Environmental Technology Program

Aquaculture Lab Manual
2022

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LIST OF ABBREVIATIONS

ammonia-N: ammonia-nitrogen

ave.: average

bw/day: body weight per day

°C/°Cent.: degree Centigrade

CMC: carboxy methyl cellulose

CSM: cottonseed meal

cu.ft.: cubic foot

dia.: diameter

DO: dissolved oxygen

°F: degree Fahrenheit

g: gram

gal: gallon

gpm: gallons per minute

GPS: global positioning system

HSB: hybrid striped bass

kg: kilogram

kWh/A: kilowatt Hours per annum/year

L: liter

mg: milligram

mg/L: milligram per liter

mins.: minutes

mL: milliliter

N: nitrogen

nitrate-N: nitrate-nitrogen

pH: potential of hydrogen

ppm: parts per million

ppt: parts per thousand

RAS: recirculating aquaculture system

sq.ft.: square foot

SRAC: Southern Regional Aquaculture Center

TAN: total ammonia nitrogen

temp.: temperature

VTR: volumetric tan conversion rate

Lab 1
Pond Construction Lab

Name ____

Goal: To demonstrate site selection activities, including soil testing for clay content, soil sample coring, topographical map and soil survey use, pond area measurement.

Materials Needed:

- Soil core auger, plastic bags, marker
- Soil test kit (dispersant, sieve, blower, drying tray)
- GPS – Garmin unit for plotting routes and calculating area
- Transit

In the Lab:

1. Clay content test. Use materials provided to measure. Then, suspend the soil. After waiting the prescribed interval, read the amount of soil component in each tube. **(Normally, dry soil overnight before weighing.)**

    a. Weigh 20 g of finely crumbled soil.
    b. Fill a tube with 20 g of soil. (Record the volume after gently tapping the tube to settle voids.)
    c. Add water to 40 mL mark. Then, add 5 mL dispersant solution.
    d. Cap and shake for 5 minutes.
    e. Allow to settle for 5 minutes. Then, read the amount of settled material.
    f. Calculate the amount of settled material as a percentage of the dry material. This is the percentage of sand and silt. Silt may stand out above the sand layer as a dark layer.

2. Using a transit, determine the water line of the proposed pond and mark with flags.

3. Garmin operation:
    a. Turn on power.
    b. On **Main Menu**, find **Area Calculation**.
    c. Press **Start**.
    d. Walk the exact area (using the flags from the previous exercise).
    e. Select **Calculate** when finished.
    f. Record pond area in the table provided on the next page.

Pond Construction Lab
Report Form

Name ____

Sample and Depth	mL Dry Soil	mL Silt and Sand	mL Clay (By Difference)	% Clay	Thumb Test Length
6 ? inches deep					
18 ? inches deep					

Discuss the findings in relation to depth to clay and clay content of the samples, **including results from thumb test for these two samples:**

Method for pond area calculation:

Using the Garmin, plot the waypoints around three ponds. Report the area calculated. Then, calculate the average of these numbers.

Pond Area			
Water surface elevation			
Dam elevation			
Spillway elevation			
Freeboard	**4 feet**		

One acre equals 43,560 sq.ft.

Lab 2
Dissolved Oxygen

Name ________________________________

Goals:

1. To demonstrate the operation of an oxygen meter and use it to measure oxygen under different conditions.
2. To demonstrate oxygen consumption of fish and aerator efficiency.

Materials Needed:

- Oxygen meter
- Three buckets
- Measuring container
- Scale
- Aerator
- Air stones

A. Show how water contains oxygen:

TASK 1: Measure oxygen in three types of water: municipal water, RAS water, and farm pond water.

Municipal water often is low to moderate in oxygen. In addition, municipal water contains chlorine which must be removed through carbon filtration, aeration, or treatment with sodium thiosulfate.

RAS tank water should have high amounts of oxygen (close to 100% saturation). This is due to the addition of oxygen either through aeration or pure oxygen generation.
Most ponds have low oxygen in the morning and higher oxygen in the afternoon. The difference between the morning and afternoon amounts usually is an indication of the density of the phytoplankton in the pond.

B. Show how oxygen drops when fish are placed in a container:

TASK 1: Fill three containers with 0.5 L of water. Measure dissolved oxygen.

Water Type	Oxygen mg/L	Temperature ^{O}C	Convert to ^{O}F
City water			
Moderate Bloom Pond 1			
Heavy bloom Pond Pond 9			

TASK 2: Place 5 small fish in the water in **each of two containers** (one with aeration and one without aeration).

- After 5 minutes, measure the oxygen again.
- Repeat every 10 minutes until oxygen drop of 50% is measured.
- Record the time and oxygen and temperature at each interval.
- Replace catfish in aerated water after the experiment.

