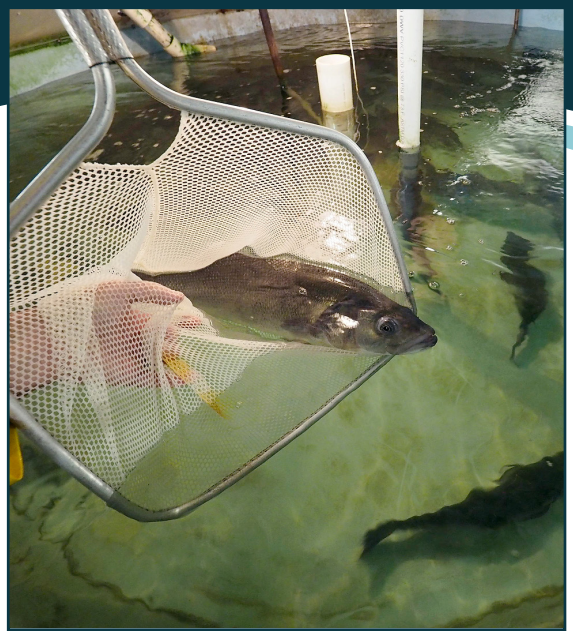
Answer the following questions about the article and alkalinity:

1. Levels of pH, carbon dioxide, alkalinity, and hardness can have a profound effect on what aspects of an aquatic system?
2. Define pH as described in the article.
3. What is the recommended range for pH in a pond?
4. What is the pH of fish blood?
5. Using an equation, explain why pH in a pond is lowered after dark. Name the acid produced.
6. When are fish susceptible to carbon dioxide poisoning?
7. What is a normal carbon dioxide reading?
8. How can high carbon dioxide levels be controlled chemically? Why can this practice be dangerous?
9. Copy Table 1 from the article.
10. Define alkalinity.
11. What are common bases found in ponds and water systems?
12. How is alkalinity measured?
13. What minimum alkalinity is necessary for a healthy aquatic system, and what is the normal range?
14. Using equations and pH, explain what causes the formation of calcium and magnesium salts (described as "water hardness").
15. Define buffering capacity.
16. Why is water with good buffering capacity considered hard?
17. Why is pond water with good alkalinity usually slightly basic? What is the range?
18. Use Table 2 from the article to answer the following question. Calculate the mg/L of carbon dioxide at a pH of 7.4 at 30°C. The total alkalinity was 96 mg/L.
19. Draw Figure 2 from the article.
20. What is the normal range of a well-buffered system?
21. How can alkalinity benefit microscopic plants (phytoplankton)?
22. Name two benefits of phytoplankton's use of carbon dioxide. Copy the equations from the article.
23. Explain how the release of carbonate converted from bicarbonate by plant life causes pH to rise dramatically.
24. Define hardness, how it is measured, and the units used.

25. Why are alkalinity and hardness confused when discussing water quality?
26. How is it possible to have low hardness and high alkalinity?
27. List reasons calcium and magnesium are essential in the biological process.
28. Why is calcium most essential for fish survival?
29. What salts found in fish blood are essential for normal heart, nerve, and muscle function?
30. What is the recommended range for free calcium?
31. Explain why a high hardness value might not indicate high calcium concentrations.
32. Explain how the factors 2.5 and 4.12 are related to hardness of calcium and magnesium.
33. Calculate free calcium if the calcium carbonate value is 150 mg/L and the hardness is caused by calcium.
34. Calculate free magnesium if the calcium carbonate value is 150 mg/L and the hardness is caused by magnesium.
35. What common compound can be used to increase calcium and calcium carbonate concentrations?
36. What compounds can be used to raise calcium concentrations in soft water?
37. What happens to ammonia levels as pH increases?
38. How can high alkalinity cause ammonia problems?
39. How can high acidity cause metal toxicity of copper and zinc?
40. What effect does hardness have on metal toxicity?
41. Describe the water quality parameters of an ideal pond.

UNIT 5:

Handling Fish & Data



5.1 Background

Many species of fish continue to grow throughout their lives. Rapid growth often indicates an abundant food source and other favorable environmental conditions, whereas slow growth generally indicates the opposite (Moyle and Cech, 1982). Apart from food availability, water temperature is probably the single most important factor affecting fish growth (Piper et al., 1982).

Fish growth rates can be determined by measuring changes in size (typically length or weight) over time. For example, a fish may be measured at the beginning of an experiment, marked, and released. The fish can be recaptured later and remeasured. The growth rate is the change in size over that time.

The purposes of this experiment include monitoring the growth of individual fish (or an average growth rate if fish tags are not available); identifying differences in growth rates among fish fed different diets (if separate tanks on the same filtration system are available); examining the relationship between fish length and weight; and determining food conversion ratios.

5.2 Objectives

1. Construct a fish measuring board using a ruler or meter stick and a piece of wood or Styrofoam.
2. List three ways of measuring fish length.
3. Set up and conduct an experiment to examine fish growth rate.
4. Determine the proper concentration of fish anesthesia to use to relax fish prior to handling. (See Section 5.4.1: Fish Anesthesia and MS-222 for details)
5. Determine the appropriate amount of food to feed fish based on a variety of factors.

6. Determine the food conversion ratios and condition factors of the fish and explain the significance of this measurement.
7. Construct graphs (using graph paper or computers) showing increase in fish length and weight over time.
8. Construct a length-weight curve for fish used in the experiments and discuss why such a curve might be useful to scientists.
9. Synthesize information obtained during this experiment into a written report detailing the results and answer analysis questions.

5.3 Classroom Resources

5.3.1 Fish Anesthesia and MS-222

Fish biologists often need to handle fish to conduct routine scientific research. For many fish, the stress associated with being handled can result in injury or death. Anesthetics are commonly used to reduce fish stress. Anesthetics work by reducing a fish's metabolic activity, which relaxes the fish and makes it easier to handle while measuring or weighing.

