Mycobacteriosis in Aquarium Fish[1]

by Diana Walstad (Revised May 2017)

In the summer of 2004, I had been keeping and breeding Rainbowfish without problems for almost 20 years. After adding four new fish to one tank, the new fish over a period of about six months slowly died off one-by-one. When symptoms appeared on tank mates, 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 (**Fig**). However, the histological exam showed that the internal organs of both fish were riddled with granulomas containing "acid-fast bacteria." My fish had mycobacteriosis–a common bacterial disease of fish and reptiles that is incurable. Distressing! My fish were all going to die.

Symptom of MB

The jaw ulceration shown in this diseased Angelfish is similar to what I saw in my Rainbowfish that was diagnosed with MB.

(Photo courtesy of Stan Chung.)

MB (mycobacteriosis or "Fish TB") is considered to be the number one chronic disease in aquarium fish {4}. It was first documented in carp in 1897. Over the years, the disease has not abated. It causes half of fish deaths due to unknown causes. Because diseased fish show no consistent or defining symptoms, hobbyists underestimate its prevalence. If a newly purchased fish stops eating and dies after a few weeks or months, most hobbyists do not suspect MB (much less know what it is!). Additionally, chronic MB weakens the fish's immune system making the 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?

MB outbreaks have been reported in pristine scientific laboratories, zoos, commercial fish farms, ponds, etc. Wild fish in nature can and do get the disease. However, MB is a much more common health problem in aquarium fish than wild fish in nature. {20} Hopefully, this article will help hobbyists and fish breeders keep the disease out of their tanks and—if fish do become infected—deal with the problem more effectively.

Disease Presence

Fish may die within days or weeks from a major assault by the bacteria involved. Typically, though, fish develop a chronic disease manifested by a variety of symptoms: skin ulcers that don't heal, emaciation, abnormal swimming, lethargy, pop-eye, abdominal swelling (dropsy or ascites), "black head" disease, reduced reproduction, spinal deformities, etc.

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[1] Abbreviations: EM = environmental mycobacteria; gal = gallon; MB = mycobacteriosis; µg = micrograms (1/1,000 of a mg); mg = milligram; ml = milliliter; mm = millimeter; ppm = parts per million (e.g., mg/liter)

MB can only be confirmed by an autopsy and various lab methods. Upon autopsy, granulomas (whitish nodules of 0.05 to 4 mm diameter) can be seen visually in the spleen and kidney where these usually smooth, red-brown organs now have a pale, lumpy texture. A histological exam with "acid-fast staining" 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 generally indicate MB.

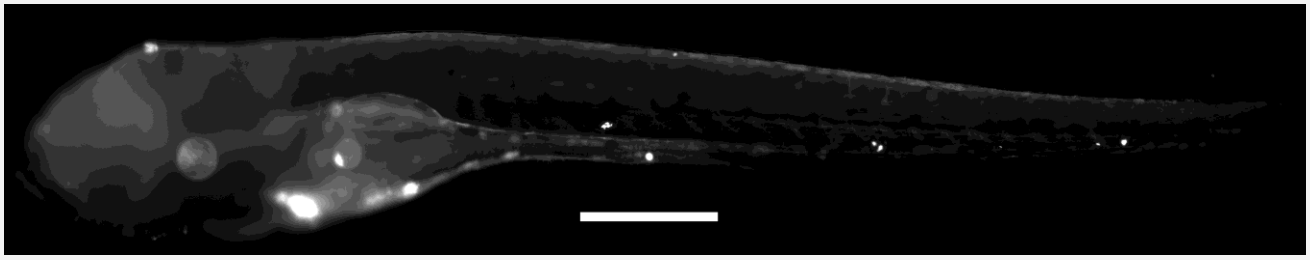
A variety of investigators have—via various culture and genetic methods—attempted to quantify the extent of MB in aquarium fish. Czech investigators {27} found that of the 70 fish that hobbyists had brought to their vet clinic because of unexplained death, 63% had MB.

***Betta splendens* with MB**

[Photo courtesy of NC State Veterinary College (Raleigh)]

Italian investigators {45} surveyed 387 diseased fish (representing 32 freshwater species and 12 marine species) from separate hobbyists' aquaria. The study revealed that 47% of fish (i.e., from homogenates of their liver, spleen, and kidneys) contained large numbers of the bacteria that cause MB.

