

Mycobacteriosis– the Stealth Disease

by Diana Walstad (January 2009)

Author of *Ecology of the Planted Aquarium*

MB (mycobacteriosis) is considered to be the number one chronic disease in aquarium fish. MB is responsible for about half of fish deaths due to unknown causes. Because MB often has no obvious or defining symptoms, hobbyists underestimate its prevalence. If a newly purchased fish stops eating and dies after a few weeks, most hobbyists do not suspect MB (much less know what it is). Additionally, chronic MB weakens the fish's immune system making infected fish highly vulnerable to other diseases. I wonder how many hobbyists have attributed their fish's death to other pathogens, when the underlying problem was chronic MB? How many hobbyists have attributed their fish's death to old age or inbreeding, when the real problem was MB?

No fish is safe from this scourge. MB outbreaks have been reported in scientific laboratories, zoos, commercial fish farms, natural ponds, oceans, etc. Since the disease is incurable, the consequences to fish breeding can be devastating. As a fishkeeper, my own experience with MB was most unpleasant. Fortunately, there was a satisfactory outcome.

MB Outbreak in My Rainbowfish

I have kept Rainbowfish for over 20 years with few problems. However, in 2004 after adding new fish to my aquaria, the new fish died or developed abnormally. Antibiotics did not help. When abnormalities appeared on previously healthy tankmates, I suspected an infectious disease.

A fish veterinarian examined two Goyder Rainbows that I had raised from eggs in 2000 and had had no problems with up until this time. One fish had a slightly eroded lower jaw, and the other had no symptoms. However, a histological examination showed that the internal organs of both fish were riddled with granulomas containing acid-fast bacteria. My fish had "Fish TB" or mycobacteriosis– a bacterial disease that is highly contagious and incurable. Moreover, the causative mycobacteria could infect me via any skin cuts whenever I put my hands in the water. "Fish-tank syndrome" is not that common, but I still dreaded even the possibility of getting painful, slow-healing sores on my hands and arms. Distressing!



MB Symptoms. The jaw ulceration shown in this diseased Angelfish is similar to what I saw in my Goyder Rainbow that was diagnosed with MB in 2005. Other MB symptoms reported in the literature are skin ulcers, emaciation, swollen bodies (dropsy), abnormal swimming, lethargy, pop-eye, abdominal swelling (ascites), abnormal skin blackening ("Black Head" disease), reduced reproduction, spinal deformities, and unnatural weight gain. (Photo courtesy of Stan Chung.)

The recommended course was to tear down the tanks, disinfect everything, and start over. However, my three established tanks contained fish and plants that I had had for many years. Unsure of what to do next, I decided to add a UV sterilizing filter to each of the three tanks (45, 50, and 55 gal). My reasoning was that even if I could not save the fish, I could protect myself from infection.¹ I set up the UV filters so that water from the biofilter flowed through the UV filter around the internal 8-watt UV lamp before returning to the tank. I kept the UV sterilizers on 24 hr a day with a gentle flow rate, thereby maximizing the water's exposure to the sterilizing UV light.

Results from the UV sterilizers were unexpected and amazing. Fish deaths stopped. A couple fish with symptoms actually recovered. Whether the UV sterilizers were killing the bacteria responsible for MB or were killing pathogens causing secondary infections was irrelevant to me. My fish were getting better!

To see how contaminated the tanks were, I purchased 8 new Rainbowfish from a trusted source. Except for one death, the fish did fine. After 8 months, a fish veterinarian examined 3 of the new fish (all *Melanotaenia boesemani*), one from each tank. A histological exam showed no tell-tale granulomas. The older fish had not infected the new fish. The fact that I had removed the UV sterilizers a few months beforehand made these results even more impressive.

Nevertheless, I assume that the fish that survived the 2004 MB outbreak are chronically infected and have slowly transmitted disease to some of their tankmates. Last summer (2008), one fish died and another developed a curved spine and swollen abdomen. To prevent another possible MB outbreak, I reinstalled the UV sterilizing filters and quickly removed the suspect fish. I also started a policy of removing surface water biofilms from the tanks.