Several types of fish anesthetics are available. One of the most used is methane tricainesuffonate (MS-222). In this lab, we will test the effects of different concentrations of MS-222 (brand name Finquel) to anesthetize fish. The appropriate concentration of anesthetic must often be determined through trial-and-error for each fish species, because factors such as water temperature and water chemistry can impact its effectiveness. MS-222 is also known to lower the pH of water. Some fish species have specific pH requirements for survival. For example, koi (a type of goldfish) prefer water with a pH between 7 and 8. It is important to consider this when exposing them to water containing MS-222. Before any anesthetic is used, we recommend testing its impact on several different fish.

Research suggests concentrations of 50-60 mg/L of MS-222 are effective for anesthetizing striped bass (*Morone saxatilis*). The purpose of this experiment is to examine the effects of different concentrations of MS-222 on goldfish and determine the correct concentration needed to anesthetize this species.

High School Strategies for the Fish Anesthesia with MS-222 Lab

Cooperative Learning: Place students in mixed ability groups so they can support each other. Have them complete the guided worksheets and laboratory activities together.

Lab Team: During the Fish Anesthesia Lab, each student on a team (four students per team) has a specific job and responsibility.

a. Make anesthesia solution and oversee clean up

b. Capture fish and measure mass and length of fish

c. Run timer and return lab materials

d. Record data

Use of Guided Worksheet: Students complete a worksheet as a group, one step at a time. Students work in pairs when making calculations. "Think, Pair, Share" can be used to help the students with problem solving.

5.4 Classroom Activities

5.4.1 Fish Anesthesia and MS-222 Lab

Objectives

1. Correctly answer questions pertaining to fish anesthesia with MS-222 (Finquel) instructions.
2. Demonstrate the ability to convert measurements typically used in aquaculture (i.e., milligrams to grams, gallons to liters, etc.) to prepare specific concentrations of fish anesthesia.
3. Utilize scientific equipment to set up and conduct a lab experiment.
4. Create a graph (on graph paper or graphical analysis) that presents the results of the experiment.
5. Compare and contrast the results of different concentrations of anesthesia on fish.
6. Extrapolate the results of the experiment and make predictions on what would happen with fish exposed to higher concentrations of anesthesia.
7. Compile and synthesize information into a report detailing the results of the experiment and answering analysis questions.

Materials

Procedures

- Four containers that hold approximately 10 liters of water
- One liter beaker to measure water
- MS-222 (Finquel) fish anesthesia
- Finquel instruction sheet
- Scoopula for measuring chemicals
- Electronic balance or other scale
- Ruler or measuring board with millimeter increments
- Calculator
- Fish nets
- Four aquarium pumps, airline tubing, and four air stones
- pH test kit
- Thermometer
- 12 goldfish of various sizes

Preliminary Research

1. Read MS-222 (Finquel) label and answer the following questions:
    a. What three factors might slow the action of MS-222 on fish?
    b. Given this information, why do you think it is important to perform preliminary tests on small numbers of fish first?
    c. If you wanted to idly anesthetize a brown trout, what is the recommended concentration of Finquel?
    d. If you wanted to anesthetize a channel catfish at a moderately rapid rate, what is the recommended concentration of Finquel?
    e. What are some of the initial physiological symptoms that would indicate a fish is affected by anesthesia?
    f. What safety precautions do people need to take when making solutions of Finquel?
2. Determine how to prepare specific concentrations of Finquel:
    a. If we were interested in making a 50 mg/L concentration of Finquel in a seven liter container of water, how many grams of Finquel would we need to add to the seven liters of water? Show work.
    b. If we were interested in making a 65 mg/L concentration of Finquel in a five gallon bucket of water, how many grams of Finquel would we need to add to the five gallons of water?

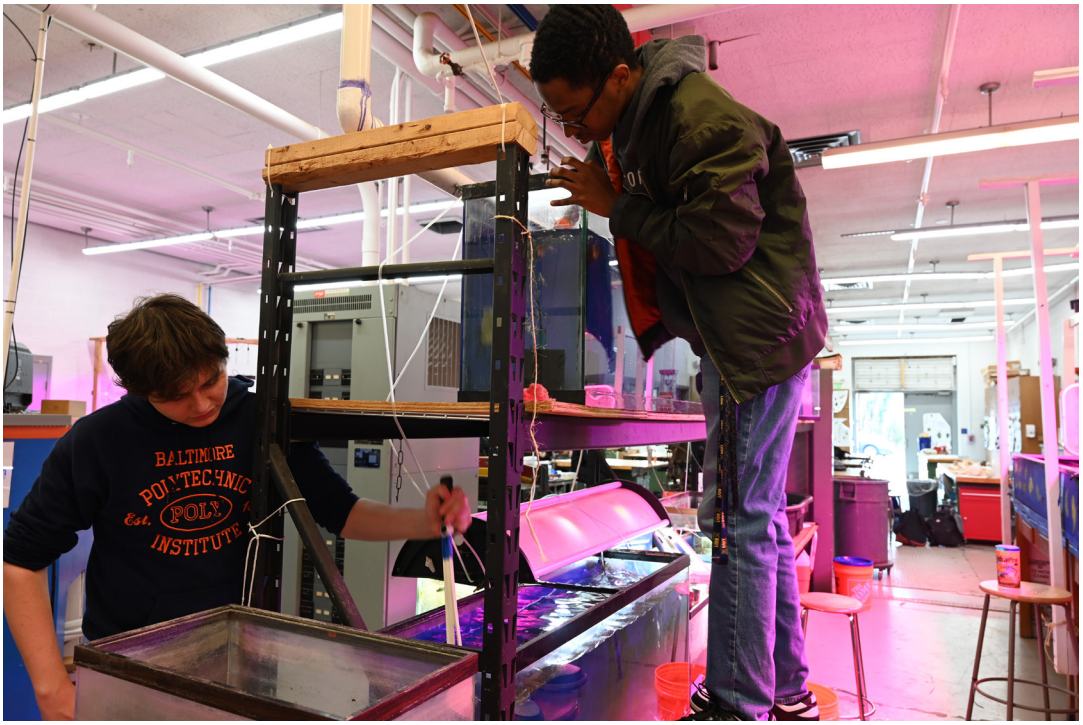
Lab Experiment Procedures

1. Prepare four containers of water with MS-222 (Finquel) concentrations of 20, 40, 60, and 80 mg/L.
2. Mix water and Finquel thoroughly.
3. Turn on air pumps and insert one air stone into each container of water.
4. Measure water temperature and pH in each container.
5. Insert one goldfish into the 20 mg/L solution of Finquel. Start the stopwatch. Record the length of time it takes for the fish to lose equilibrium and flip upside down. Remove the fish from the anesthetic solution.

