



The Process of Nitrification in Aquaria



Grade Level:

5-12

Subject Area:

Aquaculture, Biology,
Chemistry

Time:

Setting up aquarium: 45 minutes
*Daily water quality
sampling/testing:* 20 minutes (for
at least 2 weeks)

Student Performance Standards (Sunshine State Standards):

03.01 Employ scientific measurement skills (SC.912.E.7.8; SC.912.L.14.4; SC.912.S.3.1, 9; MA.912.A.1.5; MA.912.S.4.2; MA.912.S.5.1, 3; MA.912.S.5.2, 3, 4, 5).

03.02 Demonstrate safe and effective use of common laboratory equipment (LA.910.1.6.1, 2, 3, 4, 5; SC.912.L.14.6; SC.912.L.16.10; SC.912.L.17.12, 14, 15, 16; MA.912.A.2.1, 2).

03.06 Interpret, analyze, and report data (SC.912.L.16.1; SC.912.N.1.1, 2, 3, 4, 6, 7; SC.912.N.2.2, 5; SC.912.N.3.1; SC.912.N.4.1; MA.912.S.3.1, 2; MA.912.S.4.2; MA.912.S.5.1, 2, 3, 4, 5).

04.01 Research how different climactic and geological activity influences agriculture (SC.912.E.6.1, 4; SC.912.E.7.1, 4, 6, 7, 8; SC.912.L.17.4, 7, 8, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20; SC.912.L.18.12).

11.10 List and describe the major factors in the growth of aquatic fauna and flora (LA.910.1.6.1, 2, 3, 4, 5; SC.7.L.17.1, 2, 3).

12.01 Recognize and observe safety practices necessary in carrying out aquaculture activities (LA.910.1.6.1, 2, 3, 4, 5).

13.01 Identify and describe the qualities water should possess for use in aquaculture (LA.910.1.6.1, 2, 3, 4, 5; SC.912.L.17.In.a).

13.02 Explain how changes in water affect aquatic life (LA.910.1.6.1, 2, 3, 4, 5; SC.912.L.17.2, 3, 7, 10).

13.05 List and explain sources of aquaculture pollution and methods of preventing and/or correcting these pollution problems (SC.912.L.17.9, 11, 12; MA.912.G.2.7; MA.912.A.10.2).

14.01 Identify factors to consider in determining whether to grow an aquaculture species (LA.910.1.6.1, 2, 3, 4, 5; MA.912.G.2.5; MA.912.G.8.3, 6; SC.7.L.17.3)

16.04 Identify water quality problems (SC.7.E.6.6).

Objectives:

1. Students will be able to select and use testing equipment to determine water quality.

2. Students will be able to describe the natural progression of ammonification and nitrification.
3. Students will be able to explain the impact of ammonification and nitrification on marine organisms.

Abstract:

Fish or invertebrates maintained in aquaculture systems must be fed, and as a result they excrete metabolic wastes. Whenever proteins are broken down, whether by fish during digestion or bacterial action of uneaten food and fish waste, ammonia will be produced. Fish eliminate nearly all of their ammonia through their gills with a very small percentage excreted along with the fecal matter. The transfer of ammonia from the fish to the water is done by diffusion. Diffusion is defined as the net transport of molecules from a region of higher concentration to one of lower concentration by random molecular motion. Because diffusion only occurs from a higher to a lower concentration, the fish is forced to retain an equal amount of ammonia to that which is present in the water. For example, if the water has 1.0-ppm ammonia, the fish will have to retain 1.0-ppm ammonia. Therefore, the fish is only able to release that little bit of ammonia above and beyond what is present in the water. This will ultimately trap the fish in an ever-increasing ammonia level that will eventually reach critical levels.

Interest Approach:

The teacher should ask which students maintain aquariums in their homes, and whether they are freshwater or saltwater aquariums. Then a “show day” for students to bring pictures (developed into a PowerPoint) would highlight their aquaria, the plants and animals, and most importantly, what water quality monitoring do they do on their aquaria.