Fish Tank Syndrome. The EM (environmental mycobacteria) that cause MB in fish can cause painful, slow-healing sores in humans, mainly on the hands and arms. The sore on the hand of one pet shop dealer (depicted above) is a milder case. Treatment involves a lengthy and specific antibiotic regimen. Thus, I do not clean tanks if I have a skin break on my hands, and I always try to “wash up” within 30 min of contact with tank water. [Photo courtesy of Jurgen Hirt.]

Mycobacteriosis Disease

MB, the chronic disease of fish and reptiles, was first documented in diseased carp in 1897. Over the years, the disease has not abated.

¹ UV light is highly effective for killing all microbes, including mycobacteria (LeChevallier 2004). A UV sterilizing filter only kills the microbes that are suspended in the water. It does not harm the nitrifying bacteria essential to biological filtration, because they (like many other bacteria) live attached to filter media and other tank surfaces.

Fish may die within a couple weeks from a major mycobacterial assault. Typically, though, fish develop a chronic disease characterized by granulomas in the fish's tissues.

Granulomas (whitish nodules of 0.05 to 4 mm diameter) are the immune system's attempt to "wall-off" the invading mycobacteria. Upon autopsy, granulomas can be seen visually in the liver, spleen and kidney where these usually smooth, red-brown organs now have a pale, lumpy texture dotted with the sand-like granulomas. Only a histological exam (acid-fast staining of slide preps) can verify that the nodules are not due to *Nocardia* bacteria or certain parasites. However, based on the prevalence of MB in aquarium fish, granulomas suggest MB.

A chronically diseased fish may carry the bacterial pathogen for months or years. Whether it can eventually rid itself of the pathogen is a good question. The current assumption is that the fish will die of its disease. I would agree, but only if the fish is showing outward clinical signs (emaciation, swelling, etc). If an infected fish appears normal, I am not so sure. My own experience suggests that mildly infected fish can control their disease to some extent.

The mycobacteria currently responsible for most MB in freshwater aquarium fish appear to be *M. fortuitum*, *M. peregrinum*, and *M. chelonae* (Table 1). The prevalence of these species in diseased fish is probably due more to their wide environmental distribution than their pathogenic potency.

The actual species involved in causing MB is sometimes irrelevant. Relatively non-virulent species often cause as much devastation as more virulent species (and vice-versa). *M. gordonae* is



***Betta splendens* with MB.** [Photo courtesy of NC State Veterinary College (Raleigh NC, USA)]

TABLE 1. EM (Environmental Mycobacteria) Species Found in Aquarium Fish. Each column represents the % of each *Mycobacterium* species found in freshwater fish that contained EM. "Diseased" category represents 170 fish (21 species). In this broad survey, each fish came from a different hobbyist's aquarium and was sent to the laboratory for disease diagnosis. The "Undiseased" category represents imported batches of freshwater fish (5 similar fish/batch) that had no clinical disease signs but contained EM. The 28 batches represent 15 fish species. [Data from Zanoni 2008].

<i>Mycobacterium</i> Species	Species Composition in Fish:	
	Diseased	Undiseased
<i>M. fortuitum</i>	50%	18%
<i>M. peregrinum</i>	25%	21%
<i>M. chelonae</i>	10%	18%
<i>M. abscessus</i>	5.3%	14%
<i>M. gordonae</i>	3.5%	3.6%
<i>M. nonchromogenicum</i>	2.9%	14%
<i>M. marinum</i>	2.9%	3.6%
<i>M. fortuitum</i> & <i>M. chelonae</i>	0	3.6%
<i>M. chelonae</i> & <i>M. marinum</i>	0	3.5%
# of Samples examined	170	28

not considered to be a fish pathogen. Yet, it was recently identified as the culprit behind heavy mortalities in several guppy farms [Sakai (2005)].

In another study [Watrall 2007], investigators predicted that an *M. peregrinum* strain responsible for destroying an entire colony of research laboratory Zebrafish would be highly virulent. However, when tested experimentally, it turned out to be much less virulent than an *M. marinum* strain that had caused only moderate disease problems in another research laboratory.

Prevalence of MB in Aquarium Fish

MB causes more problems than most hobbyists realize. In one survey of 70 aquarium fish that had died from unknown causes, investigators [Lescenko 2003] found that 63% had MB.

