WATER QUALITY FOR FISH

(Adapted from North American 2006 Veterinary Conference Proceedings)

Fish health is profoundly affected by the environment in which fish live. Any deviation outside of an acceptable range may result in disease and even mortality; poor water quality is the most common cause of illness in fish. The astute veterinarian will make a basic water quality analysis part of every sick fish examination.

WATER SAMPLE COLLECTION

When requesting a water sample, explain to the client that the water from a bag of submitted fish is not an acceptable sample. At least 500 mL of water from the tank or pond, and a separate 500 mL sample of the source water (the water used to fill the tank or pond), should be submitted. Both samples should be collected in a manner to prevent air being trapped in the sample. This may require submerging and capping the clean sample container below the water surface. If the water samples cannot be directly analyzed, they should be immediately placed in a refrigerator for temporary storage. If the water samples must be shipped to a clinic or lab for analysis, the samples should be placed on ice in a cooler or styrofoam fish box and shipped for next day arrival.

Cooled water samples should be allowed to reach room temperature before analysis. Dissolved oxygen should be measured immediately after opening the container; the other parameters may then be measured.

TEST KITS

A variety of test kits are available, some as test strips, some use wet reagents and others dry reagents. While the test strips are handy for quick checks, they are not as reliable as those kits which use dry and wet reagents. Before purchasing a kit, make sure it contains reagents that have an expiry date. Some test kit manufacturers offer bulk reagents which is more affordable than buying an entire kit every time reagent supplies become depleted or go out of date.

WATER QUALITY PARAMETERS

While it is easy to focus on one parameter that is out of range, the thorough clinician will measure all parameters listed below, as each parameter is a piece of the whole. Some parameters, such as pH and temperature, have an important influence on others. To only measure one or a few of the parameters may result in a false impression and inadequate advice to the fish owner.

Table 1 lists typical water quality parameters and acceptable ranges for warmwater freshwater and marine fishes.

Table 1.

	Acceptable Range		
Parameter	**Warmwater Freshwater Fish**	**Warmwater Marine Fish**	**Comments**
Dissolved Oxygen	≥ 5 mg/L	≥ 5 mg/L	
Carbon Dioxide	< 20 mg/L	< 20 mg/L	
pH	6.0-9.0	7.8-8.4	Some fish require specific pH levels.
Alkalinity	≥ 100 mg/L	> 175 mg/L	
Ammonia, unionized (NH_3)	< 0.05 mg/L	< 0.05 mg/L	
Nitrite (NO_2^-)	< 0.10 mg/L	< 0.10 mg/L	Centrarchids are tolerant of higher levels.
Nitrate (NO_3^-)	< 100 mg/L	< 100 mg/L	Some marine invertebrates may be less tolerant
Hardness	50-200 mg/L	NA	High hardness can affect hatchability of the eggs of fishes such as tetras.
Chlorine, free or total	0	NA	
Temperature	22-28°C	22-28°C	

DISSOLVED OXYGEN (DO)

Dissolved oxygen is the most important limiting factor for in ponds.1 Mortalities due to low DO are quite common in the summer. A low DO event may be triggered by several events. One, water temperature affects oxygen solubility in water; so as the temperature increases, oxygen solubility decreases. Two, phytoplankton blooms are common in ponds in the summer. These phytoplankton blooms, while producing oxygen during the day, consume oxygen after sunset. If a pond is heavily stocked, the DO level may be too low in the pre-dawn hours to support the fish. Typically, DO fish kills due to heavy phytoplankton blooms are discovered by the pond owner in the morning. Three, summer rainstorms may precipitate a DO crash, either due to phytoplankton loss (cloud cover) or a phenomenon called “turnover”.

Water in ponds tends to stratify during the summer, forming thermoclines. Turnover occurs when heavy rainfall mixes the thermoclines within a body of water. The lower cool anoxic water mixes with the upper warm layer of water, dramatically decreasing the DO.

There are several means to manage low DO in ponds: supply supplemental aeration in the form of a fountain, paddlewheel aerator or other aeration device especially at night, flush the pond with well water that is directed into the air before the water hits the pond surface, reduce feeding, and reduce stocking density if pond is overstocked.

In recirculating systems or tanks, DO levels are rarely below the minimum level unless the life support system is not running (power failures, maintenance, etc.) or the system is overstocked. However, treatment chemicals containing formalin are frequently used in such systems, and formalin has a negative effect on the oxygen level. For each 5 mg/L of formalin, 1 mg/L of oxygen is removed.

The nitrifying bacteria that live in the biological filter are aerobic; as such they are also adversely affected by DO less than 2 mg/L.

CARBON DIOXIDE (CO_2)

Just as in terrestrial animals, excessive carbon dioxide can impact the erythrocyte’s ability to deliver oxygen to the cell. Low DO exacerbates this effect. Carbon dioxide is more soluble in water than oxygen, however increasing water temperature decreases CO2 solubility.[2]

Typically carbon dioxide is high in well water, and if not gassed off before entering a system, the carbon dioxide in well water can affect oxygen delivery and also decrease water pH.

In recirculating systems poorly designed for gas exchange, the carbon dioxide from respiration can accumulate to the point that toxicity occurs. A packed column (or degassing column) is an efficient and low cost method of providing gas exchange.

In ponds, the pH may vary as much as 2 units or more during the diurnal cycle due to the varying carbon dioxide levels produced by both fish and plant respiration. Phytoplankton is a significant contributor to overall carbon dioxide level.

High CO2 commonly occurs during transport of fishes. Fishes transported in bags are subjected to high CO2 level especially if packed in high density. Likewise, hauling tanks, if tightly closed, can prevent gas off of CO2 even if the water is vigorously aerated with oxygen.

pH

pH is the logarithmic measure of hydrogen ions, in other words, the measure of acidity. Carbon dioxide is the most important influence on pH changes due to the production of carbonic acid when carbon dioxide enters water via fish and aquatic plant respiration.