In a separate survey (Spain), Gomez {15} randomly collected 200 debilitated fish (representing 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). Of the 200 fish, 41% had MB. Of the 24 fish species represented in the study, all species had some members with MB. Half of 34 debilitated guppies had MB.



Infected Zebrafish Embryo {10} shows the random nature of MB infection and helps explain why symptoms vary so greatly in aquarium fish. Investigators injected a zebrafish embryo, 32 hours post-fertilization, with 9 fluorescent-tagged *Mycobacterium marinum*, a species that causes MB. The 9 bacteria quickly infected macrophages (cells of the fish's immune system) that then carried the bacteria—via the blood stream—randomly from the caudal vein injection site. This fluorescence picture was taken 5 days later, giving time for the *M. marinum* to proliferate. It shows the bacteria (as glowing spots) distributed throughout the embryo rather than concentrated at the original injection site. (Bar shown is 400 μm in length.)

M. marinum infections in Zebrafish are used to study the pathogenesis of human tuberculosis and other diseases due to members of the *Mycobacterium* genus.

[Image used with permission of the primary authors {10} and Elsevier Publishing]

Environmental Mycobacteria

The bacteria that cause MB are all members of the genus *Mycobacterium*, which is divided 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 feed on organic matter and are found everywhere—in soils, natural waters, tap water, bottled water, etc. {12}[2]

In nature, EM are typically present in very small numbers. The exceptions are swamps and peat soils {24}. (The acidity, low oxygen, etc. in these "marginal environments" inhibit competing bacteria, thereby allowing the EM to proliferate unfettered.)

TABLE 1. Characteristics of EM

Many of the characteristics listed below stem from the fact that lipids make up almost 60% of the *Mycobacterium* outer membrane. [In contrast, the percentage is only 1-4% in Gram-positive bacteria and 20% in Gram-negative bacteria {44}]. This hydrophobic outer coating makes EM impervious to water-soluble compounds (antibiotics, disinfectants, stomach acid, dyes, etc). It also explains why EM are found preferentially at the water surface and in biofilms {12}.

- Gram positive, acid-fast staining, aerobic, non-motile rods
- Resistant to chlorine-based disinfectants
- Grow *very* slowly (population doubling times for EM range from 2 to 48 hours {33})
- Extreme tenacity under starvation conditions (e.g., can grow for a year in distilled water {33})
- Do not form spores, but can survive for years within the cysts of infected amoeba {30}
- Survive and multiply in protozoa

As of 2010, there were reportedly 140 species of EM {16}, but taxonomists continue to discover more. EM have characteristics (**Table 1**) that set them apart from other bacteria. For example, they grow much more slowly. Even the "fast growing" *M. fortuitum* has a sluggish "population doubling time" of 4.6 hours, while that of *Escherichia coli* is only 20 minutes {19}.

One survey of diseased fish showed a variety of EM species associated with MB (**Table 2**). *M. fortuitum* was the most common EM isolated from diseased fish. However, its prevalence in diseased fish may be due more to its widespread environmental distribution than its virulence.

In practice, the actual species involved in causing MB may be irrelevant. Less-virulent species often cause as much devastation as virulent species (and vice-versa). For example, most scientists do not consider *M. gordonae* to be a fish pathogen. Yet, it was the culprit behind heavy mortalities in several Asian guppy farms {36}.

One *M. peregrinum* strain destroyed an entire colony of valuable research zebrafish. Investigators predicted it would be highly virulent. However, when tested experimentally, it turned out to be much less virulent than an

TABLE 2. EM Species in Diseased Fish {45}

Table shows the percent of each EM species found in freshwater aquarium fish showing disease symptoms.

Data was drawn from 170 fish (21 species) that hobbyists had sent to the laboratory for disease diagnosis. (Each fish came from a different aquarium.)

***Mycobacterium* Species**	**% Presence in Diseased Fish**
M. fortuitum	50%
M. peregrinum	25%
M. chelonae	10%
M. abscessus	5.3%
M. gordonae	3.5%
M. nonchromogenicum	2.9%
M. marinum	2.9%

[2] EM are also called NTM (nontuberculous mycobacteria).

