

**UMD-CLFS
Tropical Aquaculture Facility
(aka THE FISH HOUSE)
Biology/Psychology Building, Sub-basement**

**Standard Operating Procedures
for Cichlid Fishes**

August, 2007

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Daily Checks

- Water flow to all tanks. Check water levels in each tank.
- Air flow to all tanks
- Water level in sump
- Temperature of room and aquaria (80-86 degrees F)
- Clean tank outlet and pump inlet screens as necessary
- Remove large accumulations of detritus by siphon when observed.
- Remove dead fish immediately. Freeze for later disposal.
- Record all feedings, births/deaths and movements of fish among tanks on log sheets.

Daily Maintenance

Each day every tank should be observed for brooding females, sickness, and aggressive behavior.

Tanks with brooding females should be marked with a sticker: '*DO NOT CLEAN - BROODING*' with the date clearly marked.

Dead fish should be removed from the tanks immediately and the tank should be labeled with a sticker that indicates the date, and number of fish found dead. The dead fish should then be placed in the freezer for later disposal. If the dead fish is part of an active experiment a finclip should be taken, and the dead fish should be placed on a labeled plastic dish before being put into the freezer.

If extremely aggressive behavior is observed, it should be moderated by removal of the aggressive individual, by the addition of physical structure, and/or the addition of dither animals to the tank.

Foods and Feeding

Types of food

- Green Flakes: a flake made out of spirulina, high in plant protein and fiber.
- Yellow Flake: made out of egg yolk, high in fat and simple protein.
- Red Flake: made out of animal protein, i.e. earthworms, shrimp etc. high in animal protein and carotene, but low fat and low fiber.
- Small and large commercial fish pellets: very high in animal protein.
- Hikari Artificial Plankton – very small pellet high in HUFA (Highly Unsaturated Fatty Acids). For baby fish only!
- Daphnia: live food stimulates breeding and they can be fed with a variety of nutritional supplements that are unavailable in dried foods (easily degraded vitamins & mineral, HUFA, color enhancers). Daphnia shells are indigestible, so fish should only be fed Daphnia 1-2 times a week. Inappropriate for small fish.

Feeding:

Adult fish are fed depending on the breeding status and nutritional needs of the individual fish. Breeding groups are fed three times a day. Single adults are fed once a day.

- Adult **Tilapia** are fed the large commercial pellets. Feed only what they will finish eating within 10 minutes.
- Lake Malawi '**Mbuna**' species are fed a few pinches of green flakes (what they will eat within 10min). Breeding Mbuna are fed a meal of yellow flake once per week. Mbuna can also be fed a meal of live Daphnia once or twice per week.
- **Note: If Mbuna are fed too much animal protein they will develop Malawi bloat, an often-fatal intestinal swelling. This disease is very difficult to treat, so prevention is key.**

Juveniles fish are fed three times a day.

- **Mbuna** are fed a pinch of green flake.
- Small **Tilapia** are fed a large pinch of red flake.
- Medium **Tilapia** are fed a handful of small commercial pellets.

Fry should be fed once their egg sacks have been fully absorbed into the body. Initially they can be fed two or three times a day with the Hikari Artificial Plankton. They can be transitioned to with crushed red and green flakes.

Carotenoids

There are numerous ways to increase the intake of carotenoids to enhance the color of the fish. It can be as simple (and cheap) as feeding the fish more fresh vegetables. Peas, zucchini, romaine lettuce are all high in carotenoids. These can be used in conjunction with frozen foods like plankton (actually a small shrimp) and bloodworms (Chironomidae larvae). Using plankton can be difficult long term, since diets high in animal protein lead to 'Malawi bloat'. In addition there are any number of dry foods designed to increase fish color (Wardley's Total color, Vibragrow, Bio Blend Color Enhancing, Tetra Color Bits, Tetra Color, Hikari Tropical Discus Color Enhancer, Hikari Cichlid Gold, OSI Blood Red Parrot Pellets, AZOO Red discus pellet, AZOO Enhanced Color Peller, Omega One super color. As with all things, they vary in cost and effectiveness and simply include larger proportions of shrimp and carotene containing vegetables. While these are readily available from any aquarium supplier or pet shop, most have been designed for carnivorous cichlids (ex. discus) and again may be inappropriate as a staple diet for mbuna.