In a separate survey [Gomez 2008], investigators randomly collected 200 debilitated fish (24 different species) from various pet shops and private aquaria. All fish showed signs of chronic disease (persistent skin lesions, poor body condition, swollen abdomens, etc). MB was diagnosed in 81 (or 41%) of the 200 fish. Of the 24 fish species represented in the study, all species had some members with MB. For example, half of the 34 debilitated guppies had MB.

A recent comprehensive survey of 387 diseased fish revealed that 181 (47%) were infected with mycobacteria (Zanoni 2008). The fish, all from separate hobbyists' aquaria, represented 32 freshwater species and 12 marine species.

Potentially Pathogenic EM are Everywhere

The EM (environmental mycobacteria) that cause MB are everywhere. The 91 currently identified species [Primm 2004] have many characteristics that set them apart from other bacteria (Table 2).

Unlike *M. tuberculosis*, an obligate pathogen that does not live outside its human host, EM are found throughout the natural environment- soils, lakes, oceans, tapwater, bottled water, fish, invertebrates, etc.

EM typically live by feeding on decaying organic matter, but they can become pathogenic. Indeed, one investigator [Adekambi 2006] tested 26 different EM species for virulence by seeing if they could infect amoebae. [The ability to infect this phagocytic protozoan that ordinarily kills and feeds on bacteria is analogous to infecting macrophages, which are phagocytic cells important to fish immunity.] All 26 EM species survived and multiplied within amoebae suggesting that all 26 are potential pathogens.

TABLE 2. Characteristics of EM (Environmental Mycobacteria). Many of the characteristics listed below (acid-fast staining, resistance to disinfectants, etc) stem from the fact that lipids make up almost 60% of the *Mycobacterium* cell wall. In contrast, lipids make up only 1-4% of the cell wall in gram-positive bacteria and 20% of a gram-negative bacteria's cell wall [Wolinsky 1973].

- Gram positive, acid-fast staining, aerobic, non-motile rods
- Resist Clorox and many other water-soluble chemicals
- Grow slowly putting EM at a competitive disadvantage with other bacteria in nutrient-rich environments
- Extreme tenacity under starvation conditions (can grow for a year in distilled water)
- Non-sporulating, but EM can survive for years within the cysts of infected amoebae
- Readily incorporate themselves into surface water biofilms, a potential EM reservoir in aquaria
- Survive and multiply in phagocytic cells (e.g., amoebae and macrophages) that kill ordinary bacteria

Healthy aquaria contain a rich and diverse EM microflora. Beran (2006) surveyed 6 well-established, apparently normal aquaria for EM. The investigators isolated numerous species from the tank environment (Table 3). Notably, the two tank environments have a very different EM microflora. For example, *M. chelonae* was found in 18% of the Show Tank’s environmental samples, but none of the Breeder Tanks’ samples.

EM Presence in Fish

None of the 19 sampled fish from the Six Normal Aquaria had MB [Beran 2006]. However, some of the fish contained EM in their tissues. The EM species found in these fish were the same EM species found in the fish’s environment (Table 3).

One comprehensive survey [Zanoni 2008] documented the prevalence of EM in imported fish being sold in Italy. Fish (directly from the vendor) were pooled into batches of 5 similar fish (same species & source) for the analysis. Approximately 30% of the 127 batches, representing 48 species of marine and freshwater fish, contained EM (homogenates of pooled livers, kidneys, and spleens were cultured for EM). Only 3 individuals out of the 635 fish had clinical signs consistent with MB disease. Nevertheless, one can conclude that many aquarium fish are carrying EM when hobbyists purchase them.

Harriff (2007) proved that EM enter fish via the mouth (not the gills or skin). Because EM are an intrinsic part of the aquarium environment and digestion does not kill EM, one would expect to find live EM in the fish intestine and feces. Indeed, one investigator [Perez 2001] detected several EM species in the feces of Silver mullets.