M. marinum strain that had caused only moderate disease problems in another research laboratory {40}. Any EM species—under the right circumstances and in sufficient numbers—can cause MB.

That said, *M. marinum* is generally considered to be the most virulent species towards fish and the one most frequently found in human infections (i.e., "fish-tank syndrome"). It is virulent enough to jump the species barrier.

EM are Not *Necessarily* Pathogens

Beran *et al* {6} screened 6 well-established, apparently normal aquariums for EM (**Table 3**). 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. A few fish contained EM, but the species were the same as those found in the tank—snails, plants, debris from the sediment and filter, and biofilms on the glass. Most likely, the number of EM was insufficient to cause disease. Notably, no *M. marinum* was found.

EM are part of the natural environment, so one would expect to find them in aquariums and fish. Unfortunately, many investigations do not *quantify* the EM presence in the fish or in its environment. They only list species found. This stems from the tradition that if a clinical lab found just a single colony, it was sufficient for disease diagnosis. Moreover, standard culture methods inadvertently kill off ≈99.9% of the EM actually present {2}.

EM enter fish via the mouth (not the gills or skin) {17}. Digestion does not kill EM {12, 33}, so one would expect to find live EM in the fish's intestines, fecal matter {32} and tank debris.

Investigators {17} 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) 1 to 20 colonies and only from their intestines. One fish, though, yielded 400 colonies from his intestine and had some *M. fortuitum* 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 makes one wonder if this particular fish was at risk for later developing MB?

TABLE 3. EM in Normal Aquariums [6]

Mycobacterium cultured from the fish and environment of non-diseased aquaria

***Mycobacterium* Species**	**Percentage Presence**	
	Show Tank	**Breeder Tanks**
M. fortuitum	36%*	22%*
M. chelonae	18%*	0
M. gordonae	9.1%	5.6%*
M. terrae	0	5.6%*
M. triviale	0	5.6%
M. diernhoferi	0	5.6%
M. celatum	0	5.6%
M. kansasii	0	5.6%
M. intracellulare	0	5.6%
M. flavescens	4.5%*	0
Unindentified*	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 normal intestinal microflora. Other bacteria help keep potential EM pathogens in check by depriving ingested EM of nutrients and attachment sites within the intestine. Disease might occur when the intestinal microflora is suddenly disrupted, for example by antibiotic treatment.

Disinfection and Cleanliness Enrich for EM

Ironically, conscientious fish breeders greatly increase the risk for MB by disinfecting tanks. EM are much more resistant to chemicals (antibiotics, detergents, Clorox, etc) than other bacteria. For example, EM are about 10 to100 times more resistant to chlorine and chloramine than the ordinary bacterium *Escherichia coli* {26}.

EM readily form biofilms, which look like slime to us, but they are actually organized communities of algae, protozoa, and bacteria. Once established in a biofilm, EM are more resistant than when they are suspended in the water (**Table 4**). Thus, a 30 minute treatment with 800 ppm chlorine (1/4 cup Clorox™/gal water) might eradicate *M. marinum* but not *M. fortuitum* residing in a biofilm.

Table 4. Bleach Treatment for Eradicating EM {5}

EM Species	Condition of Bacteria	Treatment Time	
		30 min	2 hours
M. fortuitum	Suspended	53 ppm	53 ppm
	In biofilm	2,000	500
M. marinum	Suspended	<13	<13
	In biofilm	26	26

Protozoa also provide protection for mycobacteria. EM holed up in the cysts of infected amoeba were able to survive a 24 hour exposure to free chlorine gas (15 mg/liter) {1}.

The laboratory techniques required to isolate and culture EM provide a perfect example of how disinfectants and antibiotics enrich for EM. Because EM grow much slower than ordinary bacteria, laboratory cultivation of EM from diseased fish generally requires weeks and months {4,13,36}. Lab workers must kill faster-growing microorganisms that often contaminate these tissue samples. Otherwise, bacteria, fungi, and molds will grow over the entire culture dish within days, thereby making detection of any EM impossible. Lab workers treat (i.e., "decontaminate") the fish sample with a potent chemical cocktail (mixture of sodium hydroxide, malachite green and detergent) before plating the sample onto culture dishes. Then, the culture medium itself usually contains antibiotics to further kill contaminating bacteria. Inevitably, many EM are killed. However, those that survive can now multiply freely on the culture dish, such that lab workers can detect an EM presence.