Fish should be maintained within their optimum range. A low pH affects ion exchange, and below optimum pH can result in osmoregulatory failure. Low pH can also result in solubilization of heavy metal ions, if a source is present in the water. Fishes should be maintained within their optimum range. It is also important to note that the nitrifying bacteria also have an optimum range (6.5-9), and do not function below pH 6.5.[3]

ALKALINITY

Alkalinity is the measure of the buffering capacity or acid neutralizing capacity. In fish culture waters, alkalinity consists primarily of bicarbonate, carbonate, and hydroxide ions. High alkalinity buffers high pH swings due to phytoplankton respiration in ponds. Alkalinity is also used to determine productivity in ponds (the ability to support aquatic life). The nitrifying bacteria that reside in biological filters consume bicarbonate, at the rate of 7.14 g of alkalinity for each gram of TAN oxidized.[4] Alkalinity must be determined before using copper as a treatment in freshwater systems.

TOTAL AMMONIA NITROGEN (TAN)

Ammonia is the most important limiting factor for fish in kept in tanks and recirculating systems.[5] The primary source of ammonia is the protein metabolism of the fish. Ammonia is the primary waste product, and it is passively excreted across the gills.[6] A lesser amount is excreted in feces and urine. Uneaten food and decomposing organic matter are also sources of ammonia.

Total ammonia nitrogen consists of two forms, ionized ammonia (NH4+) and unionized ammonia (NH3). Unionized ammonia (UIA) is 100 times more toxic than ionized ammonia. At a pH of 7.0, most of the TAN is present as ionized ammonia. As pH and temperature increase, the percentage of TAN that is unionized ammonia increases. Since ammonia toxicity is primarily associated with UIA, the veterinarian must know pH and temperature of the water or pond to accurately determine how much TAN is unionized ammonia.

Example: A submitted water sample has a TAN of 0.5 mg/L. The client states that the pH is 8.2 and the temperature is 82oF. Using the pH and temperature, locate the percentage factor in Table 1 (0.0998). Multiply the percentage factor by the TAN to find how much UIA is present; 0.0998 x 0.5 = 0.0499. In this example, the UIA is at a dangerous level.

A UIA of 0.05 mg/L causes gill damage and reduced growth, and as UIA increases, mortality may result.

Total ammonia nitrogen may be measured by two methods, Ammonia Salicylate and Nessler's. Seawater may be analyzed using the Nessler's method by adding of 1.0 mL of mineral stabilizer to the sample before analysis. The mineral stabilizer complexes the high magnesium concentrations found in sea water, but the sensitivity of the test is reduced by 30 percent due to the high chloride concentration. The Ammonia Salicylate method is more reliable for marine samples.

Water treated with formalin or one of the ammonia locking compounds (e.g. AmQuel, AmmoLock, etc.) will have a falsely elevated TAN level when using Nessler's method. This reaction does not occur if the Ammonia Salicylate method is used.

It is important to note the Nessler's reagent contains mercury, and water samples treated with Nessler's reagent must be disposed as a hazardous material.

Table 1. Fraction of unionized ammonia in aqueous solution at different pH values and temperatures.

	Temperature														
	42.0	**46.4**	**50.0**	**53.6**	**57.2**	**60.8**	**64.4**	**68.0**	**71.6**	**75.2**	**78.8**	**82.4**	**86.0**	**89.6**	**(F°)**
pH	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**(C°)**
7.0	.0013	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080	.0093	
7.2	.0021	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.0126	.0150	
7.4	.0034	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198	.0236	
7.6	.0053	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0236	.0271	.0310	.0369	
7.8	.0084	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0423	.0482	.0572	
8.0	.0133	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0654	.0743	.0877	
8.2	.0210	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0880	.0998	.1129	.1322	
8.4	.0328	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1495	.1678	.1948	
8.6	.0510	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422	.2768	
8.8	.0785	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362	.3776	
9.0	.1190	.1368	.1565	.1782	.2018	.2273	.2546	.2836	.3140	.3456	.3783	.4116	.4453	.4902	
9.2	.1763	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599	.6038	
9.4	.2533	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685	.7072	

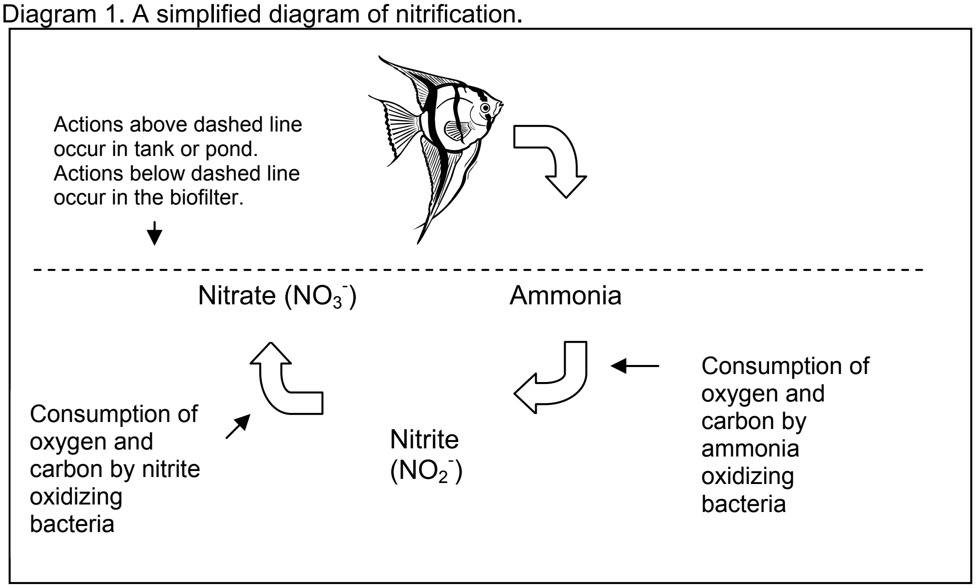
NITRITE

Nitrite is a byproduct of ammonia oxidation by the nitrifying bacteria. (See diagram 1 for an explanation of the nitrogen cycle.) It is colorless and odorless, and can result in signs of toxicity as low as 0.10 mg/L. Nitrite oxidizes the iron in hemoglobin, and the oxidized hemoglobin (methemoglobinemia or brown blood disease) is unable to bind oxygen and deliver it to cells. Consequently fish suffering from nitrite toxicity will exhibit clinical signs of hypoxia.