Fish use oxygen as they respire. In a closed container, they eventually will remove all the available oxygen. In aquaculture, oxygen is replenished by the action of phytoplankton photosynthesis and by mechanical aeration.

What are some of the dangers of having a heavy phytoplankton bloom in a fishpond?

TASK 2: Fish Weight _______ Average fish weight = (total weight/5) ___________? grams

Tank 1 (5 fish but no aeration) Stop when oxygen is 50% of the initial reading.

Dissolved Oxygen **Initial =**	**mg/L Oxygen**	**Temperature (°Cent.) and Any Observations of Fish Behavior**
Dissolved oxygen **after 5 mins.**		
Dissolved oxygen **after _____ mins.**		
Dissolved oxygen **after _____ mins.**		
Dissolved oxygen **after _____mins.**		
Dissolved oxygen **after _____ mins.**		

Using the average fish weight, what is the rate of oxygen consumption per fish weight in mg oxygen per gram per minute?

Oxygen used mg/L x L = mg used

Mg used/grams fish/minutes = oxygen consumption in mg/g/min

Tank 2 (5) fish with air stones for aeration) __________? g

Initial Dissolved Oxygen	**mg/L Oxygen**	**Temperature (°Cent.) and Any Observations of Fish Behavior**
Dissolved oxygen after 5 mins.		
Dissolved oxygen after ____ mins.		
Dissolved oxygen after ____ mins.		
Dissolved oxygen after ____mins.		
Dissolved oxygen after **25** mins.		

What is the aeration efficiency of the air stone aerator?

- Find the wattage of the aerator.
- Calculate the difference between tank 1 and tank 2 over time.
- Results should be in mg oxygen per watt per minute.

Oxygen used in tank 1 minus oxygen used in tank 2 (with aerator) = oxygen supplied by aerator

Oxygen supplied by aerator/ watts/ minutes = aeration efficiency

Tank 3 (Control tank without any fish or aeration)
______0_________?gram
Stop when you stop readings in Tank 1.

Initial Dissolved Oxygen	**mg/L Oxygen**	**Temperature (°Cent.) and Any Observations of Fish Behavior**
Dissolved oxygen after 5 mins.		
Dissolved oxygen after ____ mins.		
Dissolved oxygen after ____ mins.		
Dissolved oxygen after ____mins.		
Dissolved oxygen after ____ mins.		

Did oxygen drop over time in Tank 3? Why or why not?

Did temperature change in Tank 3? Why?

Complete and submit by next week.

Lab 3
Aquaponics Lab

Goal: To understand the basics behind recirculating aquaculture systems so that small scale systems can be constructed for teaching or demonstration purposes. This lab will cover one month of time between September and October.

Resources: Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture. James E. Rakocy, Michael P. Masser and Thomas M. Losordo. 2006. SRAC. 454.

Materials:

- An aquaponics system will be set up in the greenhouse.
- Bluegill will be used as the fish.
- Lettuce and chards will be used as the plants.
- Feed will be Purina Aquamax fingerling starter 300

The system:

- Fish tank **(measure and calculate water volume)**
- Solids separator **(measure and calculate water volume)**
- Biofilter **(measure and calculate water volume) has been operating with fish for 1 month**
- Plant floating rafts **(measure and calculate water volume and plant area)**
- Sump for return water **(measure and calculate water volume)**

- Sump pump **(record horsepower and calculate power requirement and water volume per day)**
- Air pump **(record horsepower or watts and calculate power requirement)**
- Water heater **(Record watts and calculate power consumption) however we will not use it during the warm weather we will have until the project is completed**.

Procedure:

1. Choose people to check water quality and feed each day**. It is your responsibility** to make arrangements with the instructor to **cover weekends.**

2. **Calculate the amount of feed to be offered each day**.
   - Begin feeding at 1% of the fish weight per day.
   - Increase feed to 2% after 3 days and 3% after 6 days. (However, observe feeding and do not increase if the fish fail to eat the feed in 5 minutes.)

3. Temperature for the water in the culture tank should be measured each day.
   - No water heating will be used in this period (September to early October).
   - The best temperature for catfish is between 25° and 28° Cent.

4. Water chemistry will be determined for oxygen (once daily), temperature (once daily), pH (every two days), total ammonia (every week), and nitrite (every week).
   - Nitrate and ammonia will be measured the first of each week.
   - Record feed amount each day.
   - If pH in the fish tank is less than 7.0, increase aeration and add more base. (See the instructor for guidance.)

Planting:

1. Holes are pre-drilled in a cone shape.

2. Move select plants from flats to net ponds, insert in holes in foam.

3. Measure plant height with ruler. Record average plant height for each foam piece.

Plant Group	Initial Average Height	Average Height After 3 Weeks
Raft 1		
Raft 2		
Raft 3		
Raft 4		

Lab Report
Recirculating Aquaculture System

Name ___________________________________

Initial readings

Parameter	Holding Tank	Culture Tank	Plant Tank
Oxygen			
Temperature			
Ammonia (Total)			
Nitrite			
pH			
Nitrate-N			

1. Calculate the number of fish to add:

   Volume of tank: Radius (ft.) squared x 3.14 x depth (feet) = _______

   cu.ft.______________ / 7.48 gal./cu.ft. = ____________ gal.