6. Measure the fish length and weight and return it to a freshwater container.
7. Monitor the time it takes the fish to: a) recover equilibrium and b) return to normal swimming and schooling activities.
8. Repeat steps 4-7 until three goldfish have been tested in each of the Finquel concentrations. Try to use fish of different sizes within each concentration. For example, in the 20 mg/L, try to use a large fish and small fish.

Student Questions

1. Make a prediction: if you exposed fish to a 100 mg/L solution of Finquel, how much time would you predict it would take them to lose equilibrium and swim upside down? How about a 10 mg/L solution? Consider having students make a graph of their prediction.
2. Explain why we measured and recorded water temperature and pH.
3. Synthesize the information obtained in this lab experiment into a short, 1-2 page scientific report. Include the following information:
    a. The data table and a graph of your results. Discuss whether your graph suggests any trends between the anesthesia concentration and time.
    b. Predict and explain what might happen to the time it took for a fish to lose equilibrium if the fish had been in containers that were 10°C cooler than the water temperature you used.
    c. What are the pH recommendations for goldfish or for koi (a fish similar to common goldfish)? How did this compare to the pH in each of the anesthetic solutions? Given this information, would pH of the water be a concern when using a particular solution?
    d. How did the size of the goldfish relate to the time it took the fish to lose equilibrium?
    e. What precautions did you take to ensure the goldfish did not die in the MS-222 (Finquel) solution?
    f. Explain how the concentration of Finquel affected recovery time of the fish once it was returned to a freshwater tank.
    g. In your opinion, what would be the best concentration of Finquel to use to anesthetize a goldfish? Explain your answer.



Preliminary research

1. Read MS-222 (Finquel) label and answer the following questions:

   a. What three factors might slow the action of MS-222 on fish?

   Answer: *Cooler water temperature, soft water (< 10mg/L $CaCO_3$), larger fish*

   b. Given this information, why do you think it is important to perform preliminary tests on small numbers of fish first?

   Answer: *To determine the desired concentration and rates of anesthesia and optimal length of exposure.*

   c. If you wanted to idly anesthetize a brown trout, what is the recommended concentration of Finquel?

   Answer: *80-135 mg/L*

   d. If you wanted to anesthetize a channel catfish at a moderately rapid rate, what is the recommended concentration of Finquel?

   Answer: *70 mg/L*

   e. What are some of the initial physiological symptoms that would indicate a fish is affected by anesthesia?

   Answer: *1) Sedation: decreased reactivity to visual and vibrational stimuli, opercular (gill) activity is reduced 2) Total loss of equilibrium: the fish turns over and locomotion ceases.*

   f. What safety precautions do people need to take when making solutions of Finquel?

   Answer: *Avoid inhaling or getting in eyes, use goggles and respirator when measuring*

2. Determine how to prepare specific concentrations of Finquel.

   a. If we were interested in making a 50 mg/L concentration of Finquel in a seven liter container of water, how many grams of Finquel would we need to add to the seven liters of water? Show work.

   Answer: *50 mg/L x 7 L water = 350 mg of Finquel= 0.350 g*

   b. If we were interested in making a 65 mg/L concentration of Finquel in a five gallon bucket of water, how many grams of Finquel would we need to add to the five gallons of water?

   Answer: *1 gal = 3.785 L, if we want 5 gallons, that is 18.925 L*

   65 mg/L x 18.925 L = 1,230.13 mg Finquel = 1.23 g

Processing the Data

1. Make a prediction: If you exposed fish to a 100 mg/l solution of Finquel, how much time would you predict it would take them to lose equilibrium and swim upside down? How about a 10 mg/L solution?

   Answer: *Responses will vary depending on the results of the data. Students should be able to extrapolate the data and make predictions.*

2. Explain why we measured and recorded water temperature and pH.

   Answer: *Water temperature can have an impact on the length of time it takes for a fish to show symptoms of anesthesia. Many fish have specific pH requirements for survival. Since Finquel has been found to lower the pH of water, you want to be sure not to endanger the fish by placing them in water with a pH outside their tolerance limits.*

3. Synthesize the information obtained in this lab experiment into a short, 1-2 page scientific report. Include the following information:

   a. The data table and a graph of your results. Discuss whether your graph suggests any trends between the anesthesia concentration and time.

      Answer: *There should be a direct relationship.*

   b. Predict and explain what might happen to the time it took for a fish to lose equilibrium if the fish had been in containers that were 10° C cooler than the water temperature you used.

      Answer: *It should take longer for a fish to be anesthetized in water that is 10°C cooler because of declines in their metabolism.*

   c. What are the pH recommendations for goldfish or for koi (a fish similar to common goldfish). How did this compare to the pH in each of the anesthetic solutions. Given this information, would pH of the water be a concern when using a particular solution?

      Answer: *Koi prefer water with a pH range of 7-8. Results will depend on the pH of the sample waters.*

   d. How did the size of the goldfish relate to the time it took the fish to lose equilibrium?

      Answer: *It should show that bigger fish take longer to become anesthetized. However, results may vary depending on the size differences of the sample fish.*

   e. What precautions did you take to ensure that the goldfish did not die in the MS-222 (Finquel) solution?