Channel catfish (Ictalurus punctatus) are especially sensitive to nitrite. On the other hand, the centrarchid family (bluegill, largemouth bass, etc.) are tolerant of nitrite levels that would be fatal to non-centrarchids.

The uptake of nitrite at the gill can be blocked by addition of chloride, either as sodium chloride or calcium chloride, at the rate of 10 mg/L of chloride for each 1 mg/L of nitrite ion.

Diagram 1. A simplified diagram of nitrification.



NITRATE

Nitrate is the byproduct of nitrite oxidation by nitrifying bacteria. Older literature states that nitrate is non-toxic. More recent work indicates that nitrate may alter hematologic factors in striped bass at levels as low as 200 mg/L. Nitrate is utilized by plants, and high levels may result in algae blooms. The easiest way to reduce nitrate is to do frequent, large water changes.

HARDNESS

Hardness is the measure of the divalent cations, primarily calcium and magnesium. Freshwater fish constantly lose electrolytes to the water column, and it is easy for them to take up calcium and magnesium as needed for osmoregulation. Hard water can have an adverse effect on hatchability of some fish eggs, especially the fish that originate from soft water, i.e. tetras. Water hardness greater than 150 mg/L minimizes toxicity of heavy metals.

The Environmental Protection Agency developed a guideline for hardness of natural water:

- Soft 0-75 mg/L
- Moderate 75-150 mg/L
- Hard 150-300 mg/L
- Very hard ≥300 mg/L

CHLORINE

Chlorine and chloramine are widely used by public municipal water suppliers to deliver safe drinking water to consumers. Typically chlorine arrives at the consumer's tap at 1.5-2.0 mg/L. However, chlorine at 0.05 mg/L can be toxic to many fish species. Chlorine is easily neutralized with sodium thiosulfate at 7.6 mg/L for each mg/L of chlorine. Activated carbon will also remove chlorine, but it requires frequent replacement. The practice of collecting the water for dissipation of chlorine is not reliable, as 20 hours is required for each 1 mg/L of chlorine.

Chloramine, a combination of chlorine and ammonia is also toxic to fish. To eliminate chlorine, the bond between chlorine and ammonia must first be broken, and then each removed separately. There are products available, sodium hydroxymethanesulfonate and other similar products, which neutralize chloramine.

TEMPERATURE

Fish are ectothermic, so temperatures outside their optimal range have deleterious effects. Increases in temperature result in an increase in metabolism, thus an increased demand for oxygen. However, oxygen solubility decreases as temperature increases. Ammonia toxicity is increased by rising temperatures. High temperatures also promote more rapid growth and reproduction of parasites and bacteria. At the other extreme, low temperatures slow metabolism and depress immune response, and digestion is impaired.[7] Temperature changes should be gradual, ideally no more than 2oC per hour.

OLD TANK SYNDROME

After the novelty of keeping fish has worn off, it's not unusual for many tank owners to decrease the frequency of performing routine maintenance chores such as detritus removal and water changes. Some even resort to just topping of the tank as the water level drops due to evaporation. It's not uncommon to have clients relate stories of how doing a water change sickened or killed their fish, or the difficulty they have in maintaining the optimal pH in an older aquarium. Since most tank owners tend to focus on pH more so than other water quality parameters, they can miss important pieces of information crucial to creating a fish friendly environment. So what is really going on?

The nitrogen cycle is defined as the process by which nitrifying bacteria oxidize ammonia and nitrite to nitrate; this process primarily takes place in the biological filter (biofilter). It is an acidic process, as bicarbonate is consumed by the nitrifying bacteria resulting in decreased alkalinity, and organic acids such as nitric acid are produced in addition to nitrate. Consequently pH slowly declines over time if no water is removed from the aquaria and alkalinity is not replaced.

If tank owners are topping off tanks instead of removing old water and adding new water, other parameters are affected. In addition to high nitrate and low alkalinity, hardness is usually increased since the divalent cations do not evaporate. Hardness is a useful test to determine if a tank owner is actually performing water changes.

The nitrate accumulation, acid production and alkalinity depletion occurs over weeks, and even months if the tank is lightly stocked. Eventually this scenario may develop:

- pH 5.0
- Total Ammonia Nitrogen (TAN) 2.0 mg/L
- Nitrate (NO3-) >400.0 mg/L
- Alkalinity 0.0 mg/L
- Hardness 1000.0 mg/L

So why are the fish alive when the pH is so low and the TAN is high? It probably took months for the pH to reach 5.0, and the fish were to adapt. The TAN is elevated because the nitrifying bacteria in the biofilter function poorly or not at all at pH ≤6.5. The low pH means that no unionized ammonia, the most toxic form of ammonia, is present. The hardness and the nitrate levels are indicative of little or no water change. The alkalinity is depleted due to the function of the biofilter. If the tank owner did not measure these parameters before doing a water change, the resident fish could become ill or even die after the water change is performed. Methods to manage this scenario so that the fish are not harmed include doing small (~10%) water changes daily and adding an ammonia locking compound such as sodium hydroxymethanesulfonate until normal parameters are measured. Frequent, large water changes will prevent this scenario from happening again.