All of this is good to promote color and maintain normal coloration. Dainichi markets an excellent, but expensive cichlid pellet which costs about \$25.00 a pound. (info@dainichi.com, or see their website <http://www.dainichi.com/home.html>). Their food was originally designed for Tropheus which are very prone to Malawi bloat. For custom orders, they will spray their cichlid staple with Cyclop-eeze (a decapod that is extremely high in carotenoids and astaxanthene). The manufacturer tested the food on yellow *Labidichromis cauruleus* and turned them orange! The product is available as is a flake made of Cyclop-eeze (<http://www.cyclop-eeze.com/>).

Filtration System – Main Fish Room

The main fishroom has been constructed as a single-pumped recirculating biofiltration system. A 'single system' design was chosen for its robustness and ease of maintenance. In theory, multiple separate systems might reduce the spread of disease. In practice, multiple systems running in the same space offer little protection, since water, fish, and nets are constantly exchanged among systems.

Particulate removal

Water overflows each tank into a common drain. The wastewater passes through an automated drum screen filter, which removes waste particles larger than 25 microns. An automated backwash system is activated by a float switch to wash the screen and flush the waste to the sewer.

Biofilter

The water then flows into a 100 cubic foot sump containing approximately 30 cubic feet of Kaldnes beads. These beads offer a large surface area for the growth of nitrifying bacteria, which convert ammonia first to nitrite, and then to nitrate. The biofilter is sized to process the nitrogen waste from 25 lbs feed/day. The beads are vigorously stirred with compressed air from a rotary vane compressor. Collisions between the beads remove excess biofilms, making the system self cleaning. Note that biofilms on tank walls and inside of pipes provide significant additional area for the growth of beneficial bacteria.

Pumps

Water is drawn from the sump by an array of 1hp centrifugal pumps. At present it appears that 3 pumps provide sufficient flow. 2 additional pumps are in place as backups.

UV filtration

The water next flows through an array of UV sterilization filters. When all six are running they should provide 100,000 microwatts/second treatment at 240 gpm flow. (The industry standard is 33,000 microwatts/second). Bulbs should be replaced on a rotating schedule at 12 month intervals.

Aquaria

The water is then delivered to the aquaria. Airstones in each aquaria are supplied by a low-pressure blower. Some fine particulates inevitably flock and settle in the aquaria, and are removed as necessary by manual siphon.

Water Quality Management

The ammonia cycle

Standard: 0ppm ammonia and nitrite

Remedy: Suspend feeding. Determine reason for biofilter failure

The most important aspect of water quality in a recirculating system is the conversion of ammonia waste to nitrite, then nitrate, and its subsequent flushing from the system. The following discussion is adapted from <http://www.thetropicaltank.co.uk/cycling2.htm>

Ammonia

The ammonia level should always be zero (that is, undetectable by conventional test kits) in a mature aquarium. Fish waste, uneaten food and decaying plant matter will all contribute to the level of ammonia in the tank. However, in a mature system, there are usually enough ammonia-converting bacteria to ensure that it never rises to detectable levels. However, there are situations which may result in a temporary rise in ammonia levels, even in a mature tank. These include:

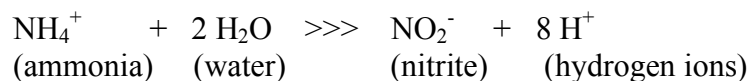
- Filter failure, or lack of maintenance
- Use of medications
- The addition of a large number of fish at the same time
- Over-feeding
- Over-enthusiastic cleaning of 'biological' filter media.

In such circumstances, the bacterial population will need time to increase or recover to cope with the demand. If fish appear unwell, testing for the presence of ammonia should be a priority.

The total ammonia in an aquarium will be present in two forms: ammonia (NH₃) and the ammonium ion (NH₄⁺). The proportion will depend mainly on pH, and to a lesser extent temperature. At alkaline pH, more of the ammonia will be present as the more toxic NH₃, while at acidic pH, more of the less toxic ammonium (NH₄) will be present. Ammonia poisoning is therefore more common at alkaline pH.

Ammonia can cause damage at levels of only 0.1 ppm (which is below the level detected by many kits!). There may be haemorrhaging and destruction of mucus membranes, the gills are particularly likely to be damaged, and may appear reddened. As with nitrite poisoning, fish may appear to gasp for air at the surface, and show rapid gill movement. Higher levels, of several ppm, can be fatal.

In a mature aquarium, ammonia is oxidised by bacteria to form nitrites. The chemical reaction which occurs is shown below:

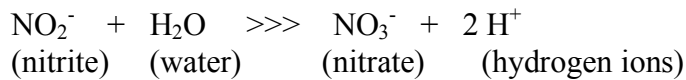


For many years the bacteria responsible were thought to be *Nitrosomonas* species, but more recent research indicates that these bacteria may do little or nothing in freshwater aquaria, and that bacteria known as *Nitrosococcus* may be the true ammonia-oxidisers in our system.