   Gal. of water x 0.125 = number of fish: ______________________

   Example: If you have 1,000 gallons of water, the maximum weight would be 125 pounds. If you have fish averaging 1 pound each at harvest, you need to stock 125 fish initially.

Calculate the amount of feed:

Weight of fish x feeding rate (0.03 per day) = daily feed rate

Weight of fish __________g. x 0.01 (1% per day at first) = ____________g.
(Start with 1% and increase to 3% over first week.)

Determine Ammonia load:

Maximum feed per day x ammonia produced/lb. feed = Ammonia load/day

0.022 lbs. of ammonia/ lb. feed

We will assume the fish will increase by ½ during the 2 weeks of feeding. Feed will be adjusted during the trial to reflect that increase. Use the final weight of fish (1.5 x initial weight) in order to calculate the amount of feed. The maximum feed rate will be used to estimate square feet of biofilter.

Feed rate x maximum fish weight = maximum feed per day (use this below)

0.03 x final weight of fish in pounds ___ lbs. = ___________

Ammonia load = ______________________________

Ammonia load can be used to estimate biofilter surface area. However, we will use maximum feed rate.

Calculate amount of filter media needed:

There are two methods for estimating the amount of square feet of surface needed for biological filtration: 1.) from amount of feed uses, or 2.) from amount of ammonia produced.

Maximum feed per day/ pounds of feed per day per square foot of media = sq.ft. media

_______Max. lb. of feed per day/ 0.0029 lb. feed/day/sq.ft. biofilter = _________ sq.ft.

Example: 0.29 lb. of feed/ 0.0029 lb./d/sq. ft. = 100 sq.ft.

Square feet of media/ sq.ft. per cu.ft. (from chart) = cubic feet of media needed

________________sq.ft. filter area / 150 sq.ft. per cu.ft. biofilm media = _______ cu.ft.

Measure cubic feet of media we have in the system. Note: One cubic foot has 1,728 cubic inches.

Culture Tank Volume: ______cu.ft.___________

Sediment Tank Volume: ______cu.ft._________

Plant Tank Volume: __________cu.ft.________

Sump Volume: ______cu.ft.________________

Total System Volume (not counting pipes) :_______________

Examples of media and surface area:

Media	Surface area (sq.ft/cu.ft)
Bio barrel, 1 inch	64
Biofilm media, ½ inch	**150**
Sand (0.05 inch dia.)	5070
Gravel (0.25 inch dia.)	183

2. **Feeding**: Feed each day the amount needed to supply 3% of fish weight. However, the first three days, feed only 1% or one third of the calculated feed. Step up feeding if the fish eat their feed in 5 minutes or less.

3. **Water quality**: Measure weekly ammonia, pH, nitrite, but daily oxygen and temperature.

(See next page for Week 1 table.)

Week 1

Parameter	Ammonia	Nitrite	Temp.	pH Culture	pH plant	Oxygen	Feed
Day 1							
2							
3							
4							
5							
6							
7*							
Nitrate							**Total feed =**
Phosphate							

*Check nitrate and phosphate. Then, drain solids from settling tank and biofilter, recharge water to 8-inch line.

Change feeding rate at the beginning of each week:

____________ lb. feed fed / 1.8 = __________ lb. weight gain

____________ lb. weight gain + ___________ lb. initial weight = ____________ lb. fish

____________ lb. fish x .03 = ____________ lb. feed for new rate.

Week 2

Parameter	Ammonia	Nitrite	Temp.	pH Culture	pH plant	Oxygen	Feed
Day 1							
2							
3							
4							
5							
6							
7*							
Nitrate							**Total feed =**
Phosphate							

*Check Nitrate and Phosphate. Then, drain solids from settling tank and biofilter, recharge water to 8-inch line.

Change feeding rate:

____________lb. feed fed / 1.8 = __________ lb. weight gain

____________lb. weight gain + ___________ lb. initial weight = ____________lb. fish

____________ lb. fish x .03 = ____________ lb. feed for new rate.

Week 3

Parameter	Ammonia	Nitrite	Temp.	pH Culture	pH plant	Oxygen	Feed
Day 1							
2							
3							
4							
5							
6							
7*							
Nitrate							**Total feed =**
Phosphate							

*Check Nitrate and Phosphate. Then, harvest fish and measure plant height.