Water treatment, like decontamination during laboratory cultivation, selects for EM and inevitably increases their numbers. This "EM enrichment" is a common occurrence in drinking water systems {26}. Chlorine/chloramine treatment at one water treatment plant reduced the number of EM in raw, incoming 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 {11}. Water treatment kills bacteria, including EM. However, the surviving EM readily form biofilms in the water distribution pipes, which then constantly shed EM into drinking water. Investigators found–on average—a 25,000-fold increase in the numbers of EM immediately downstream from 8 different treatment facilities {11}.

EM survive and thrive in nutrient-poor (i.e., "clean") environments that starve ordinary bacteria. Steinert *et al* {37} 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 have been quite the opposite. For 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, 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!

Routine disinfection controls ordinary diseases, but with MB, it can backfire. One zebrafish breeding facility {29} started with sterilized eggs, used a UV sterilizing filtration system, and disinfected the tanks every 6 weeks. Despite all the cleaning, the facility experienced disruptive outbreaks of *M. marinum* for years.

Disinfected tanks with clean water are deprived of nutrients for normal bacteria growth. They provide a perfect environmental niche for generating large numbers of EM. This may explain why zebrafish breeding facilities, where the fish are provided with optimal care and ultra-clean conditions, have had numerous outbreaks of MB {4,22,29,40,42}.

Any situation that inhibits "ordinary" bacteria will almost surely increase the numbers of EM. Even if disinfection successfully kills all EM, tanks will be reseeded by EM from the environment.

Pool Study

Angenent's study {2} of a hospital's warm-water therapy pool documents not only how incredibly enriched EM can become in a "clean," disinfected environment. It also quantifies the EM presence in various parts of the pool—filters, biofilms, water, and air above the pool. Notably, the investigators used a detection method (direct counting of bacteria combined with genetic analysis) that they found to be ≈1,000 times more sensitive than the standard lab culture method.

Despite the pool's being outfitted with a "state-of-the-art" disinfection system and monitored according to public health standards, life-guards and other pool workers were coming down with respiratory infections. (Water was filtered with multiple pressurized sand filters followed by UV sterilizing filters and then dosed with hydrogen peroxide.)

Eventually, Angenent {2} proved that EM in the pool water had caused the respiratory infections. A winter sample of the pool water had a low total bacteria count (400,000 per ml). However, EM made up an incredibly high 5% of its total bacteria population. (The percentage in natural waters is more likely to be *less than* 0.1%.)[3] Moreover, the bacteria population in biofilms lining the sides of the pool near the water surface contained 30% EM. In the air above the pool water,[4] EM made up 80% of the total bacteria population. No EM were found in air outside the pool house or in the pool's filters.

Based on the evidence, it seems that the copious EM in the biofilms lining the pool were constantly shedding EM into the water faster than the UV sterilizers and hydrogen peroxide could kill them. EM released from the contaminated pool water as an aerosol into the air had infected the pool workers.

The absence of EM in the sand filters is probably because the filters collected debris, and therefore, contained enough nutrients to support a normal bacteria population. (The filter's bacteria were the predictable *Sphingomonadaceae*, γ-Proteobacteria, and β-Proteobacteria). When the investigators later re-examined the filters with more sensitive genetic probes, they did find EM genetic material, but only traces. Competition from ordinary bacteria had reduced the EM presence in the filters to virtual insignificance {3}.

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[3] The number of EM typically found in most natural waters is 0.1 to 500 per ml {9}. The total bacteria count of natural waters ranges from 500,000 to 4 million per ml {41}. [My calculations: 500 EM ÷ 500,000 bacteria X 100 = 0.1%]

[4] The air was sampled ~20 cm (~8 inches) above the water surface. The EM species found in the sampled air were identified as *M. avium, M. asiaticum, M. fortuitum, M. gordonae*, and *M. diemhoferi*. The respiratory infections in the pool workers were attributed mainly to *M. avium*, a human pathogen.