NITRATE TOXICITY?

Older literature states that nitrate is not toxic to fish except at very high levels, 1000-3000 mg/L (96 hour LC_{50}), though eggs and fry are much more sensitive. However, growth of channel catfish is affected when nitrate is >200 mg/L.[8] High nitrate levels affect the oxidation of nitrite to nitrate in the biofilter, so nitrite, which is much more toxic, may become a problem. Nitrate is used by plants, and if no plants are present in the tank, algae blooms frequently arise.[9]

One study of hybrid striped bass exposed to elevated nitrate (200 mg/L) documented hematological and immunological changes, but was unable to prove conclusively that the nitrate was responsible.[10]

THE SOLUTION TO POLLUTION IS DILUTION

There are no good general recommendations for how often or how much water should be changed as stocking density, quality of replacement water and feedstuffs vary from tank to tank. Usually the more water changed and the more often, the better the resulting environment is for the fish. If the pH and the temperature of the replacement water are the same as that of the tank water, ≥50% may be changed with no ill effects on the fish. By measuring nitrate levels routinely a recommendation can be developed for a specific tank. The website below has a water change calculator that may be useful:

http://www.practicalfishkeeping.co.uk/pfk/pages/water_change_calculator.php

REFERENCES:

1. Neill WH, Bryan JD, Responses of fish to temperature and oxygen, and response integration through metabolic scope, in Brune DE, Tomasso JR (eds.) Aquaculture and Water Quality, 1991; 30-57.
2. Noga EJ, Fish Disease: Diagnosis and Treatment, 2000; 224-225.
3. Hochheimer JN, Wheaton FW, Understanding biofilters – practical microbiology for ammonia removal in aquaculture, in Engineering Aspects of Intensive Aquaculture, 1991; 57-79.
4. Yanong, RPE, Fish health management considerations in recirculating aquaculture systems - Part 1: introduction and general principles, http://edis.ifas.ufl.edu/pdffiles/FA/FA09900.pdf, 2003; 1-9.
5. Wedemeyer GA, Interactions with water quality conditions, in Physiology of Fish in Intensive Culture Systems, 1996; 61-90.
6. Witters HE, Disorders associated with environmental pH in Woo PTK (ed.), Fish Diseases and Disorders; 1998; 187-206.
7. Cecil TR, Environmental disorders, in BSAMA Manual of Ornamental Fish 2001; 205-11.
8. Wedemeyer GA, Physiology of fish in intensive culture systems. 1996, 80-82.
9. Cecil TR, Environmental disorders, in VSAMA Manual of Ornamental Fish 2001; 205-11.
10. Hrubec TC, Smith, SA, Robertson JL, Nitrate toxicity: a potential problem of recirculating systems. Successes and Failures in Commercial Recirculating Aquaculture Volume 1 1996; 41-48.

FOR MORE INFORMATION:

Yanong, RPE, Fish health management considerations in recirculating aquaculture systems - Part 1: introduction and general principles, http://edis.ifas.ufl.edu/pdffiles/FA/FA09900.pdf

SRAC 4601 Measuring Dissolved Oxygen Concentration in Aquaculture
http://srac.tamu.edu/getfile.cfm?pubid=167

SRAC 4603 Managing Ammonia in Fish Ponds http://srac.tamu.edu/getfile.cfm?pubid=169

SRAC 461 Water Quantity and Quality Requirements for Channel Catfish Hatcheries
http://srac.tamu.edu/getfile.cfm?pubid=109

SRAC 462 Nitrite in Fish Ponds http://srac.tamu.edu/getfile.cfm?pubid=110

SRAC 463 Ammonia in Fish Ponds http://srac.tamu.edu/getfile.cfm?pubid=111

SRAC 464 Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds
http://srac.tamu.edu/getfile.cfm?pubid=112

SRAC 468 Carbon Dioxide in Fish Ponds http://srac.tamu.edu/getfile.cfm?pubid=115

COMMON PARASITES OF FISHES

The most common fish parasites are ectoparasites, which mean they're found on external tissues such as skin, fin and gill. The best way to diagnose these parasites is to perform skin scraping, gill and fin biopsies, and then view the tissues as wet mounts under a light microscope. Most of the ectoparasites are easily viewed with a 4X objective. No special stains are required.

Questions to ask if a parasite is observed are:

- Is it a simple (single celled) or complex (many celled) organism?
- Where is it located on or in the fish?
- Is its life cycle direct or indirect?

These questions are important as the answers help to identify the parasite and choose the appropriate treatment.

Parasites can be divided into two major categories: protozoans (single celled organisms) and metazoans (many celled organisms).

Protozoans include ciliates, flagellates, microsporidians, and sporozoans. Of these, the **ciliates** are the most common ectoparasites of fish. Examples of ciliates are *Ichthyopthirius multifiliis, Cryptocaryon irritans, Trichodina, Chilodonella, Brooklynella, Tetrahymena, and Uronema.*

Ichthyophthirius multifiliis (Ich) in freshwater fishes and *Cryptocaryon irritans* (SW Ich) in marine fishes are just a few of the ectoparasites that can be seen with the naked eye. Both of these parasites have a complex life cycle and are obligate fish parasites. They are typically found on external tissues such as skin, fin and gills. Their life cycles are similar: the mature stage (trophonts) burrows under the skin of the fish, which makes it impossible to treat as it is protected by the fish's skin. While in the fish, the trophonts greatly enlarges, causing severe damage to fish tissues. After maturing, the trophonts emerge from the fish to become tomonts and swim to the bottom or tank decorations where each tomont produces a cyst wall that makes it impervious to treatment. While in the cyst, the tomont divides into as many as one thousand tomites. After development, the cyst wall ruptures, releasing theronts. The theronts swim in an erratic corkscrew manner. If they bump into a fish, they immediately begin to burrow below the fish's skin. If they do not find a host in 12 hours, they die.