Change feeding rate:

___________lb. feed fed / 1.8 = _________ lb. weight gain

___________lb. weight gain + __________ lb. initial weight = ___________lb. fish

___________ lb. fish x .03 = ___________ lb. feed for new rate.

Final fish weight: _____ Initial fish weight: _____ Gain: _______
Basil plant height gain: ____________ Plant losses: _________
Broccoli plant height gain: __________ Plant losses: _________
Brussel sprouts height gain: ________ Plant losses: _________

4. Use power parameters given for the pumps and heaters and blower to calculate an operating cost per day.

Electricity cost is $0.15 per kilowatt hour.)
Assume that the heater operates 12 hours per day, or during nighttime when sunlight does not contribute heating.

One horsepower is approximately 760 watts. One thousand watts running for one hour is one kilowatt hour.

Fill in the following table:

Power User	Watts	Time Running Per Day	Kilowatt Hours	Cost Per Day
Air pump	25			
Return pump	250			
Water heater	4,500			
Total Cost Per Day				

Lab 4

Economics Laboratory

Name____

This lab will use the catfish pond economics spreadsheet to check the relationships that changing variable in system design can have on a catfish farming operation. See the instructor about obtaining the spreadsheet.

The original assumptions are in the EXCEL file.

This sheet has been prepared to reflect a 40-acre catfish farm with 8 ponds of 5 acres each.

After making certain changes, return the spreadsheet to the original values before proceeding to the next situation. Please fill in the table that starts on the next page:

Situation	Variable Change	Total Cost Per Pound	Revenue Per Pound	Increase or Decrease RISK?
1	Original spreadsheet			Low Risk
2	Adjust harvest size of catfish from 2.0 to 1.5 lbs.			
3	Adjust the fish losses from 20% to 30%			
4	Use harvest equipment and a seine and 128 hours harvest labor instead of custom harvest			
5	Change feed cost from $425 to $300 per ton			
6	Change stocking density to 6,000/acre and harvest size to 1.0 lb. per fish			
7	Change aerators to 2 per pond or 16 for the farm, increase stocking to 7,500 per acre, increase electricity to 1,500 kwh/A			
8	Increase chemical costs to $300/acre			
9	Change feed to $600 per ton			
10	Change feed conversion to 2.0			

1. Describe a change to this catfish farm (choose one of the 10 we tried). Would you recommend that would **reduce risk** at an acceptable profit? Explain your choices.

2. What are some **other changes** in catfish farm operation that might improve net revenue? Explain how you would make the changes and **what effect they would have on the amount of fish produced, amount of feed, aeration, and labor needed, and harvest size of the fish**.

3. If the sales price were increased to $1.40/lb. (increase returns by $96,000), list three things that you might add to the enterprise that would improve farm conditions or increase production. In other words, if you had a promise of higher returns, would you spend more on certain items? If so, what would they be? Explain.

Lab 5
Marketing Aquaculture Products

Lab Report

Name____

Difference between Marketing and Sales:

Marketing identifies potential buyers for a product, while sales delivers a specific product to a specific buyer for a specific price. Sales must be achieved for any business to succeed.

Market estimation:

1. Identify a geographical area or population of people as the target.
2. Determine what information is needed.
3. Develop a means to obtain that information.
4. Obtain the information.
5. Analyze the information obtained.
6. Apply the conclusions to create sales.

In this lab, we will prepare and taste several aquaculture products. After answering questions about the product, we will make a sales plan.

Products:

- Catfish (bone-in)
- Catfish (fillet)
- Tilapia (fillet)
- Salmon, wild (fillet)
- Salmon, farmed (fillet)
- Shrimp, wild and farmed
- Caviar

Preparation: (Fillets will be cut into two or three pieces per fillet.)

- Catfish - Breaded and fried or baked.
- Tilapia - Fried or baked
- Salmon - Grilled or baked
- Shrimp – Boiled with seasoning
- Caviar – On cracker with cream cheese

The proper time will be used to fry, bake or boil the product.

- Overcooking of the fish is not desirable.
- Fish are usually fried until *golden brown* or *floating* in the oil.
- Fry temperatures are approximately 350° to 375°F.
- Fry time depends on the thickness of the product.
- Avoid using frozen or partially frozen product for frying.
- Bake seasoned fillets for 20 min at 350°F .
- Seasoning will be olive oil drizzle with lemon or blackened seasoning.

Tasting: After each product is cooked, set up a testing table. Each student will taste and answer the survey questions.

- You do not have to taste the product if you do not want to.
- If you have medical reasons of not eating seafood, please do not eat any of these products.

Survey – Individual

Name____________________________________

Product	**Taste:** Good Bad Fishy Bland Cardboard Other	**Texture:** Firm Soft Oily Grainy	**Would You Buy It?** Yes or No	**What Would You Pay for It?** $2.00 or less $4.00 or less $6.00 or less $8.00 or less $10.00 or less
Catfish fillet (fried), farmed				
Catfish fillet (blackened Cajun), farmed				
Tilapia fillet (broiled), South America				
Tilapia fillet (fried), South America				
Swai (fried), Vietnam				
Shrimp, wild, U.S.				
Shrimp, farmed, Thailand				
Caviar, on cream cheese				

Summary of Data:

- Discuss individual comments to complete this section.
- After a tally of the comments, list the top two comments and the number of people with the same comment.