Protozoa as a Disease Reservoir

Microscopic protozoa within the tank's debris and biofilms are potentially a concentrated reservoir of pathogenic EM. Rotifers, paramecia, and amoebas all feed on bacteria, including EM. However, unlike most ordinary bacteria which are killed and digested by the protozoa, many EM survive after ingestion.

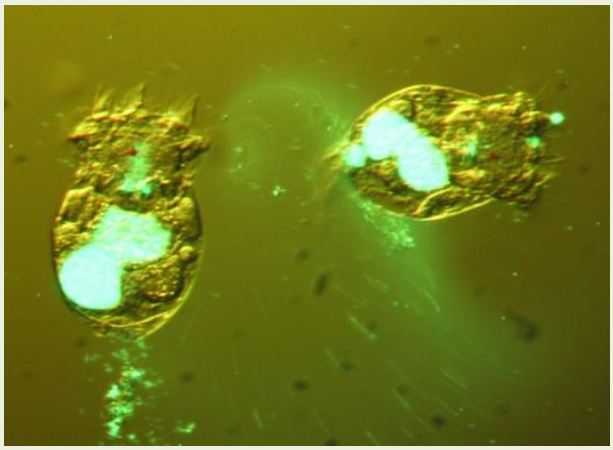
The lowly amoeba is sometimes used to study virulence factors of EM. [Both amoebas and macrophages (blood cells involved in protective immunity) engulf and kill "ordinary" bacteria, but virulent EM are unique in that they can survive and multiply within amoebas and macrophages.]

One investigator {1} screened 26 different EM species—mostly clinical isolates—for their ability to infect the amoeba *Acanthamoeba polyphaga*. All 26 EM survived and multiplied within the protozoan. Another investigator {8} found that *M. avium*, *M. marinum*, and *M. fortuitum*—all clinical isolates from diseased human patients—could survive and multiply in the amoeba *A. castellanii*. However, the amoebas quickly killed an environmental isolate of *M. smegmatis*, confirming its relative lack of virulence.

M. avium survived for over three years in an experimental culture of amoebas, suggesting that protozoa can provide a long-term reservoir for EM {30}. Amoebas infected with EM produce cysts infected with EM. The EM inside of cysts have been found to survive chlorine exposure {1} and antibiotic treatment {8}.

Live food cultures of paramecia and rotifers can also become infected. For example, one zebrafish breeding facility attempting to trace the source of an *M. marinum* outbreak, found *M. marinum* in the rotifers (*Brachionus plicatilis*) that were being fed to the baby zebrafish. The incoming rotifer cultures (from life food vendors) were "clean." Apparently, the rotifer cultures became infected *after* coming into the fish-breeding facility (**Fig**). {29}

Evidence suggests that EM are more virulent following survival in a protozoan. One investigator {8} tested this by growing an EM (*M. avium*) in the amoeba *A.castellanii*. The EM that had been grown in amoebas (for 2-3 days) multiplied 5 times more in macrophages than control EM (those cultured in broth). And when tested in mice, the amoeba-grown EM were able to colonize the intestine and grow significantly better in the mouse's liver and spleen than control EM.



Rotifers as a Disease Reservoir

Rotifers readily ingest EM. Here, *M. marinum* (labeled with green fluorescent protein), is shown inside the rotifers.

(Fig. 3 from Mason {29})

The EM that are released from an infected protozoan would be more virulent than the EM still innocently feeding on organic debris. For a basic tenet of pathogenic microbiology is that *bacterial growth within an animal increases its virulence*. In this case, the animal is not a fish, but a protozoan. Thus, while many bacteria lose virulence without an animal host, EM maintain their virulence. Whether an aquarium contains fish or not, EM will be busy infecting protozoa, thereby maintaining and sharpening their virulence.

That said, EM do not seem to multiply vigorously in amoebas {8} or snails {28}. In discussing his results, Cirillo {8} explains that the EM's enhanced virulence is a transient phenotypic change, that is, a temporary alteration in gene expression. Thus, it would not be due to genetic mutations, which are permanent changes, and therefore, more worrisome.

EM Presence in Aquarium Trade

Several surveys have found EM in aquarium fish from wholesale distributors and pet shops.

