

## **Mycobacteriosis- the Stealth Disease**

(by Diana Walstad)

I have kept Rainbowfish for 20 years. Except for a single bout with Ich (the common “white spot” disease), the fish have been remarkably problem-free. However, in 2004 after adding new fish to my aquariums, the new fish died or developed strange body sores. Antibiotics did not help. When symptoms appeared on tankmates, fish I had raised from eggs and knew were healthy, I suspected an infectious disease.

A fish veterinarian examined two fish, the only symptoms being tissue erosion of one fish’s jaw. However, a histological examination showed that the internal organs of both fish were riddled with granulomas containing acid-fast bacteria. My fish had “MB” or mycobacteriosis– a common bacterial disease in fish that is highly contagious and incurable. Distressing! My fish were all going to die. Moreover, the causative bacteria could infect me (“fish-tank syndrome”) via open wounds or cuts whenever I put my hands in the water, resulting in painful, slow-healing sores.

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 couldn’t save the fish, I could save the plants and protect myself. 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 permanently removed the UV sterilizers a few months beforehand made these results even more impressive.

### **Background**

MB (mycobacteriosis), the chronic disease of fish and reptiles, was first documented in diseased carp in 1897. Over the years, the disease has not abated. It probably causes many more problems than hobbyists realize. For example, in a random survey of 312 aquarium fish at various fish distribution centers, Praero (2004) found MB in 12% of the fish. In a survey of 70 dead aquarium fish, Lescenko (2003) found that 41% had MB. Many experts consider MB to be the most common chronic disease in tropical aquarium fish [Astrofsky 2000].

Because MB has no clear symptoms and can only be confirmed by a histological examination, 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. For example, when Talaat (1998) experimentally induced MB in goldfish, the infected fish developed the parasitic disease Ich (control fish without MB developed no Ich). I wonder how many hobbyists have attributed their fish's death to parasites and other pathogens, when the underlying problem was chronic MB?

The genus *Mycobacterium* is roughly subdivided into two major groups: (1) human pathogens like *M. tuberculosis* and *M. leprae*, which cause tuberculosis and leprosy, respectively, and do not live outside their human hosts; and (2) EM (environmental mycobacteria), which live everywhere in the natural environment (soil and water). EM can, under the right circumstances, cause disease, but that is not the norm. Three EM species (*M. marinum*, *M. fortuitum*, and *M. chelonae*) are commonly cited as causing MB. However, investigators using new genetic methods frequently isolate other EM species from diseased fish [Herbst 2001; Kent 2004; Poort 2006; Rhodes 2004; Sakai 2005; Whipps 2003]. Almost any EM species can produce MB— provided it is present in sufficient numbers.

MB involves the “hi-jacking” of a major player of the animal's immune system— the macrophages. These large white blood cells ordinarily engulf and digest invading bacteria via phagocytosis. However, macrophages cannot kill pathogenic EM. Instead, the engulfed EM survive and multiply inside the macrophages. Infected macrophages travel throughout the animal's body starting infections in new tissues.

Fish may die within 2-8 weeks from large numbers of *M. marinum*, a particularly virulent EM. Typically, EM infections cause chronic disease. After the fish's macrophages capture the bacteria, granulomas (round nodules of 0.05 to 4 mm diameter within the fish's tissues) develop after a few weeks. Inside the granulomas, bacteria, macrophages, and other cells wage a lengthy war of attrition. If the animal's immune system diminishes, the bacteria may break out of the granulomas, multiply unchecked, and kill the fish. On the other hand, mildly infected fish may be able to eventually rid themselves of disease. Thus, Gauthier (2003) found healed granulomas in mildly infected fish.

### **EM are *Not* Necessarily Pathogens**

The same species that cause fish MB are also found throughout nature— in soils, lakes, and oceans. For the most part, they feed on decaying organic matter.

Healthy aquariums have a natural EM flora. Beran *et al* (2006) screened 6 well-established, apparently normal aquariums for EM. The investigators isolated numerous EM species (e.g. *M. fortuitum*, *M. chelonae*, etc) from the environment (snails, filters, surface water biofilms, plants, fish, etc). None of the 19 fish autopsied had the granulomas characteristic of MB. However, the investigators did find EM in the fish's tissues.

Other investigators [Harriff *et al* 2007] found *M. fortuitum* in the intestines of 9 out of 18 presumably healthy Zebrafish. The numbers of this potential pathogen were very small in 8 of the 9 fish; only 1 to 20 colonies were cultured from the intestines and none from the liver or spleen. The exception was one fish whose intestine yielded 400 colonies and whose liver and spleen also yielded *M. fortuitum*. The investigators found no evidence of MB disease (granulomas or inflammation) in any of the 18 Zebrafish.