Student Materials:

1. Aquarium with undergravel filter
2. Airline tubing
3. Airstone
4. Aquarium gravel
5. Aquarium air pump
6. Aquarium test kit
7. Assorted laboratory glassware
8. Selected fish
9. Graph paper

Teacher Materials:

<i>Material</i>	<i>Store</i>	<i>Estimated Cost</i>
Aquarium air pump	Aquatic Ecosystems, WalMart, PetsMart	\$9 and up
Airline tubing	Aquatic Ecosystems, WalMart, PetsMart	\$1.20/ft. and up
Aquarium with undergravel filter	WalMart, Aquatic Ecosystems, PetsMart	WalMart kit-\$25 Larger tanks-\$100 and up
Airstone	Aquatic Ecosystems, WalMart, PetsMart	\$1 and up
Aquarium gravel (inoculum)	WalMart, PetsMart	\$3 and up
Aquarium test kit (ammonia, nitrite/nitrate)	Aquatic Ecosystems	\$180 and up
Selected fish	WalMart, PetsMart	NA
Assorted laboratory glassware	NA	NA
Graph paper or EXCEL access	NA	NA

Student Instructions:

1. Establish an aquarium (freshwater or marine) with an undergravel filter.
2. Inoculate the gravel with some “live gravel” from a pre-existing aquarium to establish *Nitrosomonas* and *Nitrobacter* bacteria.
3. Introduce 2 or 3 fish to the tank.
4. Record water quality parameters every 24 hours:
 - a. Temperature
 - b. pH
 - c. Ammonia
 - d. Nitrite
 - e. Nitrate
5. Graph your results.
6. Continue for at least two weeks.
7. Discuss the role of nitrifying bacteria on the progression from ammonification through nitrification.

Teacher Instructions:

Preparations:

1. The teacher will need to obtain sufficient water for the aquarium, equipment, and gravel (preferably from a running aquarium as an inoculum)

Activity:

1. The teacher should be monitoring the students to ensure proper use of the

test reagents.

Post work/Clean-up:

1. Wash glassware
2. Put away reagents and test kit

Anticipated Results:

1. Students will identify the major water quality parameters using the designated testing equipment.
2. Students will express the parameters using the proper units of measure.
3. Students will correlate the interactions of each parameter as they relate to each other.

Support Materials:

1. *Water Quality* notes (to be used for notes, reading, etc.)
2. *Nitrogen Cycle* diagram
3. “Water Quality in Ponds and tanks” – PowerPoint presentation – UF Tropical Aquaculture Laboratory
4. University of Florida online publications:
 - a. Ammonia (UF Fact Sheet FA-16)
5. Southern Regional Aquaculture Center online publications:
 - a. Ammonia in Fish Ponds (SRAC 463)
<http://srac.tamu.edu/fulllist.cfm>
 - b. Managing Ammonia in Fish Ponds (SRAC 4603)
<http://srac.tamu.edu/fulllist.cfm>
 - c. Nitrite in Fish Ponds (SRAC 462)
<http://srac.tamu.edu/fulllist.cfm>
6. Francis-Floyd, Ruth and Watson, Craig. 1996. *Ammonia*. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida
7. Noga, E.J. 1996. *Fish Disease Diagnosis and Treatment*, Mosby – Year Book, St. Louis, Mo.
8. Tomasso, J. XXXX. *Water Quality*. World Aquaculture Society, Baton Rouge, LA. XX pp.

Explanation of Concepts:

This activity introduces students to the concept of biological oxidation of metabolic products produced by fish or uneaten food in aquaria or tank systems. It should be coupled with background related to the toxicity of ammonia and nitrite/nitrates to marine organisms. (these are explained under the subheadings “ammonia” and “nitrite” in the *Water Quality* notes).



Support Materials



Water Quality Notes

Water quality is THE most important production component in aquaculture but often the most neglected. It is always the responsibility of the aquaculturist, whether a beginning hobbyist or a professional caretaker, to control all the critical water quality parameters.

The major water quality parameters that need to be controlled are as follows: OXYGEN, TEMPERATURE, AMMONIA, NITRITE, pH, ALKALINITY, HARDNESS, and SALINITY. While any of the above mentioned parameters, when far enough away from their accepted ranges, can certainly cause acute mortality, the real problem arises in long term fluctuations. Over time the stress experienced by the fish, which are constantly trying to acclimate to fluctuating parameters, can result in both increased disease problems as well as chronic losses.

The following explanations of the above mentioned parameters are designed to familiarize the reader with the definition of each water quality parameter as well as the interactions between each parameter and with the fish in general.