      Answer: *Closely monitored the fish so it could be removed from the anesthesia solution immediately after it lost equilibrium. Also, monitored the pH.*

   f. Explain how the concentration of Finquel affected recovery time of the fish once it was returned to a freshwater tank.

      Answer: *May vary depending on the results.*

   g. In your opinion. what would be the best concentration of Finquel to use to anesthetize a goldfish. Explain your answer.

      Answer: *Will vary depending on the results.*

5.4.2 Calculating the Weight of Fish

Weight of Fish: Initial Weight Calculations

Weighing your fish is optional at this point but must be completed if you plan to calculate current ammonia production and required filter surface area. You will not weigh every fish in your tank, unless you have fewer than 20 fish. For more than 20 fish, you will need to weigh 20% of your total. For instance, if you have 50 fish, 0.20 x 50 = 10, so you would weigh 10 fish. Fish need to be handled as minimally as possible and with wet hands. Using an anesthetic to sedate fish will help with some species (see Unit 5.3.1). After weighing your fish, place them in a second aerated container until all your fish have been weighed. Then, return all fish to their tank.

Species: _____ Date of weighing: _____

Number of fish in tank: _____ Number of fish to be weighed: _____

FISH	WEIGHT
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Total	
Average	

Food/day = (average weight) (total number of fish) (feeding percentage)

Example: 3.4 oz/fish (average weight) x 50 fish x .03/day (three percent) = 5.1 oz food/day

Food/day: _____

Questions

1. Think of a way you could estimate (or calculate) the amount of feed related to the fish growth rate.

2. Name three things you could do if your filter media amount was insufficient.

Weight of Fish: Next Weight Calculations

Species: _____

Date of weighing: _____ Date of last weighing: _____

Time (T) (number of days since last weighing): _____ days

Number of fish in tank (n): _____ Number of fish to be weighed (n_{weigh}): _____

FISH	WEIGHT
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Total	
Average (W_c)	

Average weight gain in fish (W_{gain}) = current average (W_c) – previous average (W_p)

W_{gain}: _____ oz/fish

Percent weight gain (% gain) = (W_{gain} / W_p) x 100

% gain: _____

Total ounces (oz) of food fed in T (F): _____ g

Estimated weight gain of all fish ($W_{totalgain}$) = (% gain)(W_c)(n)

Food conversion = F / $W_{totalgain}$ = oz food:1 oz fish

Food conversion: _____ oz food : 1 oz fish

Now, we need to calculate the new feeding amount for the fish based on the mass and growth rate.

New feed amount (F_{new}) = (W_c)(n)(feeding percent)

**Note: Ounces are used in these formulas. If you are using grams as your unit of weight, the formulas can be used the same way.*

Use the following table to keep track of your daily measurements and feeding regiments.

Sample Daily Feeding Record Chart

DATE	% BODY WEIGHT FED	AMOUNT FED (oz or g)	FEEDING RESPONSE

5.4.3 Comparing Feed Quality

Science research always involves gathering and comparing data. In aquaculture research, which involves living organisms, many factors can influence the outcome of an experiment. As a result, it can be difficult to determine whether the results and conclusions are meaningful or simply due to chance variations caused by unknown or uncontrolled factors.

In professional research, complex statistical methods are often used to account for these variables, but these methods can be confusing for beginner scientists. The following activity introduces a relatively easy way to evaluate whether the results of an experiment are significant (reflecting changes caused by experimental factors), rather than random variation resulting from uncontrolled conditions, inaccurate measurements, or other experimental errors.

Feed Quality Comparison Activity

Consider the following aquaculture experiment. To determine the optimal percentage of protein in fish feed for maximum growth, three identical tanks are stocked with populations of genetically identical fish. The fish are fed diets containing different amounts of protein. Tank 1 receives food with 30% protein, tank 2 receives food with 40% protein, and tank 3 receives food with 50% protein.

Each tank is fed the same amount of food based on the ratio of grams of food per gram of fish biomass. Every effort is made to keep the physical conditions of all three tanks identical. The fish are weighed periodically throughout the experiment so feeding can be adjusted to maintain the same food-to-biomass ratio in each tank. At the end of the experiment, the percentage weight gain of each fish is calculated, and the results are recorded. The following chart shows an example of possible data from this experiment.

Tank 1 (30% protein)		Tank 2 (40% protein)		Tank 3 (50% protein)	
Fish	% Weight Gain	Fish	% Weight Gain	Fish	% Weight Gain
1	12	1	3	1	25
2	6	2	22	2	21
3	8	3	10	3	29
4	10	4	31	4	31
5	4	5	7	5	29
6	9	6	27	6	39
7	8	7	24	7	19
8	7	8	19	8	25
9	11	9	14	9	55
		10	17	10	10
		11	37	11	45
		12	60	12	42

What are some possible reasons tank 1 has fewer fish? Could this affect the results of the experiment?

To determine which food was best for fish growth and whether the results are truly significant, rather than the result of random error or uncontrolled variation, the data must be displayed in a way that makes patterns easier to recognize. To do this, first rearrange the data in each column in descending order.

Tank 1 (30 % protein)	Tank 2 (40 % protein)	Tank 3 (50 % protein)
% Weight Gain	% Weight Gain	% Weight Gain
12	60	55
11	37	45
10	31	42
9	27	39
8	24	31
8	22	29
7	19	29
6	17	25
4	14	25
	10	21
	7	19
	3	10

The data may now seem to show the differences between the three groups more clearly, but as is often the case with data from living organisms, there can still be a lot of variation within each tank. To simplify the data, we can select five values from each tank to represent the overall trends. This method can be important for looking at trends, even when dealing with large data sets. The five values are the high, upper quartile, median, lower quartile, and low. No matter how many data points exist for each tank, these five values can be determined.

The high and low values are easy to identify. They are the numbers at the top and bottom of each column, once the data points have been arranged in descending order.