It is surprising that apparently undiseased fish were found to contain EM, including documented fish pathogens like *M. fortuitum* and *M. chelonae*. However, I would argue that the

mere presence of EM does not mean incipient MB disease. Most likely, the number of EM was insufficient to cause disease. Because EM are part of the natural environment, one could reasonably predict that EM might be present within fish, especially the intestine. I would further argue that a small number of EM could benefit fish by stimulating their immune systems.

### **Disinfection and Cleanliness Enrich for EM**

Ironically, fish breeders can increase the chances 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 10 to 100 times more resistant to chlorine and chloramine than the ordinary bacterium *Escherichia coli* [in contrast, UV sterilization kills EM and ordinary bacteria equally (LeChevallier 2004)].

The laboratory culture techniques required to isolate EM provide a perfect example of how disinfectants enrich for EM (i.e., selectively boost the EM portion of the total bacteria population). Because EM grow much slower than other bacteria, laboratory cultivation of EM from diseased fish generally requires weeks and months [Astrofsky 2000; Herbst 2001; Sakai 2005; Yanong 2003]. Lab workers must kill faster-growing bacteria that often contaminate these tissue samples. Otherwise, these 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, the dye 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.

Water treatment, like decontamination during laboratory cultivation, selects for EM and often increases their numbers. This “EM enrichment” is a common occurrence in drinking water systems [LeChevallier 2004]. For example, chlorine/chloramine treatment at one water treatment plant reduced the number of EM in raw water from 55 per ml to 0.04 per ml. However, downstream in the distribution network, the EM population had dramatically increased to 700 per ml [Falkinham 2001]. Water treatment kills bacteria, including EM. However, the surviving EM form long-term biofilms in the water distribution pipes and constantly shed small quantities of EM into drinking water.

One investigation [Angenent 2005] of a hospital’s therapy pool, documents how incredibly enriched EM can become in a clean, disinfected environment. Water in the therapy pool was filtered with multiple pressurized sand filters followed by UV sterilizing filters, and then dosed with the disinfectant hydrogen peroxide. Despite being maintained and monitored according to public health standards, the pool was causing respiratory infections in pool lifeguards. The investigators were eventually able to prove that EM (mainly *M. avium*) in the pool water had caused the respiratory infections. They found that the pool water contained 20,000 EM per ml, which is *grossly* higher than the EM range (0.1 to 500 EM per ml) found in natural waters [Dailloux 1999]. The pool water had a low total bacteria count (400,000 per ml) suggesting EM enrichment in an environment that inhibited ordinary bacteria.

Interestingly, the investigators [Angenent *et al* 2005] found only minute traces of EM in the pool’s sand filters. Because filters collect debris, the filter environment was more nutrient-rich and “bacteria friendly” than the pool water. It supported a more normal bacteria population. The investigators identified the filter bacteria as mostly *Sphingomonadaceae*,  $\gamma$ -Proteobacteria,

and  $\beta$ -Proteobacteria. Apparently, filter conditions were favorable for normal bacteria growth, which reduced the EM population to virtual insignificance.

EM survive and thrive in nutrient-poor (i.e., “clean”) environments that starve ordinary bacteria. Steinert *et al* (1998) showed this experimentally when they placed *E. coli* and an EM (i.e., *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* bacterium 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!

EM enrichment under starvation conditions may explain why laboratory breeding facilities, where fish are maintained in ultra-clean tanks and otherwise receive ideal care, have sometimes had devastating MB outbreaks [Astrofsky 2000; Kent 2004; Sanders 2001]. I believe that disinfected, clean tanks can become enriched with large numbers of EM. Unfortunately, investigators focus considerable energy on identifying EM species in diseased fish instead of simply monitoring the EM concentration (# of EM per ml) in the tank water. Since all EM are potential pathogens, the number of EM that fish are exposed to is critical.

Disinfection is essential when a grossly infected fish colony has reached the “melt-down” stage. However, fish breeders need to understand that, over time, routine disinfection selects for EM and disinfectant-resistant EM, such as *M. fortuitum* (Table 2).

Disinfected tanks with clean water are deprived of nutrients and organic matter for normal bacteria growth. They provide a perfect environmental niche for EM. Bottom Line: The cleaner the tank, the more EM and chances of MB.

### **MB in Aquariums and Fish Hatcheries**

In my opinion, mycobacteriosis in home aquariums like mine is not due to the normal EM (environmental mycobacteria) microflora within the aquarium suddenly becoming pathogenic and preying on a weak fish. I also doubt that the disease occurs when healthy fish are subjected to a brief stressful event (EM grow so slowly that they would not have time to multiply within the fish to threatening levels during a brief immunity-suppressing event). Rather, MB outbreaks in healthy tanks result from the introduction of chronically infected fish. In my case, the newly introduced fish showed no symptoms for 3-8 months. For many months then, the infected fish were constantly shedding and excreting [probably from the intestine (Harriff 07)] enough EM into the water to infect vulnerable tankmates. By the time I realized there was a problem, other fish and tanks were infected.