Catfish Fillet (Pond), Fried	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
Tally. Then, list the top two comments and the number of people with the same comment.		

Catfish Fillet (Pond), Broiled	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
Tally. Then, list the top two comments and the number of people with the same comment.		

Salmon, Wild, Fillet	Comment About Lemon-Seasoned Salmon	Comment About Blackened Salmon	Number
Taste - good			
Taste - bad			
Texture - good			
Texture - bad			
Would you buy it? yes			
Would you buy it? no			
What would it cost?			
Tally. Then, list the top two comments and the number of people with the same comment.			

Tilapia Fillet (Fried)	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
Tally. Then, list the top two comments and the number of people with the same comment.		

Salmon, Farmed	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
Tally. Then, list the top two comments and the number of people with the same comment.		

Salmon, Wild	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
Tally. Then, list the top two comments and the number of people with the same comment.		

Shrimp, Wild	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
After a tally of comments, list the top two comments and the number of people with the same comment.		

Shrimp, Farmed	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
After a tally of comments, list the top two comments and the number of people with the same comment.		

Caviar	Comment	Number
Taste - good		
Taste - bad		
Texture - good		
Texture - bad		
Would you buy it? yes		
Would you buy it? no		
What would it cost?		
After a tally of comments, list the top two comments and the number of people with the same comment.		

Name ____

1. Choose the top three seafood products that the class preferred.

2. Develop a marketing plan, including products to sell, prices to ask, and promotional materials.

   a. **Products:** Name the products. You may choose a new or more appealing name.

      1.

      2.

      3.

   b. Wholesale Pricing:

   - The wholesale price is 40% less than the retail price.
   - Wholesale price divided by 0.6 equals retail price. The 40% markup is retained by the grocer/ distributor. (Start with the wholesale price or work backward from the retail price.)

Example: A product selling for $5.00 per pound wholesale would cost $8.33 retail.

$5.00/0.6 = $8.33.

Therefore, if you want to get $5.00/ lb. for shrimp, you need to sell the retail product for $8.33.

Product: Name	Wholesale Price	Retail Price
1.)		
2.)		
3.)		

c. Promotional plan:

- Name and describe three items you would use to promote the products.
- Use the class opinion of the products we tasted to help develop this promotional plan.
- What have you seen used for other food items that you are familiar with?

1. Product 1:

2. Product 2:

3. Product 3:

Lab 6
Feed Formulation Experiment

Making a fish bait and testing for water stability

Goal: To use cottonseed meal as the main ingredient, but add binders to make the diet more stable.

Test already-prepared baits for water stability.

Formula: Calculate the percentage of each ingredient in the diet. Record in the table below.

Ingredient	Grams Bait 1	% of Formula	Bait 2	% of Formula
Cottonseed meal	50		50	
Corn meal	25		25	
Matrix	12		12	
CMC	3		0	
Urea-based polymer	0		3	
Tallow	**10**		**10**	
Added water	35		35	
Total weight	135		135	

Procedure for making the fish bait blocks:

Mix dry ingredients, starting with smallest amount than add larger to get good mixing.

1. Mix dry first. Add 3g CMC or polymer to corn. Mix well.

2. Add the cottonseed meal to this dry mix.

3. Mix the matrix with the dry ingredients very well. No lumps should remain.

4. Add tallow and mix to eliminate any lumps.

5. Add water and mix to remove any lumps.

6. Form into a rectangle approximately 1 inch thick.

7. Dry at 80° Cent. overnight.

Water stability test:

1. Record observations about the stability of the bait in water.

2. Add approximately 300 mL of water to a 500 mL beaker for each test.

3. Weigh and drop bait into beakers of water. After 20 minutes, record the appearance of the bait block.

4. Carefully drain the water from the bait blocks along with any loose particles.

5. Dry the remaining block in the beakers overnight. Weigh to determine the remaining amount. Calculate the remaining percentage.

Diet	Initial Weight of Bait Block	Comments and Observations on Each Block	Weight After Drying Block Remnants	% Stability	
1 25% CSM 10 min					
1 25% CSM 20 min					
2 50% CSM 10 min					
2 50% CSM 20 min					
3 CMC 10 min					
2 CMC 20 min					

*The matrix contains dry molasses, clay, and tallow. It is mixed with water to make a sticky slurry that is used to coat the bait ingredients. When dry, the matrix holds particles together.

a. Discuss the effect of CMC (carboxy methyl cellulose) you observed. Discuss the amount of CSM (cottonseed meal) and water stability.

b. Discuss your observations of the diets made with 50% CSM and the two binders.

c. Describe the purpose of each of the following feed ingredients in fish diets (related to the nutrients they provide, their physical characteristics, their density, or the effect of heat on their physical characteristics). Use SRAC publications and web resources.