Zanoni {45} surveyed the prevalence of EM in imported fish sold in Italy. Fish directly from the vendor were pooled into batches of five similar fish (same species and 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 three of the 635 fish had clinical signs consistent with MB disease.

Investigators from Slovenia {25} examined 107 aquarium fish from pet shops. Autopsies with genetic testing revealed that 85 fish (almost 80%) contained EM. Nine of the infected fish contained *M. marinum*, generally considered to be the most pathogenic EM species.

Swedish investigators {18} surveyed aquarium fish from eight Swedish wholesalers. In a sampling of 90 fish per wholesaler, six wholesalers (75%) had EM in their fish. EM were present in 50% of pooled guppies, 50% of pooled neon tetras, and 25% of pooled fantail goldfish and dwarf Ram cichlids. (Each pool contained 10 fish.) Surprisingly, the most common EM was *M. marinum*.

From this data, one can conclude that many fish offered by aquarium stores are carrying EM when hobbyists purchase them. However, that does not mean that the fish will automatically develop MB.

Immunity

If healthy fish are carrying small numbers of EM, and many EM are potential pathogens, then immunity is the only thing truly protecting fish. Fish can develop substantial immunity against EM. For example, Pasnik {31} vaccinated fish so that they would produce antibodies against the Ag85A antigen, which is common to all *Mycobacterium*. 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 fish (unvaccinated) died within 3 weeks, whereas 90% of the vaccinated fish were still alive at 5 weeks.

EM infection may not automatically lead to MB.[5] Thus, one investigator {14} found healed and healing granulomas in fish that had been experimentally infected with less virulent EM species *M. shottsii* and *M. gordonae*.

Most people intuitively understand that stress compromises the immune system and makes fish vulnerable to disease. However, I doubt that a brief stressful incident (e.g., heater going off one night) would trigger MB. Any temporary immune suppression brought on by acute stress would more likely trigger infections from much faster-growing bacteria like *Aeromonas* or *Pseudomonas*. These potential pathogens are all part of a fish's intestinal microflora and the environment {34}. They would invade an immune-suppressed fish long before EM could multiply to threatening levels. I suspect that MB develops mostly in fish exposed to prolonged stress measured in weeks and months, not hours and days.

Because immunity slowly weakens with age, one would expect older fish to become increasingly vulnerable to MB. Indeed, MB is more frequently diagnosed in older fish {20}.

Every tank has its own unique microflora and contains a different composition of EM species. Under certain conditions, even a healthy fish with a robust immune system is vulnerable. A healthy fish might acquire disease when it is transferred into a new tank where it is suddenly confronted with a new EM species to which it is—immunologically speaking—unfamiliar.

Rainbowfish seem particularly susceptible to MB. One renowned Rainbowfish breeder {39}, recalled transferring half of his 30 healthy Goyder Rainbows to a normal, well-established 600 liter tank containing healthy fish. The 15 transferred Goyders developed MB, and within weeks, began dying.

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[5] In human tuberculosis, only 10% of people infected with *Mycobacterium tuberculosis*, the causative bacterium, every develop tuberculosis over their lifetime{35}.

Their only symptoms were labored breathing and getting fat. An autopsy and vet exam of the last 3 afflicted Goyders confirmed MB. Even after the Goyders were gone, MB outbreaks continued in this particular tank. Eventually, the owner tore the tank down and sterilized it. (Meanwhile, the 15 non-transferred Goyders showed no problems, even two years after the original outbreak.)

I believe that when the 15 Goyders were moved into the 600 liter tank, they had no immunity to this tank's unique EM microflora. Their established tank mates had immunity so they were still healthy at the time. Unfortunately, the transferred Goyders started an infection foci in the tank. As they sickened and died, they flooded the tank with EM—now with sharpened virulence—such that even established fish with some immunity developed MB. The tank itself became "diseased."

EM in Aerosols and Biofilms

EM are particularly abundant at the water surface and in biofilms. The bulk water is simply too polar (electrically charged) for these hydrophobic bacteria. EM attach to micro air bubbles that rise to the water surface. Once at the water surface, the air bubbles burst ejecting water droplets into the air {12}. Droplets containing EM are tiny enough for air currents to carry them off. Earlier, I described a pool study where the lifeguards were developing respiratory infections from aerosolized EM.