I was able to get the MB disease outbreak under control by using UV sterilizers and euthanizing fish showing distress. After the outbreak, I believe that normal competition from ordinary bacteria gradually crowded out the remaining EM pathogens until their numbers became insignificant. My aquariums, which all contain soil and plants, inevitably provide considerable nutrients and organic debris to support normal bacterial growth. Unlike ultra-clean tanks, my tanks do not enrich the EM population.

Commercial fish breeders justifiably dread MB and use every means possible to prevent the disease from entering their breeding facilities. Unfortunately, many facilities emphasize procedures (disinfection and ultra-clean conditions) that can enrich for EM. Disinfection kills ordinary bacteria more than EM, setting up conditions that allow the surviving EM to grow

unchecked. Even if the disinfection kills every last EM in the tank, EM from outside sources (tapwater, fish, air, etc) will gradually recolonize the tank. EM are everywhere in the environment.

There is no cure for MB and none on the horizon. Disease eradication may be less effective than good fish management. I suggest that fish breeders: (1) quarantine all new fish; (2) give fish good care to reduce stress; (3) carefully monitor fish health and promptly remove sick fish; (4) use UV sterilizing filters during quarantine and disease outbreaks; and (5) go easy on routine disinfection in order to keep a normal bacterial microflora and prevent EM enrichment.

Happily, three years after the MB outbreak in my tanks, my Rainbowfish are doing fine. I am no longer afraid of mycobacteriosis.

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## TABLES

**TABLE 1. Characteristics of EM** (environmental mycobacteria).

- Gram positive, acid-fast staining, aerobic, non-motile rods
- The cell wall is lipid-rich and contains waxy mycolic acids that protect EM from chemicals and disinfectants (e.g., EM have been found growing in hospital disinfectant solutions)
- Extremely slow growth rate puts EM at a competitive disadvantage in nutrient-rich environments where other bacteria can easily out-grow them
- Extreme tenacity and ability to grow under nutrient-deprived, starvation conditions such as a year in distilled water
- Non-sporulating, but EM can survive for years within the cysts of infected amoebae
- Readily form biofilms in water distribution systems
- Readily aerosolize, such that disease can be transmitted via the air
- Pathogenic EM can survive and multiply in phagocytic cells (e.g., amoebae and macrophages) that kill ordinary bacteria

**Table 2. Bleach (ppm) Required to Eradicate Two Species of *Mycobacterium*** [Bardouniotis 2003]. Note that a typical disinfecting solution of ¼ cup Chlorox per gal of water contains 800 ppm of active chlorine [Astrofsky 2000].

Species	Condition of Bacteria	Treatment Time	
		30 min	2 hours
<i>M. fortuitum</i>	Suspended	53 ppm	53 ppm
	Biofilm	2,000	500
<i>M. marinum</i>	Suspended	<13	<13
	Biofilm	26	26

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## FIGURES

**Fig 1. Tracing the Disease Source.** This photo was taken in the summer of 2004 when MB had, unbeknownst to me, started infecting my fish. A few months beforehand, I had added newly purchased Neon Rainbows directly (without quarantine) to this 45 tank. Nothing too disturbing, but these fish did unusually poorly over the next few months. For example, one died mysteriously and another developed a large body sore 6 months later. The smallish fish in the center of the photo is one of the 4 new Neon Rainbows. Next to him are 5 Goyder Rainbows, two of which were diagnosed with MB several months later. I suspect that the 4 Neon Rainbows were infected with MB and gradually transmitted MB disease to their tankmates, especially the vulnerable Goyders. Only autopsies of the Neon Rainbows— immediately after purchase— would have either confirmed (or discounted) my suspicions.

**Fig 2. Susceptible Fish Species - “Goyder Rainbow”.** This male *Melanotaenia trifasciata* (Goyder River strain) appears robust, but he is almost surely infected with MB. Several months later, I had to euthanize him when he began to swim erratically. This beautiful fish, along with all 4 other Goyders that I raised from purchased eggs in 2000, was wiped out by MB. I had no problems with this otherwise vigorous strain before MB symptoms first appeared in Dec 2004. Some fish species are far more susceptible than others. This was shown experimentally by Wolf and Smith (1999). The investigators injected fish with *M. marinum*, a particularly virulent EM. Striped bass either died or became severely ill within 8 days. In contrast, hybrid Tilapia developed internal granulomas but otherwise showed no outward symptoms for at least 9 months.