- **Soybean meal:**

- **Cottonseed meal:**

- **Corn meal:**

- **Vegetable oil:**

- **Wheat byproducts (middlings or bran):**

Lab 7
Phytoplankton and Zooplankton

Name____

Goals:

1. To identify and count phytoplankton and zooplankton found in aquaculture ponds.
2. To understand that living organisms are suspended in pond water and their important purposes.

Set up a microscope according to the handout. Clean only with lens paper.

Use water from samples collected to fill a counting cell with one mL of sample.

Use keys to name the algae or zooplankton to genus. Ask for help or use identification books for other organisms.

A. Phytoplankton Examination of Pond Water.

- Use microscope and counting cells to view water sample.
- Apply 1 mL of sample to slide. Allow it to set for 5 minutes before viewing. This allows floating algae to rise to below the cover slip and other algae to settle on bottom of the slide.
- Report the five most abundant algae from blue green and green algae groups.

Pond Sample	Major Blue Green Species	Major Green Algae Species	Observations on Other Algae
Pond	Genus__________ Genus__________ Genus__________ Genus__________ Genus__________	Genus___________ Genus___________ Genus___________ Genus___________ Genus___________	

Discuss the relative abundance of algae in the pond.

- Is there a diverse number of algal species?
- Why or why not?
- What effect does these algae have on the ammonia or carbon dioxide measured in this lab?

C. Zooplankton Counting:

- Collect pond water sample by pouring 4 gal. of pond water through a plankton net. Use well water to bring the captured plankton to 100mL. Now, we have the plankton from 4 gal. or 14.8 L in 0.1 L.

- Take 1.0 mL and try to count the zooplankton. If they move too fast, add a drop or two of alcohol.

- If using a sedgewick rafter cell, count the number of cells in 10 squares:

1	2	3	4	5	6	7	8	9	10	Ave.
A										
B										
C										
D										
E										

Zooplankton Identity	**Number Per Liter**
A =	**=** ________________
B =	**=** ________________
C =	**=** ________________
D =	**=** ________________
E =	**=** ________________

D. Toxic Metabolites (Ammonia, Nitrite) and Phytoplankton Effects

- The laboratory introduces the methods of total ammonia and nitrite measurement.
- pH and temperature will be measured also.

Equipment:

- Chemical test kits
- Extra graduated test tubes
- Protective eyewear and rubber gloves
- Thermometer

Materials:

- Samples from the pond
- RAS tank
- Aquaponics tank

i. **Ammonia analysis:**

Use the HACH® water test kit to measure total ammonia in water.

- Samples of water were collected from a fish pond and will be tested for total ammonia.

- Well water and an ammonia test sample will also be tested.

- Students will be evaluated on their ability to precisely measure the total ammonia in the samples.

Procedure: Nessler method from HACH® kit manual using a 5 mL sample.

1. Rinse sample tube with sample.

2. Measure precisely 5 mL into tube. Instruct about reading Meniscus.

3. Add one drop of Rochelle Salt solution.

4. Add 3 drops of Nessler's reagent. Mix and allow to react for 10 minutes.

5. Read the ammonia-nitrogen concentration in color window.

6. Calculate the total ammonia concentration from total ammonia nitrogen reading and record for lab report.

   Molecular weight of ammonia = 18
   Nitrogen is 14/18 x 100 = 77.78%

   Ammonia-nitrogen/0.7778 = concentration of Total ammonia

7. Check water pH using HACH® pH indicator method. Record for lab report.

8. Check water temperature using a thermometer. Record for lab report.

9. Calculate the un-ionized ammonia in each sample and record for lab report.

      Obtain value for percentage un-ionized ammonia from table in kit manual.

      Un-ionized ammonia = total ammonia concentration x percent un-ionized/100

ii. **Nitrite Analysis:**

Use pond water samples that were used in the ammonia test and compare them to a nitrite standard.

Procedure:

1. Rinse sample tubes with sample.

2. Accurately add 5 mL of sample.

3. Add nitrite reagent powder.

4. Mix the reagent and sample for exactly 1 minute.

5. Allow the reagent to react for 10 minutes.

6. Read the nitrite nitrogen concentration from the color wheel.

7. Report the nitrite concentrations for the lab report based on the calculation below:

   Molecular weight of nitrite = 46
   Percent nitrogen = 14/46 x 100 = 30.43%
   Nitrite concentration = Nitrite-nitrogen reading/ 0.3043

Data Report:

Sample	Temp. ºCent.	Total Ammonia-Nitrogen (reading)	*Total Ammonia	pH	Un-ionized* Ammonia	Nitrite – N (reading)
Well water						
Pond						
RAS tank						
Aquaponics tank						

*Use pH and temperature to calculate this value from the total ammonia-N reading.