Tank 1 (30 % protein)		Tank 2 (40 % protein)		Tank 3 (50 % protein)	
% Weight Gain		% Weight Gain		% Weight Gain	
High	**12**	**High**	**60**	**High**	**55**
	11		37		45
	10		31		42
	9		27		39
	8		24		31
	8		22		29
	7		19		29
	6		17		25
Low	**4**		14		25
			10		21
			7		19
		Low	**3**	**Low**	**10**

The median is the value exactly in the middle of the ordered list, with the same number of data points above and below it.

- When there are an odd number of data points, the median is the value with an equal number of values above and below it. For example, for tank 1 (fish fed 30% protein), there are nine data points. The fifth value down from the top (or up from the bottom) is the median. How many data points are above it? How many are below it? (Answer: 4)
- When there are an even number of data points, the median is calculated as the value halfway between the two values closest to the middle. In the second tank (fish fed 40% protein), there are 12 data points. The 6th value is 22% and the 7th is 19%. The median is halfway between these two values: (22 + 19) ÷ 2 = 20.5%, which rounds to 21% because all the data is shown to the nearest percent.
- In the third tank (fish fed 50% protein), the 6th and 7th values are both 29%, so the median is also 29%.

Now, we have the high, low, and median values for each tank.

Tank 1 (30 % protein)		Tank 2 (40 % protein)		Tank 3 (50 % protein)	
% Weight Gain		% Weight Gain		% Weight Gain	
High	**12**	**High**	**60**	**High**	**55**
	11		37		45
	10		31		42
	9		27		39
Median	**8**		24		31
	8		22		29
	7	**Median**	**21**	**Median**	**29**
	6		19		29
Low	**4**		17		25
			14		25
			10		21
			7		19
		Low	**3**	**Low**	**10**

Medians are one type of average. If you compare the medians of each tank, you might conclude that higher protein in food leads to better growth. But would you want to publish this result? Would you want to stake your professional reputation on it? Think about the variation in the data: the highest percent weight gain of the fish occurred in the 40% protein diet, not the 50% protein diet. To get a better idea of the trends, we need to look more closely at the data. Next, we must determine the quartiles.

Quartiles are similar to medians. While the median is the midpoint of the whole data set, the quartiles are the midpoints of each half of the data. To calculate the quartiles, use the same method as for the median: count the number of data points above the median and find the middle. Then, count the number of data points below the median and find the middle. Remember, if the number of data points is odd, the quartile will be the middle data point; if it is even, the quartile will be halfway between the two middle values. Next, determine the upper and lower quartiles for each tank. Answers are shown below.

Tank 1 (30 % protein)		Tank 2 (40 % protein)		Tank 3 (50 % protein)	
% Weight Gain		% Weight Gain		% Weight Gain	
High	**12**	**High**	**60**	**High**	**55**
	11		37		45
Upper quartile	**11**		31		42
	10	**Upper quartile**	**29**	**Upper quartile**	**41**
	9		27		39
Median	**8**		24		31
	8		22		29
	7	**Median**	**21**	**Median**	**29**
Lower quartile	**7**		19		29
	6		17		25
Low	**4**		14		25
		Lower quartile	**12**	**Lower quartile**	**23**
			10		21
			7		19
		Low	**3**	**Low**	**10**

Now, we can use these five representative values and set aside the original data. These numbers show not only the average but also the variation in the data.

	Tank 1 (30 % protein)	Tank 2 (40 % protein)	Tank 3 (50 % protein)
	% Weight Gain	% Weight Gain	% Weight Gain
High	12	60	55
Upper quartile	11	29	41
Median	8	21	29
Lower quartile	7	12	23
Low	4	3	10

5.4.4 Alternative Feeds in the Aquaculture Industry

In the previous section, we compared feed quality based on its percentage of protein. This type of analysis is regularly conducted in the aquaculture industry, but researchers look at more than just protein. Aquaculture producers must also consider cost, the quality and availability of ingredients, and the species of fish being raised. Many feeds contain fishmeal and fish oils as main ingredients, but evidence suggests this may not be a sustainable practice in the future.

Much of the fishmeal used today comes from pelagic fish—or fish found in the open ocean—like anchovies and sardines. A smaller portion comes from the waste products of fish prepared for people to eat. These fish also contain large amounts of essential fatty acids, another important component of fish feed. There is a misconception that these fish produce fatty acids. Marine phytoplankton and algae actually produce the fatty acids. Those fatty acids accumulate in fish as they eat algae and phytoplankton. This process is called bioaccumulation. Explore more about this topic in Maryland Sea Grant's lesson plan: Fish Oil, Really?

As the aquaculture industry places more attention on high-quality and sustainable feeds, developers are looking for alternatives to traditional fish-based products. Promising options include plant-based materials, yeasts, insects, food waste, and algal products. According to the National Oceanic and Atmospheric Administration, ingredients like soy, barley, rice, canola, corn and wheat gluten, and peas are already commonly found in fish feeds.

Many studies have shown up to 50% of the fishmeal in feed can be successfully replaced with plant-based products with no noticeable effects on fish growth or health. However, feeds with higher percentages of plant material may pose risks to fish due to anti-nutritional factors like phytates, saponins, and lectins, as well as high levels of indigestible proteins. Additional processing of the plant material through fermentation or heat treatments can help.

Insect meal is another alternative that provides a source of protein without the anti-nutritional factors and high fiber content of plant-based ingredients. Crickets, mealworms, and black soldier fly larvae are already being added to fish feeds. Some producers use insect meal as a supplement due to fishmeal's high cost, while others are exploring feeds with no fishmeal. Algal products are another option. Some studies have shown that algal feeds produce larger, healthier fish compared to fishmeal feeds. This makes sense, because most marine and freshwater food webs start with photosynthetic algae. Currently, algal feeds are used sparingly because they are costly to produce.

These new ingredients and innovations will play a major role in shaping how the aquaculture industry produces high-quality, cost effective, and sustainable feeds in the future.