**Fig 3. Spreading the Disease.** The Goyder Rainbow in the center of the photo probably brought disease into this tank. In Sept 2004 I had moved a pair of Goyders from the infected 45 gal into this 50 gal. At the time, I was blissfully unaware that the Goyders were probably all infected. Indeed, several months later a female *M. boesemani* in this tank became unnaturally overweight, lethargic, and had to be euthanized. Unnatural weight gain has been described in Rainbowfish diagnosed with MB.

**Fig 4. Rescue Mating.** The 4 Neon Rainbows that I now suspect brought MB disease into my tanks were dying out. In Nov 2004, I quickly set up a breeding tank when the final surviving female developed a body sore (if you look closely at the foreground fish, you can see a tumor-like mass just in front of the caudal fin). Before she and her partner died a few weeks later, she

produced 10 babies. Because I suspected she might have an infectious disease, I treated the 10 gal breeding tank with a standard dose (200 mg/day for 5 days) of the antibiotic erythromycin. While antibiotics are useless for treating MB disease, I suspect that they could possibly stop MB transmission from the diseased parents to their offspring. Indeed, antibiotics can kill EM suspended in growth media [Cirillo (1997)]. In contrast, once the EM have entrenched themselves in the fish or a biofilm or an amoeba, they are much less susceptible to antibiotics.

**Fig 5. Appearances are Deceiving- “Neon Rainbow”.** This female *Melanotaenia praecox*, a product of the “rescue mating”, gradually developed a curved spine in 2006. Although she otherwise appeared healthy, I suspected that she had chronic MB and was only now manifesting symptoms. So, in August 2006, I had this female autopsied and thoroughly examined by a veterinarian. Much to my surprise, she was found to be uninfected. The cause of the fish’s severely curved spine could not be determined, but it was not MB. Her autopsy, along with earlier ones of the 3 *M. boesemani* Rainbows (see text) proved that MB was no longer active in my tanks.

**Fig 6. Healthy Young from Diseased Fish.** These cute little Neon Rainbows, shown here waiting for dinner, are the healthy young of diseased fish. Since their birth over three years ago, they and their siblings have done well. The one female that developed a curved spine was found to be uninfected.

**Fig 7 Stopping Disease Transmission.** Centered in this May 2005 photo of my 55 gal tank is an infected Goyder Rainbow. Apparently, he did not transmit disease to the other Rainbowfish in the photo (*Melanotaenia boesemani*, *M. lacustris*, *M. herbertaxelrodi*, and *Chilatherina fasciata*), because they have had no problems. Fortunately, I had earlier added a UV sterilizing filter, which would kill EM pathogens released by the infected Goyder. While the UV sterilizer probably did not kill every single EM, it must have killed enough to protect the fish. To better gauge how contaminated the tank was, I added a “test Rainbowfish” in Oct 2005. This fish (a *M. boesemani*) was removed 8 months later, autopsied, and found to be uninfected.

**Fig 8. Back to Normal.** This is a recent (Jan 2007) picture of my 45 gal. Two years beforehand (Jan 2005), a pair of Goyder Rainbows from this tank were diagnosed with MB. Rather than tear down the tank, I added a UV sterilizing filter. The fish quickly improved. Indeed, the female Turquoise Rainbow (*M. lacustris*) shown in the center of the photo had a 4 mm body sore during the MB outbreak that gradually healed. Should I ever add new fish to my tanks, I will quarantine them for at least 3 months. My days of cavalierly adding new fish directly to established tanks are over.

**Fig 9. Granuloma Containing EM.** This photo is a close-up (400 X magnification) of a granuloma within an infected Betta fish. Acid-fast staining of this histological preparation shows the EM (environmental mycobacteria) as pink, rod-like structures walled off within the granuloma. A chronically infected fish may have many granulomas spread throughout its body. Granulomas containing acid-fast bacteria are the defining signature of chronic MB. [This photo was generously provided by the NCSU College of Veterinary Medicine (Raleigh, NC).]

**Fig 10.** This figure shows *M. marinum* (as fluorescent spots) distributed throughout a Zebrafish embryo injected with just 9 *M. marinum* bacteria a few days earlier. The infected macrophages have carried the bacteria far away from the injection site. The random distribution of bacteria (via the wandering macrophages) explains why MB symptoms are so unpredictable. Some (but not all) symptoms are reduced egg production, lethargy, sores, curved spines, swollen bodies, unnatural weight gain, emaciation, and unnatural black pigmentation.

Several investigators now use *M. marinum* infections in Zebrafish as a model for studying human tuberculosis (caused by *M. tuberculosis*), a disease that has infected one-third of the world's population and kills 3 million people each year. [Image is reprinted from Davis *et al* (2002) and used with kind permission of the primary authors and Elsevier Publishing.]