Thus, many healthy fish probably contain small numbers of EM in their intestines. For example, Harriff [2007] found *M. fortuitum* in the intestines of 9 out of 18 apparently healthy Zebrafish. None of the fish had granulomas or inflammation. Eight of the 9 fish yielded (after culturing for EM) only 1 to 20 colonies from their intestines and no colonies from their livers and spleens. One fish, whose intestine yielded 400 colonies, did have some *M. fortuitum* bacteria in the liver and spleen. The fact that *M. fortuitum* was able to penetrate this fish’s intestinal wall and invade the liver and spleen suggests that this fish was “at risk”.

TABLE 3. EM Species found in Six Normal Aquaria. Table shows the *Mycobacterium* species that were found in the algae, plants, sediment, filter, biofilms, etc) of normal, well-established aquaria. [Data from Beran 2006].

<i>Mycobacterium</i> Species	Species Composition in Tank Environment:	
	Single Show Tank	Five Breeder Tanks
<i>M. fortuitum</i>	36%*	22%*
<i>M. chelonae</i>	18%*	0
<i>M. gordonae</i>	9.1%	5.6%*
<i>M. terrae</i>	0	5.6%*
<i>M. triviale</i>	0	5.6%
<i>M. diernhoferi</i>	0	5.6%
<i>M. celatum</i>	0	5.6%
<i>M. kansasii</i>	0	5.6%
<i>M. intracellulare</i>	0	5.6%
<i>M. flavescens</i>	4.5%*	0
Unidentified species*	32%*	39%*
# of Samples	25	24

*Species found in fish tissues as well as tank environment.

EM probably make up a very tiny fraction of the fish's intestinal microflora. Other bacteria in the intestinal microflora would help keep potential EM pathogens in check by depriving ingested EM of the necessary attachment sites and nutrients.

Disease occurs when the intestinal microflora is disrupted, fish immunity is weak, and/or large numbers of an unfamiliar EM suddenly assault the fish.

Immunity

If healthy fish are carrying small numbers of EM, and all EM are potential pathogens, then the only thing truly protecting the fish is its immune system. The immunity that fish can develop against EM affords them substantial protection. For example, investigators [Pasnik 2005] vaccinated fish so that they would produce antibodies against a *Mycobacterium* antigen. The investigators waited for antibody development, which usually takes a couple weeks, and then injected the fish with live, virulent EM (*M. marinum*). All control (unvaccinated) fish died within 3 weeks, whereas 90% of the vaccinated fish were still alive at 5 weeks.

Genetic studies with Zebrafish further show how much immunity protects fish. TU Zebrafish lack the *rag1* gene such that they cannot produce functional lymphocytes critical to adaptive immunity. [In every other way, these “genetic knock-out” fish are normal.] In one survival study where investigators [Swaim 06] challenged Zebrafish with *M. marinum*, TU Zebrafish died significantly ($p < 0.0001$) more than control Zebrafish. In another research laboratory, EM infected TU Zebrafish colonies much more than other Zebrafish colonies [Whipps 2008].

Fish can be healthy and have a robust immune system, but they may not have immunity to an unfamiliar EM microflora. Table 3 suggests that every tank has its own unique EM microflora of different *Mycobacterium* species. Fish might have to produce a whole new set of antibodies for each new EM species in order to gain full protective immunity. This may explain why some healthy fish acquire the disease when they are suddenly put into a new tank and confronted with an unfamiliar EM. For example, Tappin (1999) transferred half of 30 healthy Goyder Rainbowfish to a well-established 600 liter tank containing other healthy Rainbowfish. The 15 Goyders that were kept in the original tank and not transferred had no problems. However, the transferred Goyders developed MB, and within weeks, began dying. I believe that the older tankmates had immunity to their tank's natural EM microflora, but the Goyders did not, and therefore, were vulnerable.



Susceptible Fish Species – “Goyder Rainbow”.

This male *Melanotaenia trifasciata* (Goyder River strain) appears robust, but he is infected with MB. I euthanized him soon afterward when he began to swim erratically. All my Goyder Rainbows were wiped out by MB. Other Rainbowfish (*Melanotaenia boesemani*, *M. lacustris*, *M. herbertaxelrodi*, *M. praecox*, and *Glossolepis incisus*) fared much better.