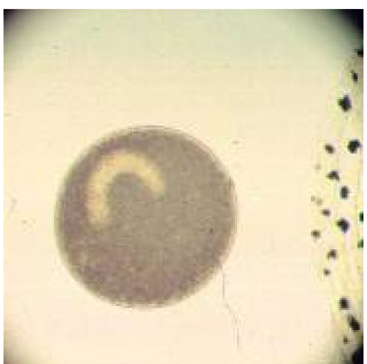
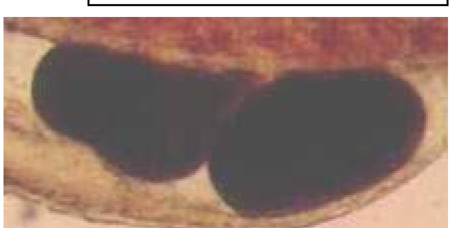
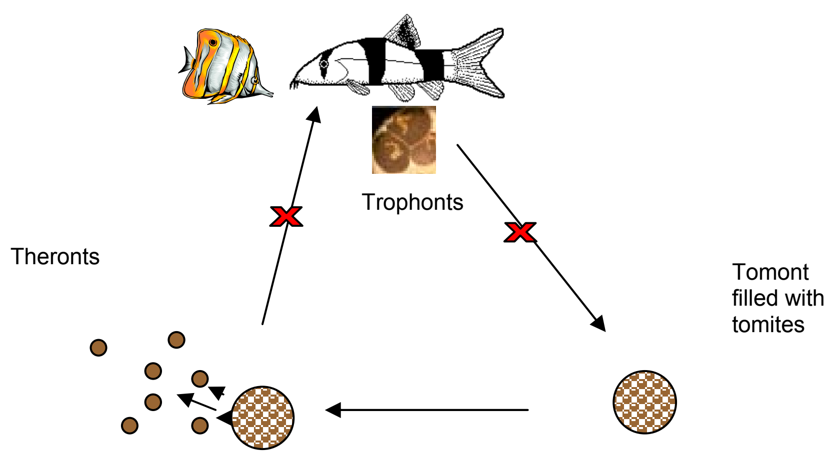


Photo 1. *Ichthyophthirius*



Two trophonts of *Cryptocaryon irritans.* Note absence of macronucleus as compared to Ich.

Life cycle of *Ichthyophthirius* and *Cryptocaryon*



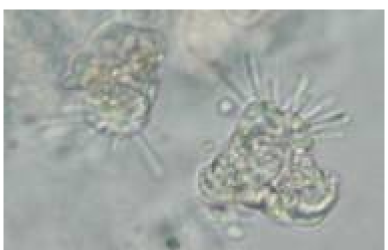
With such a complicated life cycle and with stages that are resistant to treatment, multiple treatments (usually every other day) are required to treat cases of freshwater and marine ich. Only the theronts and free swimming tomont stages are susceptible to treatment.

Treatments for freshwater and marine ich are formalin, the combination of malachite green (not for use in food fish!) and formalin, and copper. Hyposalinity is used frequently in conjunction with formalin or copper to kill *C. irritans*. Salt is sometimes recommended as a treatment for freshwater ich. However the salt concentration required to kill *I. multifiliis* is 10 gm/L, and most freshwater fish will not survive that high a salinity. Increasing water temperature is another recommendation, but that has disadvantages also. The optimum temperature range for infection with *I. multifiliis* is 18-24°C (64.4-75°F), though it can survive water temperatures as low as 2°C (35.6°F) and as high as 28°C (82.4°F). *I. multifiliis* will not survive after 5 days of water temperatures >29°C (84.2°F), but some warmwater fishes cannot tolerate such temperatures. Oxygen levels are decreased at those temperatures, and if ich infected fishes have severely damaged gills, they will die from oxygen deprivation. Bacterial activity is also increased by high water temperatures, so fishes with tissue damage are more likely to succumb to bacterial infections.

A confounding factor is that survivors of outbreaks develop a temporary immunity to subsequent infection, yet they can harbor low numbers of trophonts without showing clinical signs. Such fish may serve as vectors of infection to naïve fishes.

All of the other ciliated protozoans such as *Trichodina, Chilodonella, Brooklynella, Tetrahymena, and Uronema,* which can result in fish disease have a direct life cycle which makes them easier to control than *Ichthyophthirius* and *Cryptocaryon*.

There is a group of ciliated protozoans which are sessile ectocommensals, and do not directly cause disease in fish. Some are solitary such as *Ambiphrya, Capriniana* and *Apiosoma.* Others are colonial and include *Epistylis, Vorticella,* and *Carchesium* (and others)*.* The latter three are stalked. When any of these ectocommensals are observed on skin, fin or gills of fish, this is a strong indication that the environment is rich in organic material and consequently supports a large bacterial population. These protozoans do not feed on the fish, but instead feed on bacteria and other small organisms. While they do not directly feed on the fish, their attachment to the fish's skin can result in wounds which can become infected. Treatment of the ectocommensals must focus on managing the environment by reducing organic material, and doing frequent water changes. This will reduce the bacterial population which will have an adverse effect on the ectocommensals.



Two *Capriniana* from a skin scraping, 400X

Flagellates

Another major group of protozoans are the flagellated protozoans, or, flagellates. This group includes, *Piscinoodinium, Amyloodinium, Ichthyobodo necator,* the hexamitids, and *Cryptobia.*

Piscinoodinium (FW) and *Amyloodinium* (SW) are dinoflagellates, and are obligate fish parasites. They have a complex life cycle somewhat similar to that of Ich.

Trophont (on fish) → tomont → dinospores → trophonts

They are primarily found on gills and less commonly on skin.