Nitrite

The nitrite level should always be zero in a mature system. A temporary rise in nitrite levels may be seen for the same reasons as listed for ammonia above. However, the nitrite spike may persist longer, so if there is a delay in testing after a problem has occurred, it is more likely that nitrite will be detected. A nitrite level of only 0.1 ppm could prove harmful if exposure is prolonged. Symptoms of nitrite poisoning include gasping and rapid gill movements, which could be mistaken for a shortage of oxygen. In extreme cases, fish can actually die of suffocation because nitrite binds to the oxygen-carrying component (haemoglobin) in the blood.

In a mature aquarium, nitrite is oxidised by bacteria to form nitrate. The chemical reaction which occurs is shown below:

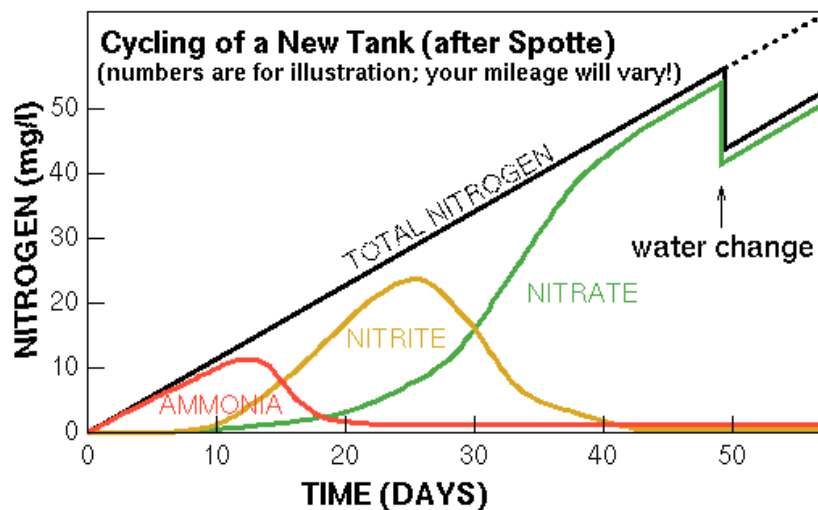


It was originally thought to be *Nitrobacter* species which were responsible for nitrite conversion to nitrate in aquaria, but again, recent research (by Dr. Timothy Hovanec and others) indicates a different group of bacteria - *Nitrospira* - are responsible.

Nitrate

In the past, nitrate was considered essentially harmless to fish; certainly it is far less toxic than ammonia or nitrite. It has been shown that levels of up to 1000 ppm may be required to cause death, but the effects of lower levels on long term health are not well understood. The sensitivity of different species to nitrate levels varies, and there may be long term effects on general health, growth and breeding ability.

Generally, many aquarists seem to agree that keeping nitrates below 50 ppm is necessary to prevent any long-term effects on fish health, but below 25 ppm is more desirable. Remember that many fish may come from a natural environment where there is little or no detectable nitrate. Fish which have been aquarium bred for generations are more likely to tolerate nitrates.



Temperature

Standard: 80-84 degrees Fahrenheit

Remedy: Check heaters in sump, and function of room air conditioning.

Although the temperature of Lake Malawi drops into the low 70's at times, the fish grow and breed best at 82F. Higher temperatures cause stress and may alter sex ratios.

pH

Standard: 7.5 - 8.0

Remedy: Add sodium bicarbonate to increase pH

The pH of Lake Malawi ranges from 7.5-8.5. As discussed above, ammonia levels are more toxic at higher pH.

Dissolved Oxygen

Standard: 90% saturation (6-7ppm)

Remedy: Increase aeration

Our system has abundant aeration, and we should always be well above 90% saturation. However, the oxygen level might become low in heavily loaded tanks without supplemental aeration (i.e. non-functioning airstone).

Conductivity

Standard: 100-300 microSiemens)

Remedy: Add salt

The conductivity of Lake Malawi is 210-220 uS. The composition is

Na	21 mg/L
K	6.4 mg/L
Ca	18 mg/L
Mg	7 mg/L
HCO ₃ + CO ₃	2.5 meq/L
Cl	4 mg/L
SO ₄	5.5 mg/L
SiO ₂	2.5 mg/L
PO ₄	20 ug/L

Freshwater fishes are under constant osmotic stress as water flows into their cells. They benefit from the addition of low levels of salt to the water. We routinely add water softener salt (NaCl) to reduce stress and prevent disease.