Stress and Disease Susceptibility

Stress compromises the fish's immune system and makes it vulnerable to MB. Poor water quality can stress fish and cause disease [Walters 1980]. However, psychological stress induced by fear also suppresses the fish's immune system. For example, investigators [Davis 2002] stressed catfish for 6 hr by lowering the water level so that the fish were submerged but unable to maintain their normal orientation ("low-water treatment"). Then the investigators added the "Ich" parasite *Ichthyophthirius multifiliis* to the tanks. Six days later, Ich infestation was 27% greater in stressed fish than control (unstressed) fish.

Stress can severely compromise a fish's immune system. Investigators [Peters 1985] stressed fish by placing two juvenile Rainbow trout in small tanks with no place to hide. The two fish fought vehemently until one established dominance. For the remainder of the 4-week experiment, dominant fish swam freely around their tanks while subordinate fish remained submissive (e.g., stayed in a corner). Subordinate fish ate, but grew to about 1/3 the size of their dominant partners. More importantly, the immune system of subordinate fish was devastated. Histological sections of spleen and anterior kidney tissue show swollen and disintegrating immune cells. In the spleen, the number of lymphocytes and neutrophils dropped 75%.

The same investigators [Peters 1988] later showed that subordinate (stressed) fish were also more susceptible than dominant fish to infection. Eleven hours after putting two fish together in 12 small tanks, investigators added *Aeromonas hydrophila* bacteria to the tanks. Then, 10 hrs later they sacrificed the fish and cultured body parts for *Aeromonas*. The investigators found that *A. hydrophila* had invaded the vital organs (spleen, liver, and kidneys) to a significantly ($p < 0.01$) greater extent in subordinate fish than dominant fish.

Many factors can reduce the damage of stressful events. For example, Salmon given a day to recover after 9 hr of shipping & handling could withstand a challenge from the bacterial pathogen *Vibrio anguillarum* significantly better than fish given only 4 hr to recover [Maule 1989]. Zebrafish subjected to crowding for 5 days without food were stressed (measured here by rapid increases in blood levels of cortisol); Zebrafish given food while crowded showed no significant stress from crowding [Ramsay 2006].

I doubt that a brief stressful incident (heater going off one night) would trigger MB. Any temporary immune suppression brought on by acute stress would most likely trigger infections from much faster-growing bacteria like *Aeromonas* and *Pseudomonas*. These potential pathogens are all part of a fish's intestinal microflora [Rawls 2004] and the natural environment. They would quickly invade the blood and organs of immunosuppressed fish long before EM could multiply to threatening levels. I suspect that MB develops mostly in fish exposed to chronic stress measured in weeks and months, not hours and days.

Disinfection Increases the Numbers of EM

Ironically, some fish breeders may increase the numbers of EM— and the risk of MB— by routinely cleaning and disinfecting tanks. EM resist most chemicals (antibiotics, detergents, Clorox, etc) much more than other bacteria. For example, EM are about 100 times more resistant to chlorine and chloramine than the ordinary bacterium *Escherichia coli* [LeChevallier 2004]. In contrast, UV sterilization kills EM and ordinary bacteria equally, so it does not enrich for EM (i.e., it does not selectively boost the EM portion of the total bacteria population).

The laboratory procedures required to culture EM provide a perfect example of how disinfectants enrich for EM. Because EM grow much slower than other bacteria, laboratory cultivation of EM from diseased fish generally requires weeks and months. Lab workers must kill faster-growing bacteria that often contaminate these tissue samples. Otherwise, the bacteria will grow over the entire culture dish making EM detection impossible. Lab workers do this by briefly treating (i.e., “decontaminating”) the fish tissue sample with a potent chemical cocktail (mixture of sodium hydroxide, malachite green and a mild detergent) before plating the sample onto culture dishes. Even then, the culture dish itself usually contains antibiotics to further kill contaminating bacteria. Many EM are inevitably killed. However, the EM that manage to survive can now multiply freely on the culture dish without being overgrown by ordinary bacteria.