I. OXYGEN

- A. Amount of Dissolved Oxygen (DO) available to fish, is measured in milligrams per liter (mg/l) or parts per million (ppm) and is directly affected by water temperature, barometric pressure, and salinity
- B. Temperature increases the amount of DO decreases
- C. Salinity increases the amount of DO decreases
 - 1. Simply stated cold fresh water holds more DO than warm salty water
- D. An increase in the barometric pressure results in an increase of the DO
- E. Dissolved oxygen levels can range from 0.0 mg/l or no DO, to 15.0 mg/l or saturation and, at times, even super saturation.
- F. A simple scale can be used to represent DO in mg/l ranging from 0---- to----15 and can be broken down as follows (**Levels (mg/l) between**):
 - 1. **0 ---- 1** Results in fish KILLS
 - 2. **1 ---- 5** Maintains life with little or no growth or reproduction and low activity and poor coloration. The closer to 5mg/l the better.

3. **5 — 15** Optimal range while striving to maintain a constant 8.0 mg/l, which correlates with the DO levels suitable for the water temperature in most tropical fish ponds culture systems.
4. **15+** Depending on the circumstances, levels of super-saturation can become problematic resulting in “gas bubble disease” (oxygen bubbles in the blood – similar to “the bends” experienced by divers who ascend from depth too quickly). In extreme cases, it can result in mortalities.

G. There are basically two methods to introduce dissolved oxygen into water:

1. Mechanical aeration (diffusion)
 - a. The movement of a gas (oxygen) from an area of higher concentration to an area of lower concentration
 - b. Can be enhanced or accelerated with the use of mechanical aeration devices, air pumps, blowers, fountains, etc.
 - c. Can be made even more efficient by using air stones, diffusers, and fine spray nozzles
 - d. For the most part an air bubble rising from the bottom to the top of an aquarium or pond does not have enough contact time in the water column to transfer very much oxygen into the water.
 - e. However, the circulation caused by the rising bubbles brings oxygen rich surface water, where there is a large surface area and efficient oxygen diffusion, down to the bottom or other areas of low dissolved oxygen.
 - f. This circulation homogenizes, or uniformly mixes the water and maintains a constant DO level.
2. Photosynthesis
 - a. The natural production of oxygen as a byproduct of living plants using chlorophyll to convert the energy of sunlight or artificial lighting into carbohydrates
 - i. Plants turn light into sugar and give off oxygen.
 - b. When these plants are under water oxygen is released into the water column in the form of DO
 - c. Aquatic plants not only look good, they also generate oxygen while the lights are on
 - d. It is also important to remember that during times of darkness, plants consume oxygen through the process know as respiration, further enforcing the need for mechanical aeration and circulation
 - e. Another benefit of aquatic plants is that they act as living biological filters

- i. The act of biological filtration will be further discussed in the following sections.

II. TEMPERATURE

- A. Has a direct effect on the amount of dissolved oxygen that water can hold
- B. Also influences many other water quality parameters
 1. It is well known that you can dissolve many substances in hot water much easier than you can in cold water
 2. High temperature combined with high pH can make a deadly combination and will be discussed under the ammonia section (see Examples #1 and #2)
- C. The temperature of the water has a lot to do with a successful aquaculture venture
- D. Important to understand the origins of your fish and ultimately maintain the proper water temperature
 1. Fish are cold blooded, or have the same body temperature as their environment, they are directly influenced by the temperature of the water
 2. The metabolism of a fish slows down in cooler water and speeds up in warmer water
 3. It is important to keep a constant water temperature that does not fluctuate more than 5° F in a 24-hour period
 4. Depending on the species of fish being kept, a constant temperature should be maintained within the accepted ranges.
- E. It should also be mentioned that water temperatures affect the life cycles of many parasites

III. AMMONIA

- A. Produced when proteins are synthesized (Figure 1)
 1. Whenever proteins are broken down, whether by fish during digestion or bacterial action of uneaten food and fish waste, ammonia will be produced.
- B. Fish eliminate nearly all of their ammonia through their gills with a very small percentage excreted along with the fecal matter
- C. Transfer of ammonia from the fish to the water is done by diffusion
 1. Defined as the net transport of molecules from a region of higher concentration to one of lower concentration by random molecular motion
 2. Because it only occurs from a higher to a lower concentration, the fish is forced to retain an equal amount of ammonia to that which is present in the water
 - a. If the water has 1.0-ppm ammonia, the fish will have to retain 1.0-ppm ammonia
 - b. The fish is only able to release that little bit of ammonia above and beyond what is present in the water