Discuss the results: Explain the ammonia and nitrite values in terms of phytoplankton presence or absence and the possible toxicity to catfish.

Discuss the relative abundance of algae in the three samples.

- Is there a difference in water clarity?
- What effect does algae have on the ammonia or carbon dioxide?

Citation of literature used for discussion:

- List at least two citations used to explain the results.
- *Visit the library* and/or use the SRAC.

1.

2.

Lab 8
RAS Design

Goal: Design an RAS for hybrid striped bass production.

Objective 1: Determine tank size based on production goals
Objective 2: Determine flow rate of an RAS
Objective 3: Choose a pump size based on required flow and system design

Resources: Selected readings at the Southern Regional Aquaculture Center website will be used in this lab https://srac.msstate.edu/

Part 1:
Assuming an output of ½ lb. per gallon, determine the required volume of tanks for the following production goals:

System 1: 1500 lbs. _____________ gal.
System 2: 2500 lbs. _____________ gal.
System 3: 5000 lbs. _____________ gal.

What is the required flow rate for each of the systems below? Use resources at https://srac.msstate.edu/

System 1 ______________ gpm
System 2_______________ gpm
System 3 _______________gpm

Use the provided pump performance curve to recommend a pump for each of the systems:

System 1 :__________________
System 2:__________________
System 3:__________________

Part 2:

Using the HSB system in the NGTC Aquaculture lab, determine the required flow rate for a 45-minute tank exchange rate. Total volume of the system is approximately 3000 gallons.

Lab 9

Brine Shrimp Culture

Goal: To set up small scale culture systems to feed saltwater fish or crustacean larvae.

Premise: Brine shrimp are cultured as food for small forms of fish, crustaceans, and mollusks. Aquaria enthusiasts need small-scale culture of algae and zooplankton (rotifers, daphnia, brine shrimp, etc.) when they try to spawn their fish. Commercial hatcheries for specialty fish and marine species focus on this aspect of nutrition for their aquatic organisms. In most of the emerging aquaculture industries, careful attention to larvae nutrition is needed. Production of live food (with a good supply of essential fatty acids and essential amino acids) is very important when exact nutrient requirements are unknown. This is usually the case for new culture species.

Materials:

- Hatching cones
- Light source
- Scale for weighing to 0.1 gram
- Air supply, tubing and stones
- Filter or small mesh screen
- Counting cells and microscopes
- Pipets
- Brine shrimp cysts
- Sea salt
- Sodium thiosulfate

- Hydrogen peroxide
- Mixing glassware: 1,000 mL beakers (two), 500 mL beakers (two), 1-gallon container
- Filter funnels (two), squirt bottle with dechlorinated tap water

Outline:

A. Treat tap water to remove chorine.
B. Hydrate brine shrimp cysts.
C. Set up hatching system.
D. De-capsulation process.
E. Counting cysts.
F. Counting algae and feeding the artemia culture.

Procedure:

1. **De-chlorinated tap water:** Use tap water to fill a container to one gallon.
   a. Calculate amount of sodium thiosulfate to add to water.

   b. Use 13.262 mg sodium thiosulfate per part per million chlorine **per gallon of water**.

   c. parts sodium thiosulfate to 1 part chlorine based on molecular weight ratio of 278 to 70.9

   d. Assume that 5 ppm chlorine is possible (or measure the actual chlorine). Calculate the amount of sodium thiosulfate:

      One gallon is approximately 3.7 liters.

      5 ppm x 13.263 mg/ 3.7 liters = ___________________
      Weigh this amount.

      Actual chlorine in tap water using test kit = ___________

      Chlorine in treated water using test kit = _____________

e. Use tap water. It has a low bacterial and algal count due to the presence of chlorine.

- Once the chlorine is removed, you have nearly-sterile water. This is suitable for production of algae and brine shrimp as food because the time of culture is short.

- For more careful culture needs, use sterile water and hygienic methods.

2. **Salt in the system**: Make sea water (30 ppt) for brine shrimp.

a. Pour 1 L of treated tap water into two 1,000 mL beakers.

b. Save the remaining treated tap water for step 3.

c. Target 30 ppt as the desired salt content.

30 ppt = 1 liter of water and _______________ g of sea salt

Note: 1 ppt = 1 g salt per L of water.
1 mL of water weighs 1 g.

d. Add the salt to a portion of the water in each 1L beaker. Mix. **Set Aside** .

e. After allowing to fully dissolve, add to hatching cones.

f. Add the brine shrimp later. Use wash water to make up the rest of the 1L volume.