UNIT 6:

Aquaponics in the Classroom

6.1 Background

How do aquaponics and hydroponics differ? Hydroponics is a soil-less culture in which plants are grown using nutrient solutions to achieve rapid growth. Aquaponics is also a form of soil-less culture, but it uses the natural waste from fish in an aquaculture system to feed the plants and promote fast growth. Aquaponics is not as common as hydroponics, because only certain plants can tolerate fish waste and having their roots continuously submerged in water. However, aquaponic systems can also be designed to periodically feed plants instead of keeping their roots submerged in recirculating water.

6.2 Objectives

1. Investigate the relationships between nutrients, waste, and plant growth.
2. Investigate modern designs of hydroponics and aquaponics systems.
3. Design and construct an aquaponics growth tube based upon a standard model.
4. Grow a variety of plants using an aquaponics growth tube and measure the growth rates of the plants.
5. Design an experiment to demonstrate the effectiveness of an aquaponics growth tube.

6.3 Classroom Resources

6.3.1 Aquaponics in the High School Classroom

Written by Robert L. Foor-Hogue, former science research teacher at South Carroll High School

For the biology teacher, teaching ecology draws together content accumulated during the year and becomes the comprehensive "glue." Something special happens when a student discovers how everything fits together. But ecology is the most difficult topic to teach with just words and pictures. Something else is usually needed to maximize the impact of ecological principles and make them come alive. Integrating aquaponics (hydroponics and aquaculture) into the classroom can provide this dynamic and effective focus.

A 100-gallon aquarium can serve as a "fish farm." Teams of students can monitor the change in biomass of the fish population. They can maintain daily records of the amount of high-protein floating fish food consumed, as well as noting feeding habits and behavior. As "aquaculturists" they try to develop a feeding regime that maximizes fish growth.

Water from the fish tank contains metabolic wastes that can be pumped every 30 minutes through a series of aquaponic tubes filled with lava rock and holding seedlings in peat pots. This soil-less garden can support about 40 plants. Growth is rapid; a crop of leafy vegetables, such as spinach or lettuce, is ready for harvest in about four weeks.

The apparatus is both biologically and pedagogically fertile. Perhaps the most basic impact it has on students is the idea of growing food in the classroom. There is ample time to allow each class to grow its own crop. Successfully grown plants include parsley, spinach, chives, cress, lettuce, nasturtiums, zinnias, and tomatoes.

There are, of course, suspicions that fish waste is lurking somewhere inside the lettuce leaves. But these fears gradually subside as a few brave students sample the produce. Eventually, with discussion of the biogeochemical cycles, the process becomes understandable.

These systems also stimulate inquiry, because they generate real problems. Try to use any issues that arise as a source of inquiry and problem-solving in the class. What can we do to get the lettuce to form tighter heads? How can we ensure effective pollination for the cucumbers? Is the humidity high enough? How many foot-candles of light are we giving the tomatoes? Will that be enough to induce flowering? How could we make a rough measurement of the rate of transpiration of the plants?

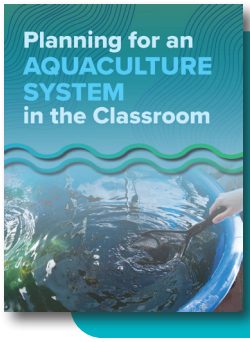
If insects become a problem, ask students to identify the insects and suggest ways to deal with them that do not require insecticides. Several of my students called a hydroponic farm in Connecticut for advice on insect control. A minor skirmish with aphids was successfully countered with a patrol of ladybugs.

Nutrient deficiencies are always a possibility. This again provides a valuable learning experience. Divide a class into groups and have each carefully examine the crop, noting any signs of nutrient deficiencies or other problems. Have each group produce its own diagnosis, along with a prescribed course of action. Next, have each group make its case to the class, and allow the class to vote on which recommendations to follow.

Iron and calcium, for example, tend to be in short supply in this kind of system. My students correctly identified a calcium deficiency and added calcium nitrate to the aquarium, producing observable improvements in a few days. Students enjoy the dynamic this type of problem-solving brings to the class.

Time at the end of the year can be used for individual projects, such as investigations into the vitamin C content of hydroponically grown lettuce or the effect of radiation on the growth of dwarf marigolds.

Finally, the system visually demonstrates many important ecological concepts. An ecology unit exam could include a diagram of the system and ask students to trace the components of the major biogeochemical cycles through the system and speculate on the effects certain changes would have on the ecosystem.



For building or constructing a small-scale aquaponics system, see the aquaculture startup guide:

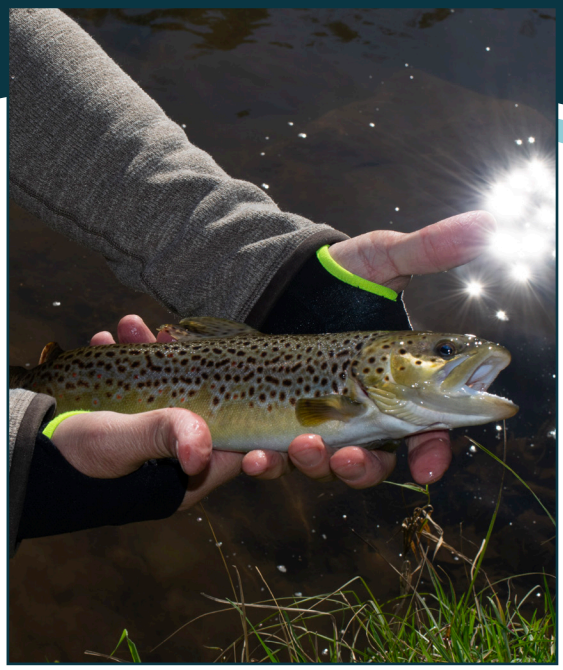
Planning for an Aquaculture System in the Classroom

6.4 Research Topics

- Research and explain aquaponics—the conversion of fish waste (ammonia) to a form of nitrogen that will provide essential plant nutrition.
- Design your own aquaponics system.
- Experiment with lift tubes made from pipes of different diameters and evaluate their effectiveness.
- Attempt to raise different types of plants from seeds, cuttings, or rooted seedlings.
- Collect, graph, and analyze data from your plant growth studies.
- Explain how aquaponics can replace or supplement an aquatic biological filter.