- c. This will ultimately trap the fish in an ever increasing ammonia level which will eventually reach critical levels
- D. Ammonia, or the total ammonia nitrogen (TAN), is the combined sum of ionized ammonia (IA) and un-ionized ammonia (UIA), with the UIA being the toxic component
1. Here is where the above-mentioned temperature / pH combination comes into effect
 2. As the temperature and the pH increase, the ratio or amount of UIA also increases resulting in a more toxic solution
 3. It is always recommended to maintain the levels of both TAN and UIA at 0.0-ppm, with levels of UIA > 0.05 ppm resulting in gill damage
 4. Example #1- As you can see, both tanks have the same pH and total ammonia but differ by about 10°F. Because of the added temperature the water in aquarium T-2 contains nearly 1.5 times more toxic un-ionized ammonia than that of T-1.
 5. Example #2- As you can see, both aquariums have the same temperature and total ammonia and only differed by one unit of pH, yet aquarium P-2 has nearly 10 times as much toxic un-ionized ammonia as P-1.

Example #1

Aquarium	pH	(TAN)	Temperature	% (UIA)
T-1	8.0	1.0-ppm	22°C (about 72°F)	4.38
T-2	8.0	1.0-ppm	28°C (about 82°F)	6.54

Example #2

Aquarium	Temperature	(TAN)	pH	% (UIA)
P-1	28°C (about 82°F)	1.0-ppm	7.0	0.69
P-2	28°C (about 82°F)	1.0-ppm	8.0	6.54

IV. NITRITE

- A. Byproduct of certain bacteria which consume ammonia (*Nitrosomonas*)
- B. Directly toxic to fish because it binds to the fish's hemoglobin or red blood cells and blocks their ability to absorb and ultimately carry oxygen throughout the body
- C. Nitrite poisoning or toxicity is often referred to as brown blood disease and because of the blood's inability to transport oxygen is commonly mistaken for oxygen depletions
- D. Nitrite is once again broken down by yet another bacteria (*Nitrobacter*) and converted into nitrate
- E. Nitrate presents little, if any, problems in fresh water systems and can easily be removed from marine systems

- F. Salt or sodium chloride can also be used as a temporary solution for nitrite toxicity
- G. Ammonia and nitrite removal can effectively be achieved with one of two methods:
 - 1. Dilution
 - a. Removing some water, which is high in ammonia or nitrite, and replacing it with water, which does not contain any ammonia or nitrite
 - b. Achieved by simply performing water exchanges or employing a flow through system
 - i. Flow through refers to a system that allows high quality water to enter run through the system and then exit out
 - ii. In a flow through system the flow rate can be calibrated to help control the water quality
 - iii. These techniques can become very difficult as the size of the aquaculture venture increases
 - iv. It should be noted that great care should be taken when doing water exchanges so as not to increase any stress on the fish
 - 2. Biological filtration
 - a. Concentrates very large amounts of the above mentioned beneficial bacteria on selected inert substrates in order to increase the contact time with the water as it is pumped through the biological filter
 - b. Some examples of biological filters are sponge, undergravel, wet / dry, trickle, rotating bio-wheel, bead, and fluidized bed
 - c. Most biological filters take from 1 to 6 weeks and at times can take over 8 weeks to become established
 - i. During this time water exchanges and great patience should be used to keep ammonia and nitrite levels in check
 - d. As mentioned above, live plants can act as living biological filters

V. pH

- A. The negative logarithm of the effective hydrogen ion concentration represented by a number from 1 to 14
 - 1. Any pH less than 7 is considered acidic
 - 2. pH readings higher than 7 are considered basic
 - 3. A reading of 7 is said to be neutral.
- B. For general fish keeping purposes an acceptable pH range is from 6.5 to 9.5

- C. It is important to remember that when the pH increases so does the amount of toxic ammonia (UIA).
 - 1. Please refer to example #2 for the effects of pH on the toxicity of ammonia
- D. At pH levels of 6.5 or lower, the beneficial bacteria, which convert nitrite to nitrate becomes very inefficient and eventually quit all together