In fish rooms with open tanks and air bubblers, one can easily imagine that disease could be transmitted via aerosols {23}.

EM have a marked nutritional preference for lipids {33}, and lipids tend to float like oil on the water surface. This fact combined with EM's hydrophobic nature means that surface scum can be a major EM reservoir. Many fish feed at the water surface, thereby ingesting copious EM with every meal.

The hydrophobic nature of EM also makes them stick to surfaces where they form biofilms {33}. Investigators point to biofilms at the water surface, on tank glass, and on detritus at the tank bottom as major reservoirs of EM in zebrafish facilities {43}.

Thus, whenever I change the tank water, I first remove any surface scum by skimming the water surface with a water pitcher. I also scrape off the biofilm crud that lines the tank glass at the water line. These are two places that one would expect to find EM {2}.

MB in Zebrafish Hatcheries

Many research labs currently use zebrafish to study virulence factors of the mycobacteria involved in human diseases. Ironically, several of the fish breeding facilities that supply these labs have had devastating outbreaks of MB. In order to prevent and manage the problem, the scientists involved have carefully documented their findings. This published information can be very useful to aquarium hobbyists grappling with a very complex problem.

First and foremost, one learns that even facilities providing the very best care for their fish are not immune from the problem. There will always be potential EM pathogens in tanks and there will always be vulnerable fish. Hatchery employees working with many tanks might not spot a debilitated fish or one with chronic MB and no outward symptoms. The chronically infected fish then starts shedding large numbers of virulent EM into the fish colony. MB can spread to other tanks via water spills and aerosolized EM from diseased tanks or from infected live foods.

M. marinum bedeviled one zebrafish breeding facility for years {29}. In 2010, the problem climaxed when the *M. marinum* infected three workers ("fish-tank syndrome"). The outbreak occurred despite the fact that the facility started its fish colonies with sterilized eggs, used a UV sterilizing filtration system,

provided excellent fish care, and employed a sentinel fish program.[6] Spot checks found *M. marinum* contamination throughout the facility—in life food cultures, hose outlets, even on a computer keyboard in the fish room.

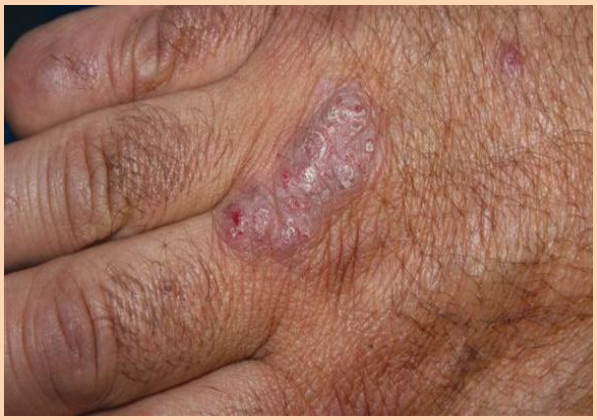
In response, the facility beefed up its entire system. Sentinel fish were autopsied sooner (within 3 months instead of 6). Live food cultures were moved away from the fish tanks to avoid accidental transmission by water splashes and aerosols. Tanks with older breeding zebrafish were moved to the bottom racks; tanks with juveniles—less likely to have MB—were placed on top racks. Elderly fish (older than two years and past their breeding age) were discarded. Tanks were torn down and heat-sterilized every 3 weeks to get rid of biofilms. The investigators carefully studied their egg sterilization procedure and found that exposing fertilized eggs to 30 ppm sodium hypochlorite for 10 minutes was highly effective. By 2015, *M. marinum* was not eliminated entirely from the facility, but the outbreak was brought under control and its impact on research minimized. {29}

Destroying all fish and starting over will not eliminate EM, but it can successfully remove an entrenched pathogen. A virulent *M. haemophilum* strain forced one facility to disinfect everything and start over with new zebrafish {43}. Within just four weeks of introducing new fish (from sterilized embryos), the investigators detected EM. Months later, five species of EM (*M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, and *M. phocaicum/mucogenicum*) had colonized the tanks and tank sumps. Two years later, the total clean-up appeared to have been successful. Occasionally, an autopsied fish revealed MB from a "garden variety" strain of *M. chelonae*. However, there were no more MB outbreaks or traces of the original *M. haemophilum* strain that had caused so much trouble.