Ichthyobodo necator is a flagellated protozoan, and can be found on both freshwater and marine fishes. There may be other species that are as yet unidentified. It is an obligate parasite, and is found on skin and gill. There are two forms, motile and attached. While it is small in size (about the size of a fish erythrocyte), it can be deadly if in heavy numbers.

The hexamitids are flagellated protozoans usually found in the lower intestine. Examples of species are Hexamita and *Spironucleus.* Low numbers do not cause significant disease, but high numbers can significantly impact the health of the fish. Some fish species seem to be more susceptible to infection with hexamitids. Freshwater angelfish, discus, other cichlids,

The *Cryptobia* spp. are also flagellated protozoans. Two species of importance to fisheries are *Cryptobia branchialis* and *C. iubilans. C. branchialis* is primarily found on the gills, and sometimes skin.

It is associated with high organics. On the other hand, *C. iubilans* is found in the stomach, and occasionally upper intestine. East African cichlids appear to be susceptible to infection with *C. iubilans.* Heavy infestations with *C. iubilans* can result in granuloma formation in the stomach that interferes with digestion. Consequently infested fish may appear to be thin.

Metazoans

The metazoans contain many parasitic species. This large group includes the Platyhelminthes (flatworms), Nematoda (round worms), Acanthocephalans (thorny headed worms) pentastomids and parasitic crustaceans. Members of these groups can frequently be found in or on fishes, but the most important group economically is the Platyhelminthes. This group contains four major classes: Turbellaria, Monogenea, Trematoda, and Cestoidea (tapeworms). Of these four classes Monogenea and Trematoda are the most important to fishes.

All monogeneans have a direct life cycle. More than 1100 species of Monogenea use fishes as hosts.

Within **Monogenea** the family Gyrodactylidae and the suborder Dactylogyrida are important parasites of fishes. Both of these groups have a variety of large and small hooks at one end that allows them to cling to their host animal. Distinguishing characteristics of members of the family Gyrodactylidae are absence of eye spots and viviparity. In contrast Dactylogyrida do have eye spots and are oviparous. This is important for both identification purposes and for treatment. For example, since gyrodactylids give birth to live young, one treatment using an effective chemical will eliminate both adults and their embryos. In contrast, multiple treatments will be required to first kill adult dactylogyrids and immature dactylogyrids that have emerged from the eggs. The eggs are impervious to treatment.

Gyrodactylids prefer to attach to skin, and dactylogyrids prefer gill; however in heavy infestations these groups of monogeneans may be found on any external tissue.

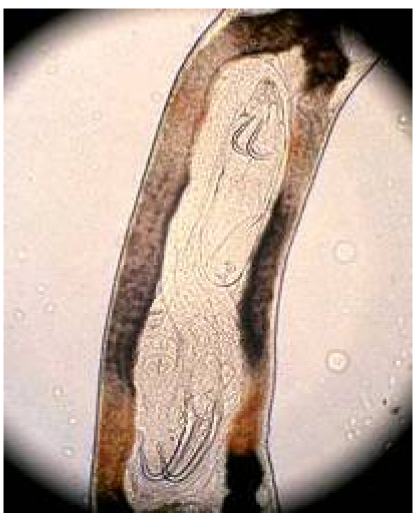


Photo at left demonstrates two embryos, one inside the other, inside an adult gyrodactylid.

Another monogenean family is the Capsalidae. Members of this group are limited to marine fishes. Some are large enough to be seen with the unaided eye. All are oviparous.

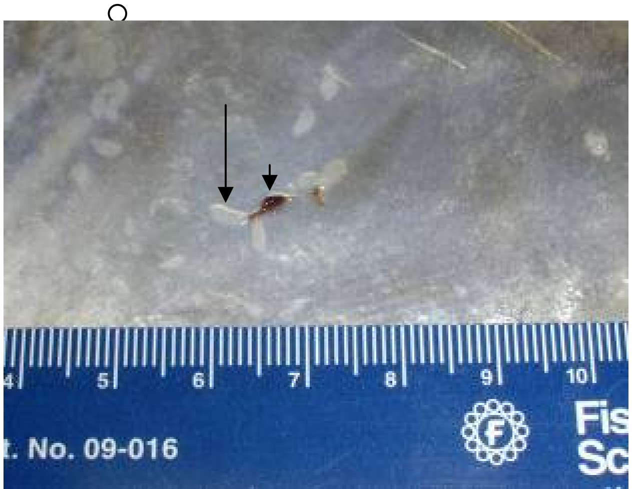
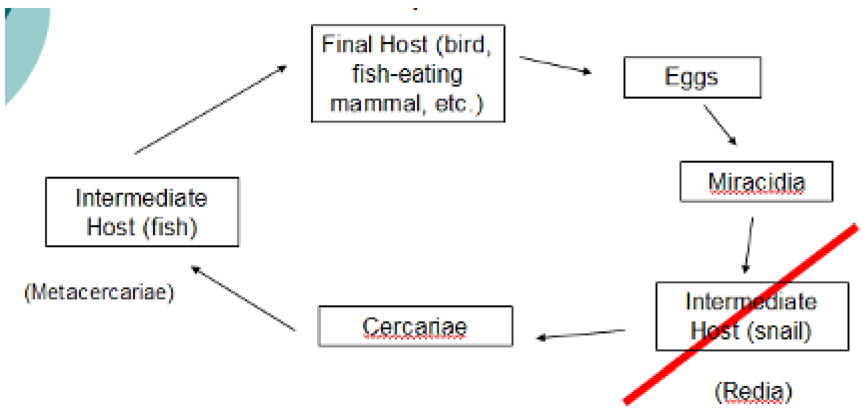


Photo at left of numerous capsalids on the skin of a lookdown. The long arrow points to the body of an adult capsalid. The short arrow points to a mass of capsalid eggs.

Another important group of flatworms is **Digenea**, the true trematodes. There are 11,000+ species in this class, many of which use fish as either intermediate or final hosts. Digeneans have an indirect life cycle.