VI. ALKALINITY

- A. Best described as the buffering capacity of the water or the measurement of the dissolved carbonate ions
- B. Though very important for the stability of a recirculating system and for certain reproduction requirements, does not really affect most fresh water fish during normal fish keeping and should be maintained at levels above 100-ppm
- C. Alkalinity and pH are directly influenced by both respiration and nitrification
 - 1. Respiration refers to the act of taking in oxygen and exhaling carbon dioxide
 - a. Respiration takes place by all the aquatic organisms including fish, bacteria, and even plants
 - b. Plants respire mainly during the hours of darkness but minimal respiration does occur during the day
 - c. When carbon dioxide is released in solution it acts as an acid, actually forming carbonic acid
 - d. Both the free hydrogen ions and the carbonic acid then try to lower the pH to acidic levels less than 7
 - e. This is where the alkalinity or buffering capacity of the water is very important
 - f. Over time the alkalinity will not be able to buffer against the increasing acid levels and eventually the pH will plummet to levels of 4 resulting in acute fish losses
 - g. This whole process is known as "Old Tank Syndrome"
 - h. You can avoid the build up of carbon dioxide by simply degassing the water
 - i. Degassing refers to the act of spraying, splashing, or bubbling the water in order to allow the trapped carbon dioxide to diffuse or escape into the atmosphere
 - 2. Nitrification refers to the process by which bacteria breakdown ammonia into nitrite and then into nitrate
 - a. During the nitrification process, free hydrogen ions (H^+) are produced.
 - b. These hydrogen ions act as an acid and, therefore, decrease both the pH and the alkalinity

- c. Periodic water exchanges (25%) will control the production of free hydrogen ions and will insure a healthy nitrification process

VII. HARDNESS

- A. Hardness, or total hardness, primarily consists of the measurement of the dissolved calcium and magnesium ions found in water
- B. As with pH and alkalinity, total hardness has little effect on general fish keeping and should be maintained at levels above 20-ppm
- C. Water hardness is very species dependent
 - 1. Especially when reproduction is attempted, not only for the broodstock but also for proper egg and fry development

VIII. SALINITY

- A. Described as total concentration of all ions present in the water
- B. These are most commonly present in the form of chloride, sodium, sulfate, calcium, magnesium, potassium, bicarbonate
- C. Rock salt (sodium chloride) is commonly used in the fresh water aquaculture for the following beneficial reasons:
 - 1. Freshwater fish are always trying to maintain their internal electrolyte (salt) levels up, a little salt in the water helps with the fish's osmoregulatory system, therefore, lessening the amount of stress involved in that process
 - 2. Salt helps with the fish's slime coat or mucus production, which ultimately aids in the fish's immune system
 - 3. A certain amount of parasite control can be achieved when constant salinity levels are maintained
 - 4. Temporary relief of nitrite toxicity is achieved when salt or sodium chloride (NaCl) is dissolved into water and the chloride ion (Cl^-) binds to the fish's hemoglobin occupying the same receptors, which the nitrite would otherwise bind, effectively blocking the negative aspects of nitrite poisoning
 - a. This is, by no means, an answer to nitrite toxicity but can, and often does, help the aquaculturist avoid a potentially dangerous situation

Table 1: Water Quality Parameters (Fresh/warm water species)

PARAMETER	GOOD	BAD
Dissolved Oxygen (DO)	5 – 15 ppm	< 4 or >25-ppm
Temperature (°F)	72 – 85 °F	
Total Ammonia (TAN)	0.0-ppm	> 2-ppm @ pH > 8
Un-Ionized Ammonia (UIA)	0.0-ppm	> 0.05 ppm Gill Damage
Nitrites (NO ₂)	0.0-ppm	>2-ppm @ Cl ⁻ < 20-ppm
pH	6.5 to 9.5	
Alkalinity (ALK)	> 100-ppm	< 20-ppm
Total Hardness (HRD)	> 20-ppm	0.0-ppm
Chloride (Cl ⁻)	100-ppm	< 20-ppm
Salinity (S ppt-Salinity measured in parts per thousand)	1 to 3-ppt	