UNIT 7:

Environmental Assessment



7.1 Background

One objective of your aquaculture project is to release local fish species back into their natural environment. To achieve this goal, you must account for two important details. First, in partnership with the Maryland Department of Natural Resources (DNR), you must find a body of water suitable for the fish. Second, you must confirm the fish are naturally found in that aquatic system. Introducing fish that are not native to a stream can cause ecological harm. Permitting for fish reintroduction is managed by Maryland Sea Grant. However, schools may need to contact DNR independently to coordinate details like release location and timing.

A variety of methods can be used to evaluate an aquatic system. There are several indices for rating aquatic insect diversity, as well as for looking at the chemical and physical parameters of water. Other stream characteristics, such as bottom composition, channel formation, and banks, also help determine the health of the aquatic system.

Two stream evaluation activities are included on the following pages with varying degrees of difficulty and complexity. Teachers and students should decide which will serve their needs and abilities best.

7.2 Objectives

1. Make observations and learn how to sample physical, chemical, and biological parameters of an aquatic ecosystem.
2. Use aquatic insect indices, chemical tests, and physical examination to rate the overall health of a stream.
3. Determine if a stream is suitable for the fish you are raising and suggest ways to correct any existing problems.

7.3 Classroom Resources

7.3.1 Assessment of an Aquatic Ecosystem

Review the US Environmental Protection Agency's (EPA) bioassessment protocol for use in streams and wadeable rivers, which can be found online or in References and Resources.

Materials for Aquatic Ecosystem Assessment

1. Water quality probes or meters and computer interfacing equipment, if using
2. Test kits: Dissolved oxygen, pH, alkalinity, phosphate, nitrate, nitrite, ammonia, calcium, carbon dioxide, chlorine (Note: Many of these are included in the *LaMotte Water Quality Test Kit*.)
3. Kick seines or other collecting nets
4. Collecting pans
5. Forceps
6. Dissecting scopes (optional but very helpful)
7. Old shoes or wading boots

7.4 Classroom Activities

7.4.1 Aquatic Invertebrate Studies

Objectives

1. Use a key to identify various aquatic insects.
2. Count a sample of aquatic insects. Apply a variety of biotic indices to the sample and to a group of hypothetical data to compare relative stream "health" or diversity.
3. Compare the index numbers to identify what each index is measuring and if the index has a bias.
4. Discuss choosing the best statistical formulas and measures for conducting research and decide which index was "best" for this study.
5. Optional: Use your most reliable index to rate the health of the stream you are considering releasing your fish into.

Procedures

1. Using sampling methods, collect aquatic insects and place them in a shallow pan of water. After collecting the insects, count the entire sample. This can be done outside or indoors. If no stream is accessible to students, the teacher may supply the sample.
2. Use the identification key in the Isaak Walton League of America "Save Our Streams" program activity to identify the insects in your sample.
3. A nice feature of these indices is that it isn't necessary to know specific insect types. Identify each insect as A, B, C, D, etc. We suggest following the order of index calculations below so the count only needs to be done once.

4. Calculate each diversity index for your class data and for the hypothetical data, following the directions and formulas given below.
5. View Maryland Sea Grant's "Biofilms and Biodiversity" lessons online to learn more about biotic indices and how to calculate biodiversity.

Methods for Analyzing Biodiversity

Sequential Comparison Index (SCI): For class data, each time you pick an insect out of the pan, compare it with the previous insect. If it is different, it is a new "run." If it is the same as the previous insect, it is part of the same "run." For example:

Species:	A B	A	B	CC	AA	C	B	D	A
Runs:	1 2	3	4	5	6	7	8	9	10

This sample would calculate to 10 "runs" and 12 species.

Organism SCI = (number of runs) / (number of organisms) = 10 /12 = 0.833

Diversity Index (DI): Multiply the SCI by the total number of species.

In the above example, there are four species (A, B, C, and D).

DI = 4 species x (0.833) = 3.33

Simpson Diversity Index, SDI

Where P_i is the proportion of each species in the population. For example, the P value for species A would be the number of occurrences of A over the total population count for all species.

$P_A = 5 / (5+3+3+1)$

To calculate $\sum P_i 2$, calculate each Pi^2 for each species and add them all together.

Species	Number of Occurrences	P_i	P_i^2
A	5	0.416	0.173
B	3	0.25	0.0625
C	3	0.25	0.0625
D	1	0.08	0.0066
		ΣP_i^2	**0.3046**

SDI = 1 / (0.3046) = 3.28

Once you have established a baseline for your specific ecosystem, the SDI values will start to take on more meaning. SDI is influenced by the richness, or number of species present, in a sample population and the equitability of the species in the population. If one species dominates what would otherwise be a rich population, it can lower the diversity index value.

7.4.2 Bank Erosion Assessment Data Sheet

Team:_____ **Site**:_____ **Date**:_____

Map:

Photo:

Main Cause:

bend at steep slope	insized stream reach	unknown
pipe outfall	headcut	other: _____
below culvert	livestock in stream	

Length:_____ ft **Max Bank Height Above Water**: _____ ft

Average Bank Height Above Water: _____ ft

Erosion Potential:

Bank Height	Low	Moderate	High
Bank Angle	Low	Moderate	High
Root Density	Low	Moderate	High
Soil Stratification	Low	Moderate	High
Particle Size	Low	Moderate	High

Present land use right side: (looking down stream)

crop field	cow pasture	paved	multiflora rose
feed lot	horse pasture	shrubs & small trees	park
hay pasture	lawn	forest	other: _____

Present land use left side: (looking down stream)

crop field	cow pasture	paved	multiflora rose
feed lot	horse pasture	shrubs & small trees	park
hay pasture	lawn	forest	other: _____