The diagram below illustrates typical life cycle for many digeneans in which fish serve as intermediate hosts.



There is no effective treatment for the metacercarial stage of digeneans at this time. Consequently efforts are focused on two stages of the life cycle: One, elimination of aquatic snails from the environment as they frequently serve as an intermediate hosts, and two, preventing aquatic birds from gaining access to ponds or outside aquaria.

COMMON BACTERIAL DISEASES OF FISH

(Adapted from 2005 Florida Veterinary Medical Association Conference Proceedings)

Columnaris disease, septicemias caused by Aeromonas spp. or Vibrio spp. and mycobacteriosis are bacterial infections frequently encountered in aquatic animal medicine. Clinical signs of bacterial disease may include hemorrhagic areas in the fins, mouth, or body, ulcerations of the skin, ascites, and exophthalmia.

FLAVOBACTERIUM COLUMNARE (COLUMNARIS DISEASE)

Members of Flavobacteriaceae that are fish pathogens are gram negative rod shaped motile bacteria that grow best on low nutrient agar such as Ordal's. All are saprophytes found in freshwater and marine water.

- *Flavobacterium columnare (*formerly *Flexibacter columnaris)*- skin disease in warmwater fishes in freshwater; sometimes causes systemic disease especially in channel catfish
- *Tenacibaculum maritimum (*formerly *Flexibacter maritimus)* – skin disease in warmwater marine fishes, bacterial stomatitis in Atlantic salmon smolts in net pens
- *Flavobacterium branchiophilum* (formerly *Flavobacterium branchiophilus*) -bacterial gill disease and may be associated with fin rot
- *Flavobacterium psychrophilum (*formerly *Flexibacter psychrophilus*) cold water disease in salmonids

Fish infected with *F. columnare* typically have a whitish or creamy exudate on the dorsal body, mouth or tail. It is primarily found on skin and gills. This bacterial disease can be easily diagnosed by examining wet mount scrapings of infected skin and fins, or infected gill biopsies. In heavily infected fish, these bacteria form "haystacks" which are visible at 100-400X.

Some fishes seem to be more susceptible to *F. columnare* than others, e.g., mollies, guppies, and swordtails. Columnaris infections can rapidly proliferate, resulting in mortality within 24-48 hours of appearance of clinical signs. Mild cases respond well to decreasing stocking density, and increasing salinity to 4 gm/L. The use of marine salts to increase salinity is not recommended as some virulent strains thrive on high calcium and magnesium levels. Severely infected fishes that are still eating may be treated with food medicated with oxytetracycline at 25-37 mg/pound of body weight/day for 7-10 days. Oxytetracycline baths at 38-380 mg/gallon for 1-3 days may also be useful. However, as oxytetracycline degrades in water, the degradation product can be harmful to humans. Gloves should always be worn when working in oxytetracycline treated water. If the water turns dark brown, a large water change ($\geq$ 50%) should be performed immediately. Potassium permanganate at 2 mg/L (higher doses are required if the water is organically rich) is effective against *F. columnare*; however infected fishes with extensive skin lesions may not survive treatment.

THE AQUATIC OPPORTUNISTS

Aeromonas hydrophila, *A. sobria* and *Vibrio* spp. are commonly encountered as bacterial infections secondary to stressors such as ammonia or nitrite toxicity, heavy ectoparasite infestations, or rough handling. *Aeromonas* are ubiquitous to freshwater aquatic systems, though it's not uncommon to culture *Aeromonas* spp. from marine fishes. *Vibrio* spp. are ubiquitous to marine aquatic systems, but certain species, e.g., *V. cholerae* and *V. alginolyticus,* are frequently cultured from moribund fishes in freshwater with salinity < 4 gm/L.

Aeromonas and most *Vibrio* species can be cultured using TSA agar with 5% sheep's blood.

Both *Aeromonas* and *Vibrio* thrive in organically rich environments. If either genus is cultured from fish, the management of the infected fish should be closely examined and any problem areas corrected.

Both genera are potentially zoonotic; however the risk is low.

NONTUBERCULOUS MYCOBACTERIA (NTM)

Nontuberculous mycobacterial infections are commonly seen especially in both captive and wild-caught freshwater and marine fishes. Older fish, such as specimens on display in public aquaria or broodstock are frequently affected. The majority of the non-tuberculous mycobacteria (NTM) are slow growing and by the time clinical disease is recognized, many animals may be exposed. The NTM that are fast growing present a significant risk to any person working with infected animals and the holding systems in which they reside. The *Mycobacteria* that can infect fish are non-tuberculous saprophytes commonly found in aquatic environments.

The two most common species cultured are *M. marinum,* found in fresh and marine tropical water, and *M. fortuitum,* found in tropical and temperate freshwater. The NTM most commonly associated with disease in marine fishes are *Mycobacteria marinum*, *M. fortuitum,* and *M. chelonae*. *M. marinum* has been cultured from farmed moribund turbot, wild rabbitfish in the Red Sea, and the

European seabass. *M. chelonae* has been isolated from salmon and yellow perch. Other mycobacterial species have been isolated from marine fishes with clinical signs of disease and include *M. montefiorense,* isolated from moray eels, a *Mycobacteria* sp. similar to *M. montefiorense* from rockfish, and *M. poriferae,* isolated from a seahorse. M*ycobacteria* thrive under conditions of low dissolved oxygen, low pH, and high organics.

Infections with NTM in fish may present as acute or more commonly, chronic disease. The chronic form is insidious and by the time clinical signs are evident the disease may be quite advanced. Such infected fish serve as a source of infection for other resident fish.

Clinical signs of disease are quite variable. In the acute form, moribund animals or sudden death may be the only sign. Emaciation, skin ulcerations and low level chronic mortalities are typically coupled with chronic NTM infection; however severely infected fish which appear to be robust are frequently diagnosed at necropsy. On internal examination, grossly visible grey-white granulomas may be present in liver, spleen and kidney.