Even though Lake Malawi naturally contains high levels of salts, it is not clear that these are necessary or optimal for the culture of the fishes. However, the following recipe has been suggested for reproducing the water chemistry of Lake Malawi:

Target Water (GH=14, pH=8,4, KH=14).

water (liters)	CaSO4	MgSO4	NaHCO3	NaCl	KCL	KI
1000	2,03	15,75	30,35	122,00	36,6	0,73

(Recipe in teaspoons!)

(<http://www.malawicichlidhomepage.com/aquainfo/salts.html>)

Chlorine:

Standard: 0ppm

Remedy: Call for service

Water for the facility is treated to remove chlorine by a commercial system (located in the double-door closet in the hallway). The media in this system is supposed to be able to remove normal levels of chlorine from the tap water at the rate of 10gpm for 25 years! High levels of chlorine, or chloramines) require longer contact times with the media. We have installed a flow restrictor to reduce the flow rate to a maximum of 5gpm (in practice it seems to be about 2.5gpm).

It is **absolutely essential that NO chlorine gets through the filtration** and into the recirculating system. It **WILL kill the bacteria in the biofilter, and create a MONUMENTAL emergency**. Although the commercial filtration system appears robust, it is essential that we test the chlorine levels in the treated water each month.

Water testing:

Temperature, pH and salinity will be continuously monitored with an electronic meter. This will be supplemented with chemical tests for ammonia/nitrite/nitrate at weekly intervals, and tests for chlorine at monthly intervals. Additional water quality tests will be conducted whenever excessive mortality is observed.

Breeding management

Aggression

The most significant challenge in rearing cichlid fishes is their high level of aggression. Males are highly territorial, particularly when in breeding condition. Females may also maintain a dominance hierarchy. Bites, nips and ramming can damage fins and scales, which are subsequently infected with bacteria and fungi. While these infections can be treated, the best practice is to prevent the injury in the first place.

The typical set up for breeding is to place a male with at least 4-5 females in a tank with considerable structure. Structure consists of bricks, ceramic tiles, PVC pipe and/or flower pots to provide a focal point for the male territory and refuge for the females.

Dither is the term for additional fish added to distract/redirect the aggression of the dominant fish. We often add juvenile tilapia (*Oreochromis* spp.) to the breeding tank for dither. We also co-culture several Malawi haplochromine cichlid species in the same tank for the same purpose.

If excessive aggression is exhibited within a tank there are a few different remedies. If the male or the dominant female is displaying aggression towards the females, then putting more dither into the tank may be necessary. If the dominant male is showing aggression towards another male then the subordinate male should be placed into a 10-gallon tank by himself. If physical damage has already been done to one of the fish they should be placed into a 10-gallon tank by themselves to heal.

Care of Eggs and Fry

Care of eggs

After a brooding female's eggs have been taken from her and placed into a glass jar to develop special care must be taken for the eggs to develop into fingerlings. Fish eggs, when left in the mother's mouth to hatch are continually swirled around the mother's mouth. This is simulated in the glass jars by placing a medium sized air stone in the bottom center of the jar. It is important that the air stone is in the center because if it gets caught on the side eggs will be pulled beneath it and killed.

Another possible problem with eggs is the development of fungus or mold. Either of these will kill eggs, and if infected eggs are left in the jar, then they will quickly infect other eggs resulting in the death of the entire brood. The preventative measure used to prevent fungus and mold growth is to put 1-3 drops of methylene blue into the glass jars containing fish eggs. Methylene blue is a very effective anti-fungal agent, which is relatively harmless to the fish in small doses. Care must be taken to not use too much because using more than a few drops can result in sterility of the fish.

For the few days that the eggs are kept in the jar it is important to change the water once a day. When doing this dead eggs can be removed with a small pipette. Eggs that have been stained blue by the methyl blue are dead and should be removed immediately.

Care of yolk-sac fry

After the eggs have hatched into small immobile fish, they can be transferred into a plastic tub. Methyl blue can still be used and a small air stone should be placed onto the middle floor of the tub. The water should be changed carefully everyday and after the fingerlings have absorbed their egg sacks they can start being fed small amounts of the baby food. One hour after feeding the water should be changed so that old food doesn't rot and contaminate the water.

Care of fingerlings

It is important when working with the fingerlings that they be handled as little as possible. The best way to move very small fingerlings is to either transfer water from one tank to another, or to use a turkey baster to suck up water and the fingerling, which can then be moved. Using nets to move small fish is something that should be avoided. The nets can strip off the fingerlings' slime and cause damage to their scales, leading to infection and death. If it is absolutely necessary to use a net, then the ultra fine net should be used (these are the white nets with the blue handles).