EM survive and thrive in nutrient-poor (i.e., “clean”) environments that starve ordinary bacteria. Investigators [Steinert 1998] showed this experimentally when they placed *E. coli* and an EM (*M. avium*) in separate containers of starvation media (no nutrients). After 10 days, the *M. avium* population increased 72 fold while the *E. coli* population *decreased* 20 fold. Under nutrient-rich conditions, the results would be quite different; on rich lab media, *E. coli* has a population doubling time of 20 minutes, while *M. avium* requires a full 15 hours. This means that after 15 hours on rich growth media, a single *M. avium* has divided into two bacteria. Meanwhile, *E. coli* has divided every 20 minutes (or 45 times) and theoretically increased its population from one bacterium to almost 40 trillion bacteria!

Disinfection and eradication may be warranted in situations where a fish colony has reached the “melt-down” stage or is infected with a particularly virulent EM. However, EM will assuredly recolonize disinfected tanks. Moreover, disinfected, ultra-clean tanks are deprived of nutrients and organic matter for normal bacterial growth. They provide a perfect environmental niche for gradually generating large numbers of EM. Since MB infection is dose-dependent, numbers count.

Surface Scum as an EM Reservoir

One investigation [Angenent 2005] of a hospital’s therapy pool quantitated the EM population found in different parts of the pool. Despite being maintained and monitored according to public health standards, pool lifeguards were getting respiratory infections. The investigators eventually proved that the pool was the source of the infection. Surface water biofilms were releasing large quantities of EM (mainly *M. avium*) into the air in the enclosed pool house. [EM are easily aerosolized.] The investigators found much smaller numbers of EM in the bulk water and almost none in the pool’s filter.

It is not surprising that EM are particularly abundant at the water surface where lipids and other hydrophobic compounds naturally accumulate. EM have a marked nutritional preference for lipids, and they are able to metabolize lipids better than other bacteria [Primm 2004]. As Falkinham [2006] explained, EM do not remain long in the bulk water; it is too polar (electrically charged) for these lipid-rich, hydrophobic bacteria. EM absorb to micro air bubbles that rise to the water surface. Once at the water surface, EM establish themselves in surface water biofilms. They multiply freely in an environment that is both aerobic and lipid-rich.

Surface scums are a potential EM reservoir whereby EM can evade the UV sterilizing filter. [In outdoor ponds, sunlight UV would kill many EM in surface water biofilms.]

Moreover, many fish feed at the water surface, thereby ingesting copious EM with every meal. Preventing or removing surface scums is a simple precaution for reducing fish exposure to EM.

Preventing MB in Hatcheries and Aquariums

Commercial fish hatcheries justifiably dread MB. Large fish colonies in tanks with stagnant water or recirculating filtration systems will always be vulnerable. Even if the hatchery provides good overall conditions, there will always be a few fish that are immunologically weak. These fish can easily develop chronic MB by the EM within their environment. Hatchery employees working with many tanks and large outdoor ponds might not spot these problem fish. The chronically infected fish then start shedding large numbers of EM into the fish colony. Moreover, the EM they shed would be much more virulent than the EM still innocently feeding on tank organic debris. For a basic tenet of pathogenic microbiology is that *bacterial growth within an animal increases its virulence*.

MB outbreaks often result from the introduction of new fish. Even if the new fish is not diseased when purchased, it is often weakened immunologically. Moreover, it often faces a brand-new microflora containing EM species for which it does not yet have antibodies.

Quarantining new fish for detecting MB requires at least 2-3 months. EM grow slowly, so the disease develops slowly. Moreover, chronically diseased fish frequently have developed some resistance to their EM pathogen, so outwardly they may appear fine for some months. Meanwhile, your fish have no immunity to this new EM and are highly vulnerable. To bring this latent disease out into the open, I would add a few fish from the main tank to serve as disease “sentinels”. If the new fish are infected, the Sentinels—not having immunity to the new EM pathogen—will begin to show problems. On the other hand, if both the new fish and the Sentinels do well during the quarantine, one is reasonably assured that the new fish will not endanger established tanks.