3. **Brine shrimp hydration – 1 hour**

   a. Add 2.5 g (approximately one tsp.) of brine shrimp cysts to 200 mL of de-chlorinated tap water in a 500 mL beaker to hydrate. Make two beakers.

   b. The dry cysts will begin to get spherical as they absorb water.

   c. Aerate during this process by putting an air stone in the beaker. Adjust air to a moderate flow.

4. **Hatching system set up**

   a. Prepare two hatching cones in the cone stand by washing with tap water.

   b. Set up aeration and air stones so that the stones are at the end of a plastic tube and in the bottom of each cone.

   c. Set up a light source behind the cones.

   d. Set up the filter funnel for the next step.

5. **Brine shrimp decapsulation – 15 minutes**

   a. After the 1-hour hydration, pour collect brine shrimp in a filter funnel.

   b. Use stock hydrogen peroxide (approximately 3% strength) to treat the cysts.

   c. After 15 minutes, wash with dechlorinated tap water 3 times.

   d. Add saltwater to two cones to approximately the 900 mL mark.

e. Let the cysts drain. Then, wash into the cone containing the saltwater.

f. Bring the volume up to 1,000 mL. Add air line.

6. **Counting brine shrimp cysts.** Pair into teams for this task.

a. Get 5 microscopes and 5 counting chambers.

b. Use a large pipet to **collect approximately 5 mL** of the suspended brine shrimp cysts.

c. Move to a test tube.

d. Place 1 mL from the collected amount on the counting cell using a plastic dropper.

e. <u>Return the remainder of the brine shrimp to the culture.</u>

f. Describe the appearance of the cysts:

____

____

g. Counts per 10 fields. Sum. Then, average (count till you have 25 cysts or 10 fields).

	1	2	3	4	5	6	7	8	9	10	Sum
Hydrated											
Not hydrated											
	Average number of hydrated cysts per field =										

Cell Ave _____x number of cells/ mL _____ = brine shrimp per mL___________

h. Algae can be fed to brine shrimp to enrich their nutritional value. Certain algae have essential fatty acids that are valuable to larval fish. When the brine shrimp is enriched, survival of larval fish can be improved.

i. Counting algae cells in the culture.

The algae culture is very dense.

- Dilute the culture 1:10 by adding 1 mL of culture to 9 mL of water in a graduated cylinder or test tube.

- Take 1 mL of the diluted culture and count with a counting cell.

Algae counts per field

1	2	3	4	5	6	7	8	9	10	Sum
Cell average =										

Cell ave. _____x number of fields/mL _____ = cells per mL ___________

Number per mL _____ x 10 = Number per mL commercial source _____

j. Assume you would add 10 mL of the commercial source of algae to your brine shrimp cone. How many cells do you add?

Number per mL commercial _____ x 10 mL = Number fed

Number fed _____ divided by 1,000 mL = density of food per mL _________

k. How many algae cells were fed for each brine shrimp?

- **Use brine shrimp per mL and food algae per mL to calculate food algae for each brine shrimp.**

Lab 10
Biofilter Design Requirements

Goals: Determine the required biofilter volume for RAS

Basic assumptions for this system:

- Feed rate: 1.5% bw/day
- Volume of tank: 3000 gal. (convert to cubic meters)
- TAN production: 0.03 kg TAN/kg of feed
- Fish density 0.3 lbs./gal. (convert to kg/cubic meter)
- DO demand: 0.288 kg of O2/kg feed
- Temperature: 15 – 25° Cent.

Step 1: Determine the total number of fish in the system: *Density x volume*

Step 2: Determine the total weight of fish in the system:

a. Weigh a sample of 10 fish to determine an average weight for each fish.
b. Multiply by the total number of fish in the system.

Step 3: Determine the total amount of feed per day given the daily feed rate

Feed rate x total fish weight of the system

Step 4: Calculate the DO requirement

DO (required) = DO demand x Feed rate (decimal) x fish density x tank volume

Step 5: Calculate the water flow requirement.
Assume: DO inlet = 15 mg/L and DO tank = 5 mg/L

Flow (L/min) = (DO required/ DO inlet – DO tank) x (1 day/1440 min) x (1000000 mg/1 kg)

Step 6: Calculate TAN production. Assume ammonia production is about 3% of feeding rate.

Example: 0.03 kg of TAN for every 1 kg of feed. If you are feeding 100 kg per day, then:
0.03 x 100 = 3.0 kg of TAN per day

Step 7: Calculate volume of media required to remove TAN

(1.0-0.3) x [(TAN production x 1000g)/350]
350 grams of N per day is the assumed volumetric tan conversion rate (VTR) of a bead filter.

ACKNOWLEDGEMENT

This material is adapted from aquaculture materials developed by Gary Burtle, Ph.D. It is based on work supported by the National Science Foundation under grant number DUE #2000444. Any opinions, findings, conclusions or recommendations expressed in this material are those of the grantee and do not necessarily reflect the views of the National Science Foundation.