Disease

Here are a few signs that indicate a fish is ill.

- Cessation of feeding
- Swimming asymmetrically
- Formation of velvet like fuzz on any wounds
- Treading in one part of the tank, i.e. at the surface, along the side, in a corner.
- Rubbing against rocks
- Formation of blisters or sores
- Swelling of the eyes
- Asymmetry of pupils
- Refusal to use appendage, i.e. a pectoral fin

If the fish is ill, then it is important to try and contain the illness, while treating the fish. The first thing to do with a sick fish is to move it to an empty tank. Then turn off the water supply, since the tanks are connected through a common system, disease can spread through the water. Since the water is turned off, there needs to be a good air supply to the tank to ensure water quality. It may be necessary to place more than one air stone into the tank. The tank then needs to be marked that the water is turned off, this will communicate to the next person who takes care of the fish that the water must be changed daily, and to not turn the water back on until the fish is better, and any antibiotic treatments have been flushed to the floor drain.

Treatment of disease begins with assessing the problem. If fuzz has developed on the sides of the fish due to a wound or irritation, then the fish has most likely developed a bacterial infection. The best way to treat a bacterial infection is to use a product which main ingredient is nitrofurazone. (Nitrofurazone is an antibiotic that is used only in veterinary medicine, and only on a few animals. It has been found to cause mammary gland cancer in mice, so it is believed that it is also a carcinogen to humans, and therefore it is important to not breath any dust in, or to allow any on your skin.) When treating fish it must be noted that often times the medicine given is also harmful to the fish, a balance must be found between giving enough medicine to kill the infecting organism, but not giving so much as to be detrimental to the fish. Therefore it is important the read the dilution instructions of the medication properly.

When medication is given:

- Feeding should be halted.
- An extra air source should be applied.
- The water supply must be turned off to the tank. **The medicated water must not be allowed to enter back into the system – it will decimate the biofilter!!**
- A half water change should be done after two days, and the fish can then be re-medicated.

Building systems

Lighting

Lights in the main lab are controlled by a digital timer (blue panel inside the big gray box – on the right as you enter the facility). The lights are programmed to turn on and off gradually to simulate dawn and dusk. The zebrafish and behavior rooms are controlled by a digital timer integrated with the light switch in these rooms.

Emergency generator

The water pumps, air compressor and lights are connected to the emergency generators in the new Bioscience Research building.

Air handling systems

The air supply comes from a grate in the internal courtyard between the new and old buildings. If you smell smoke or other strange odors, check the courtyard for smokers! The air handling system (heating and AC) for the fishrooms is located in the basement of Bio/Psych building. There are approximately six thermostats located throughout the facility, controlling the re-heaters in the ducts.

Drains

The floor drains have screens and baskets, which can be removed for cleaning.

Alarm system

We anticipate connecting a YSI monitor/alarm system to display water quality parameters and to dial designated responsible personnel in the event of a system failure.

Emergency procedures

Recirculating system failures

The system is still new, so we don't know all its failure modes. Minor system leaks do not constitute an emergency. They are compensated for by the daily addition of at least 250 gallons of fresh water from the storage tanks.

Major leaks may present the risk of electrical shock, and will drain the system until the sump runs dry. This obviously stops recirculation to the tanks, and may cause the pumps to heat up and burn out. If the sump is dry, turn off the pumps! Refill the sump from the green storage tanks, then restart the pumps.

The drum filter appears to be robust. The most likely failure mode is a failure of the backwashing system. If the screen is not being cleaned/rotated, it will clog. Water will back up in the drains, until it overflows. Check the operation of the float switch, clean the spray nozzles, and check the function of the booster pump and drum motor. In extreme emergencies you may decide to remove the screens to allow water to flow through the filter chamber.

Power outage

The system should continue to operate during a power outage, since the important pumps and compressors are connected to emergency power. In a prolonged power outage, the drum screen may clog (see above).

Air conditioning failure

Call facilities for repair.

Pipe leaks

There are a lot of 50-year old pipes overhead in the fishroom. We've already had a 4" sewer pipe repaired. Be alert for signs of leakage from all of these pipes. If you discover a leaking pipe, first determine if you can safely enter the area (be alert for electrocution hazards!). Then, try to protect the tanks using the plastic sheeting and blue tarps we have stored in the room. We want to keep sewage, chlorinated water, and treated HVAC fluids from entering the system. Then call facilities for an immediate repair!