I would also use a UV sterilizing filter temporarily in any tank that contains new fish. The UV sterilizer greatly decreases the numbers of EM in the water, thereby reducing much of



Disease Source. A few months before this photo was taken (Summer 2004), I added four new *Melanotaenia praecox* (Neon Rainbowfish) directly to this 45 gal tank. The *M. praecox* did unusually poorly (none survived the year). One is shown in the center of the photo. Next to him are Goyder Rainbows that later died from MB. The *M. praecox* might have been chronically infected when I purchased them. However, the breeder had had no problems with these fish. Perhaps then, the new fish did not have immunity to the EM microflora of this particular tank and developed MB *after* I purchased them.

the fish's immediate exposure to potential EM pathogens. The new (and often stressed) fish is given precious time to develop antibodies and acquire some immunity to the new tank's unique EM microflora.

One can do everything right, and fish can still become infected. One research laboratory carefully started their Zebrafish colonies with disinfected eggs. The fish were maintained under optimal conditions (good water, UV sterilizing filter, fed twice daily, etc). Mortality was very low, but the lab managers were alerted when a few fish showed skin lesions consistent with MB. Autopsies of 240 randomly sampled fish showed that the fish colonies had, indeed, developed a low-level, background infection of a particular strain of *M. chelonae*. Moreover, the investigators [Whipps 2008] found the same *M. chelonae* on the sides of the tank, filter inlet/outlet tubing, sediment debris, algae, etc. They concluded that this *M. chelonae* strain, while relatively non-virulent, was endemic to the entire facility. Despite a vigorous search, the investigators were unable to locate the infection source. In this instance, EM eradication would have been exceedingly difficult and most likely unsuccessful.

There is no cure for MB and none on the horizon. Prevention and good fish husbandry (Table 4) are probably more effective than trying to eradicate the EM that are so much a part of any aquarium fish's normal environment. I counteracted the MB outbreak in my tanks by quickly removing sick fish (the major disease reservoir) and using UV sterilizers. Once the MB outbreak was under control, I believe that competition from ordinary, faster-growing bacteria reduced the numbers of the EM pathogen in the tank to disease insignificance. Removing surface water biofilms, a known reservoir for EM, also probably helps. Thus, I can manage the disease without destroying all the fish and "nuking" the tanks. In my opinion, knowing how to prevent and/or manage MB is absolutely essential for successful, long-term aquarium keeping.

TABLE 4. Preventing and Managing MB

- strictly quarantine all new fish, ideally with Sentinel Fish (see text)
- use UV sterilizing filters, especially for any tanks with new fish
- provide good water quality
- remove fish that show signs of long-term stress (e.g., darkened body color, not eating, and restricted movement)
- promptly remove sick or dead fish (a decomposing fish will flood the water with highly infectious EM)
- prevent or remove surface water biofilms
- do not feed your fish "feeder fish", which are prime candidates for carrying MB
- recognize that disinfection can sometimes increase the long-term risk of MB



Back To Normal? This recent picture (Jan 2009) of my 50 gal tank shows several large *Melanotaenia lacustris* (Turquoise Rainbowfish) that survived the 2004 MB outbreak. Some may now be chronic disease carriers. To prevent future MB problems, I use a UV sterilizing filter, prevent surface scum formation, and carefully monitor fish health. I believe these measures will help prolong the lives of all depicted fish for many more years. [Other fish in photo are young *M. praecox* and *M. herbertaxelrodi* that I raised myself.]