Suspect cases should be stained with an acid-fast stain. If *Mycobacteria* are present, they will appear as red to pink rods in light green background.

Lowenstein-Jensen or Middlebrook 7H10 media are required for growth. *M. marinum* requires an incubation temperature no higher than 28^{o}C, and 28-30 days. *M. fortuitum* is tolerant of a wide temperature range and positive growth can be obtained in <7 days at 37^{o}C.

There is no treatment for mycobacteriosis. NTM are not easily eliminated; they have the ability to rapidly mutate, and their cell wall is very thick and high in lipids. This characteristic makes the cell wall almost impermeable, and resistant to drying, acids and alkalies. In addition, all mycobacteria are obligate intracellular pathogens, and as such are not easily recognized by the immune system as a pathogen. Compared to other bacterial diseases i.e., *Vibrio*, etc., the number of NTM required to cause disease is low.

Non-breeding fish populations can be managed by careful observation and removal of any fish exhibiting clinical signs. Any person working with an NTM infected aquarium should be informed of the potential risks. Mycobacteriosis is a zoonotic disease; immunosuppression increases risk of infection. If broodstock are infected with NTM, all resident fish should be euthanized, and the holding tank disinfected.

FOCUS ON SELECTED FISH VIRUSES

SPRING VIREMIA OF CARP VIRUS (SVCV)

Spring viremia of carp virus is an RNA virus, tentatively placed in the *Vesiculovirus* genus. Hosts include koi/common carp, grass carp, bighead carp, silver carp, Crucian carp *Carassius carassius*, and goldfish *C. auratus*. The range includes Europe, the Middle East, South America, China, and the U.S. (2002).

Clinical signs of disease are variable and can be compounded by concurrent infections (may be mistaken for other infections, especially bacterial). Fish may be lethargic, have decreased respiration, and exhibit loss of equilibrium. Externally they may be dark in color, have exophthalmia, pale gills, hemorrhages, ascites, protruding vent, skin ulcers. Internally edema, inflammation, and pin point hemorrhages (esp. gas bladder) may be observed on necropsy.

Histopathology findings may find perivasculitis in liver vessels, multifocal necrosis in liver and pancreas, damage to excretory and hematopoietic tissue of kidney, and perivasculitis, desquamation of epithelium, atrophy of villi of the intestine.

Transmission of virus is primarily horizontal: feces, gill and skin mucus, urine, and exudate from skin blisters. However mechanical vectors such as blood sucking parasites, birds, and fish handling equipment may also transfer virus.

Water temperature is very important as clinical signs of disease only occur when water temp is 15-20^{o}C.

There is no treatment for the virus, and it can be infective in mud for up to 42 days. It is killed by disinfection agents/techniques including gamma/UV radiation, chlorine, 500 ppm, for 10 minutes, pH <4.0 or >10.0, or heat 140 ^{O}F (60 ^{O}C) for 15 min.

Spring viremia of carp virus is identified by several methods. One method is cell culture using epithelioma papillosum of carp (EPC) or fat head minnow (FHM) cell lines. Cytopathic effect is widespread cell rounding followed by release of cells from plate surface (Goodwin). Indirect fluorescence antibody test and enzyme linked immunosorbent assay (ELISA) are used to identify the virus isolated in cell culture, but virus neutralization or polymerase chain reaction (PCR) are used to confirm infection with SVCv.

Steps to prevent introduction of SVCv infected fish are:

1. Buy from SVC-free source. To be an SVC-free source, 150 susceptible fish must be tested for SVC twice a year for two years. The fish must be collected under the supervision of an accredited veterinarian. If the tests are negative for SVCv, 150 susceptible fish must be tested yearly to maintain the SVC-free status.
2. Quarantine/Biosecurity: Keep shipments and species separate. During quarantine, the water temperature should be maintained at 15-20^{o}C and quarantine should be a minimum of four weeks. Any fish exhibiting clinical signs of disease should be tested for SVC.
3. Know the supplier.

Spring viremia of carp virus is a reportable disease. The USDA-APHIS area veterinarian in charge and the state veterinarian must be informed ASAP of positive test results.

KOI HERPESVIRUS (KHV)

Koi herpesvirus disease is caused by a DNA virus, cyprinid herpesvirus 3 (CyHV-3). In Israel, the disease is also known as carp interstitial nephritis and gill necrosis virus. The fish host is koi/common carp *Cyprinus carpio,* however other fishes may act as inapparent carriers of the virus. The range of KHV is U.S. (1998), Israel, Europe, Asia, Japan (2003).

Clinical signs are non-specific and include lethargy, swimming near the surface, and patchy white areas on the gills. The virus causes extensive damage to many tissues, but especially gills.

Histopathology may reveal fusion of secondary lamellae, hypertrophy and necrosis of epithelial cells of gills. In the spleen and kidney, focal necrosis and intranuclear inclusions may be observed.

The virus is identified by cell culture using the koi fin (KF-1) cell line, or PCR. Gill, spleen, and kidney are the best tissues for virus identification.

The virus is transmitted by contact with infected fish or contaminated water (infective for up to 3 days).

There is no treatment for KHV. Survivors of an outbreak will become carriers.

Like SVCv, KHV is a reportable disease.

Before diseases are attributed to a virus, rule out environmental problems such as water quality and temperature extremes, social stressors, nutritional mismanagement, general management, and other infectious pathogens such as parasites, bacteria, and fungi.

Koch's postulates modified by Rivers

- A particular virus is regularly associated with the disease (i.e. can be isolated from individuals with the disease)
- Virus is present as the causative agent not as a 'passenger' (may require volunteer, or animal experiments)
- Infection and the disease can be transmitted to new uninfected hosts by material free of other microorganisms