7.4.3 Ideas for Field Research Projects

1. Locate an environmentally sensitive area that impacts the Chesapeake Bay. (Examples: wetland, stream, storm water management area, wet/dry pond)
2. Obtain maps of the selected area from local, state, and national sources. Include topological, wetland designation, zoning, soil survey, and aerial photography to assess the area.
3. Survey, map, and photograph the area.
4. Determine soil types using an agar and Munsel chart.
5. Run tests to establish baseline data on the stream. These should include nitrate, ammonia, chlorine, nitrite, dissolved oxygen, phosphate, turbidity, calcium, alkalinity, and temperature.
6. Identify native plant and animal species.
7. Develop a project(s) to preserve the area, repair damage, or make the area accessible for study.
8. Estimate native fish populations in local streams by electrofishing with the Maryland Department of Natural Resources. Then, select a species to raise in an aquaculture system.
9. Research, design, and construct an aquaculture system to raise the selected fish species. Fish can be collected by electrofishing with the Maryland Department of Natural Resources or can be purchased through local hatcheries.
10. Secure funding for a project by writing a grant or raising money.
11. Photograph and produce a summary of the completed project.
12. Keep permanent logbooks and perform additional tests on the area to document long-term impacts.

REFERENCES AND RESOURCES

Unit 1:

- Cohn, A., Flight, C., Frederick, A., Gallagher, J., Harpaz, G., Hauser, E., Kenney, A., Lang, A., Rekeweg, M., Van Leuven, T., Weaver, S., Wright, C., and Yalcin, K. 2025. *Planning for an Aquaculture System in the Classroom*. Maryland Sea Grant. UM-SG-TS-2025-01.
- Maryland Department of Natural Resources. Email: customerservice.dnr@maryland.gov; Phone: 1-877-620-8367
- Maryland Department of Natural Resources. (n.d.). Eyes on the Bay: Your home for Maryland tidal water quality data. Eyes on the Bay.

Unit 2:

- TheYieldGrid. (n.d.). TheYieldGrid: Data-backed agricultural calculators, verified planting guides, and professional agronomic tools for serious growers.

Unit 3:

- Chandler, D. L. (2019). *Biofilms in space and the risks to equipment and astronauts*. SciTechDaily.
- DeLong, D. P., & Losordo, T. M. (2012). *How to start a biofilter*. SRAC Publication No. 4502.
- Gurung, K, Tang, W. Z., & Sillanpää, M. (2018). *Unit energy consumption as benchmark to select energy positive retrofitting strategies for Finnish wastewater treatment plants (WWTPs): A case study of Mikkeli WWTP*. Environmental Processes, 5, 667-681. doi: 10.1007/s40710-018-0310-y
- Maryland Sea Grant. (n.d.). *Biofilms and biodiversity introduction*. University of Maryland Center for Environmental Science.
- Stauffer, B. & Spuhler, D. Anammox. *Sustainable Sanitation and Water Management Toolbox*.
- Tal, Y., Watts, J. E. M., & Schreier, H. J. (2006). *Anaerobic ammonium-oxidizing (anammox) bacteria and associated activity in fixed-film biofilters of a marine recirculating aquaculture system*. Applied and Environmental Microbiology, 72(4), 2896-2904. doi: 10.1128/AEM.72.4.2896-2904.2006
- Van Kessel, M. A. H. J, Harhangi, H. R., Flik, G., Jetten, M. S. M., Klaren, P. H. M., & Op den Camp, H. J. M. (2011). *Anammox bacteria in different compartments of recirculating aquaculture systems*. Biochemical Society Transactions, 39(6), 1817-1821. doi: 10.1042/BST20110743

Unit 4:

- Southern Regional Aquaculture Center. *Interaction of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds*, by Wurts and Duborrow.

- Gurung, K, Tang, W. Z., & Sillanpää, M. (2018). *Unit energy consumption as benchmark to select energy positive retrofitting strategies for Finnish wastewater treatment plants (WWTPs): A case study of Mikkeli WWTP*. Environmental Processes, 5, 667-681. doi: 10.1007/s40710-018-0310-y
- Maryland Sea Grant. (2012). *Chesapeake Quarterly* (Vol. 11, No. 1).
- Stauffer, B. & Spuhler, D. Anammox. *Sustainable Sanitation and Water Management Toolbox*.
- Tal, Y., Watts, J. E. M., & Schreier, H. J. (2006). *Anaerobic ammonium-oxidizing (anammox) bacteria and associated activity in fixed-film biofilters of a marine recirculating aquaculture system*. Applied and Environmental Microbiology, 72(4), 2896-2904. doi: 10.1128/AEM.72.4.2896-2904.2006
- Van Kessel, M. A. H. J, Harhangi, H. R., Flik, G., Jetten, M. S. M., Klaren, P. H. M., & Op den Camp, H. J. M. (2011). *Anammox bacteria in different compartments of recirculating aquaculture systems*. Biochemical Society Transactions, 39(6), 1817-1821. doi: 10.1042/BST20110743
- Wurts, W. A. & Durborow, R. M. (1992). *Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds*. SRAC Publication No. 464.

Unit 5:

- Frederick, J. A., Connors, J., Belden, M., Liu, C., & Place, A. R. (n.d.). *Fish oil, really?* Maryland Sea Grant.
- Moyle, P.B., and Cech, J.J. (1982). *Fishes: An Introduction to Ichthyology*. 5th edition, 2003. Prentice-Hall; Englewood Cliffs, New Jersey; 593 pages.
- Piper, R.G. (1982). *Fish Hatchery Management*. U.S. Department of the Interior, Fish and Wildlife Service; Washington, D.C.; 517 pages.

Unit 7:

- Maryland Sea Grant. (n.d.). *How to calculate biodiversity*. University of Maryland Center for Environmental Science.
- Barbour, M.T., Gerritsen, J., Snyder, B.D., & Stribling, J.B. (1999). *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*, Second Edition. EPA 841-B-99-002. US Environmental Protection Agency; Office of Water; Washington, DC.