REFERENCES

- Adekambi T *et al.* 2006. Survival of environmental mycobacteria in *Acanthamoeba polyphaga*. *Appl Environ Microbiol* 72: 5974-5981.
- Angenent, LT *et al.* 2005. Molecular identification of potential pathogens in water and air of a hospital therapy pool. *Proc Natl Acad Sci* 102: 4860-4865.
- Beran, V *et al.* 2006. Distribution of mycobacteria in clinically healthy ornamental fish and their aquarium environment. *J Fish Dis* 29: 383-393.
- Davis KB *et al.* 2002. Effect of handling stress on susceptibility of channel catfish *Ictalurus punctatus* to *Ichthyophthirius multifiliis* and channel catfish virus infection. *Aquaculture* 214: 55-66.
- Falkinham, JO. 2006. Personal Communication.
- Gomez S. 2008. Prevalence of microscopic tubercular lesions in freshwater ornamental fish exhibiting clinical signs of non-specific chronic disease. *Dis Aquat Org* 80: 167-171.
- Harriff MJ *et al.* 2007. Experimental exposure of zebrafish, *Danio rerio* (Hamilton), to *Mycobacterium marinum* and *Mycobacterium peregrinum* reveals the gastrointestinal tract as the primary route of infection: a potential model for environmental mycobacterial infection. *J Fish Dis* 30: 587-600.
- LeChevallier, MW. 2004. Control, treatment and disinfection of *Mycobacterium avium* complex in drinking water. In: Pedley S *et al* (eds). *Pathogenic Mycobacteria in Water*. IWA Publishing (London, UK) for the World Health Organization, pp. 143-168.
- Lescenko, P *et al.* 2003. Mycobacterial infection in aquarium fish. *Vet Med* 48: 71-78.
- Maule AG *et al.* 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). *J Endocrinol* 120: 135-142.
- Pasnik DJ and Smith SA. 2005. Immunogenic and protective effects of a DNA vaccine for *Mycobacterium marinum* in fish. *Vet Immunol Immunopathol* 103: 195-206.
- Perez, AT *et al.* 2001. Presence of acid-fast bacteria in wild and cultured silver mullets (*Mugil curema* VAL., 1836) from Margarita Island Venezuela. *Interciencia* 26: 252-256.
- Peters G *et al.* 1988. Stress caused by social interaction and its effect on susceptibility to *Aeromonas hydrophila* infection. *Dis Aquat Org* 4: 83-89.
- Peters G and Schwarzer F. 1985. Changes in hemopoietic tissue of rainbow trout under influence of stress. *Dis Aquat Org* 1: 1-10.
- Primm TP *et al.* 2004. Health impacts of environmental mycobacteria. *Clin Microbiol Rev* 17: 98-106.
- Ramsay JM *et al.* 2006. Whole-body cortisol is an indicator of crowding stress in adult zebrafish, *Danio rerio*. *Aquaculture* 258: 565-574.
- Rawls JF *et al.* 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci* 101: 4596- 4601.
- Sakai M, *et al.* 2005. Characterization of a *Mycobacterium* sp isolated from guppy *Poecilia reticulata*, using 16S ribosomal RNA and its internal transcribed spacer sequences. *Bull Eur Assoc Fish Path* 25: 64-69.
- Steinert M *et al.* 1998. *Mycobacterium avium* Bacilli grow saprozoically in coculture with *Acanthamoeba polyphaga* and survive within cyst walls. *Appl Environ Microbiol* 64: 2256-2261.
- Swaim LE *et al.* 2006. *Mycobacterium marinum* infection of adult Zebrafish causes caseating granulomatous tuberculosis and is moderated by adaptive immunity. *Infection Immunity*. 74: 6108- 6117.
- Tappin Adrian. Internet article updated 2000:
<http://pandora.nla.gov.au/pan/21803/20040913/members.optushome.com.au/chelmon/Myco.htm>
- Veiseth E *et al.* 2006. Accelerated recovery of Atlantic salmon (*Salmo salar*) from effects of crowding by swimming. *Com Biochem Physiol, Part B* 144: 351-358.
- Walters GR and Plumb JA. 1980. Environmental stress and bacterial infection in channel catfish, *Ictalurus punctatus* Rafinesque. *J Fish Biol* 17: 177-185.
- Watrall V and Kent ML. 2007. Pathogenesis of *Mycobacterium* spp. in Zebrafish (*Danio rerio*) from research facilities. *Comp Biochem Physiol (Part C)* pp. 55-60.
- Whipps CM, Matthews JL and Kent ML. 2008. Distribution and genetic characterization of *Mycobacterium chelonae* in laboratory zebrafish *Danio rerio*. *Dis Aquatic Org* 82: 45-54.
- Wolinsky E. 1973. Mycobacteria. In: Davis BD, Dulbecco R, Eisen H, Ginsberg HS, Wood BW, and McCarty M (eds), *Microbiology* (2nd Ed); Harper & Row (Hagerstown MD), pp 844-869.
- Zanoni RG *et al.* 2008. Occurrence of *Mycobacterium* spp. in ornamental fish in Italy. *J Fish Diseases* 31: 433-441.