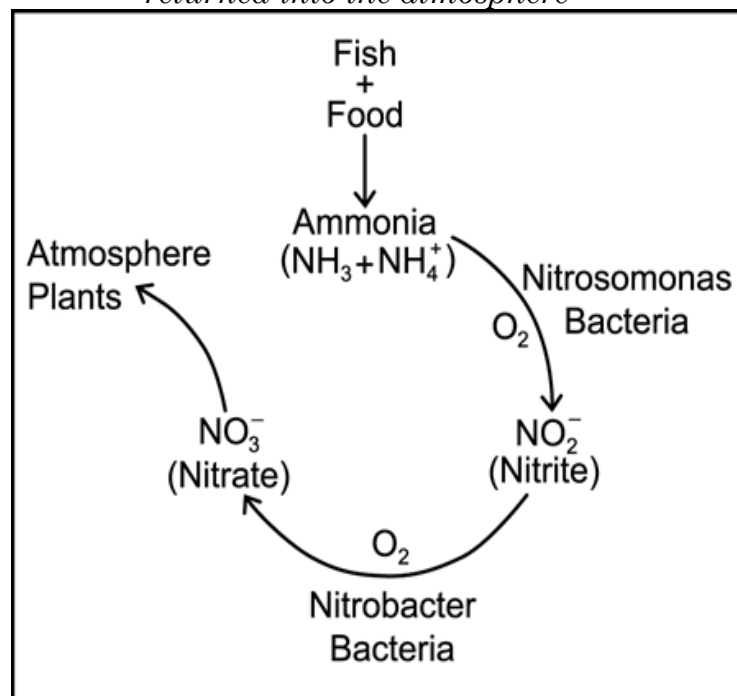
In order to make a 1-ppt solution, add 1 gram of salt per liter of water.

1-ppt = 1 gm/l or 1-ppt = 3.785 grams / gallon

***** PLEASE NOTE *****

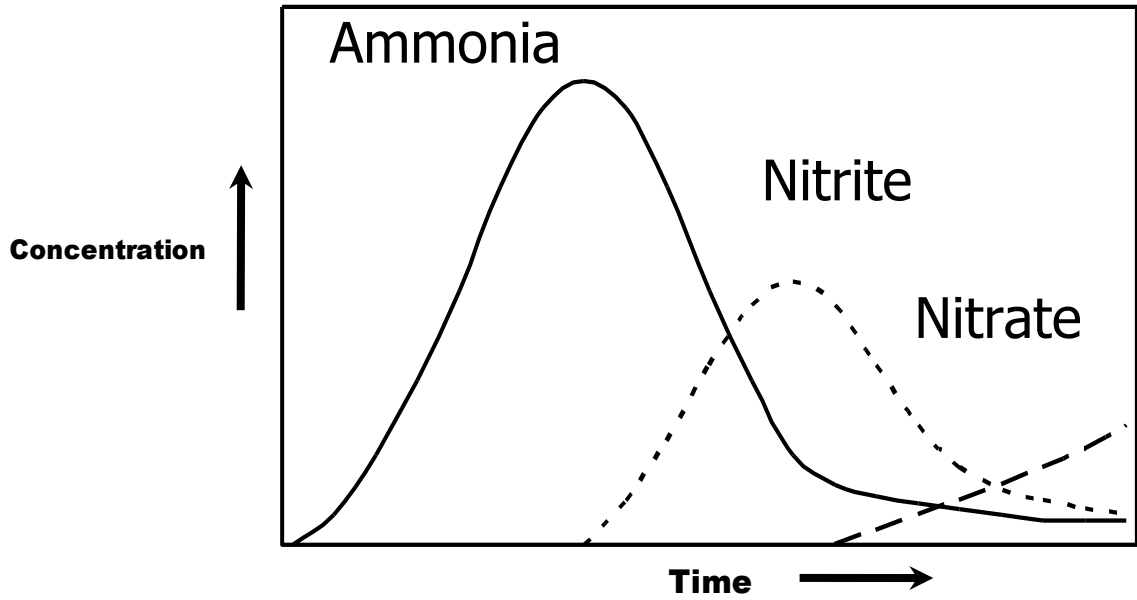
THE ABOVE WATER QUALITY PARAMETERS AND ACCOMPANYING ACCEPTABLE RANGES ARE CONSIDERED A GENERAL GUIDELINE THAT WILL NOT CORRELATE TO ALL SPECIES OF FISH.

Figure 1: Nitrifying bacteria utilize oxygen to convert ammonia and nitrite into the nontoxic by-product, nitrate (NO₃), which is used by plants as food or simply returned into the atmosphere



Nitrogen Cycle
Diagram

Nitrogen Cycle



Oxidation of Nitrogen Over Time