My MB Story (Continued)

After my fish were diagnosed with MB in 2005, I worried about my own safety. I knew that the causative bacteria (EM) could infect me via any skin cuts whenever I put my hands in the tanks. I dreaded even the possibility of getting an EM infection (**Fig**). "Fish-tank syndrome" is a disease that is fairly common among fish handlers. Any aquarium hobbyist with sores on the hands or arms that won't heal should consult a physician—or fish veterinarian. One knowledgeable hobbyist I know contracted the disease despite the fact that all her tanks and fish "looked" normal.

For my fish, things got worse. Because I had moved fish around before I was aware of the MB, the disease had spread to my other two tanks. Fish began showing symptoms and dying. I considered tearing down the tanks, disinfecting everything, and starting over. However, my three established tanks contained pet fish and plants that I had had for many years. Even if I started over, it could happen again.



Fish Tank Syndrome

The EM (environmental mycobacteria) that cause MB in fish can cause painful, slow-healing sores in humans, mainly on the fingers, hands, and arms. The sore shown here on one pet shop dealer's hand is a milder case. Treatment involves a lengthy, 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 minutes of contact with tank water.

(Photo courtesy of Jurgen Hirt)

[6] For detecting latent MB, many large hatcheries house "sentinel fish" in sump tanks that receive effluent water directly from the main tanks—before the water is filtered and UV sterilized. Investigators autopsy the sentinel fish after several months and examine them for the presence of MB and other chronic diseases. {23}.

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). Even if I could not save the fish, I could protect myself from infection. I set up the UV filters so that water from the biofilter flowed through the UV filter around the internal 8 or 9-watt UV lamps before returning to the tank. I kept the UV sterilizers on 24 hours 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. Sores on a couple fish actually healed. 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 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 MB. 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.

Breeding Diseased Fish

A few months after purchasing the Neon Rainbows in the summer of 2004, I saw that they were having problems and not doing well. In November, I quickly set up a 10 gal breeding tank. The female had a small tumor-like mass on her body. Because I suspected she might have an infectious disease, I treated the breeding tank with a standard dose (200 mg/day for 5 days) of the antibiotic erythromycin. Once she and her partner finished spawning, I quickly removed them from the breeding tank. They both died a few weeks later, but I was able to raise 10 healthy young from their eggs.

I did not suspect MB at the time, so my breeding success with this pair of Neon Rainbows was just dumb luck. While a female livebearer will transmit MB directly to her young, Rainbowfish and other egg-layers do not {4}. Any disease transmission would have to be via EM shed into the water from the infected parents or stuck to the egg surface.

I think the erythromycin treatment helped. Many people do not realize that "naked" EM (i.e., suspended and not yet established in a biofilm, amoeba, or inside the fish) are susceptible to antibiotics. Indeed, Cirillo {8} in his amoeba-*M. avium* experiments used amikacin at 100 µg/ml to reliably kill the EM that were suspended in the culture medium. (The antibiotic had no effect on the EM once they were *inside* the amoebas.)

Fig 6. Appearances are Deceiving
This female Neon Rainbow *(Melanotaenia praecox*) gradually developed a curved spine in 2006. The cause could not be determined, but it was not MB.

A couple years later, I had one of the Neon Rainbows from this spawn autopsied and vet-screened for MB. While otherwise healthy in appearance and behavior, this female had developed a curved spine (**Fig**). Based on her history, I was convinced that she had MB, but an examination proved me wrong. She did not have MB. Results from her autopsy and earlier ones from the three *M. boesemani* Rainbows, finally convinced me that the MB outbreak in my tanks was over.

What Hobbyists Can Do

First and foremost, I would be careful purchasing fish. Aquarium societies frequently hold auctions where one can buy healthy fish directly from the breeder. Raising fish from eggs is another option. Frequently, I have been able to raise healthy young from newly purchased fish that died soon afterwards (*See* above). Some of these adult fish almost surely had MB.

Secondly, I would continuously monitor for the presence of MB and deal with it as fast as possible. The sooner the infection is dealt with, the better the prognosis for the tank, fish room, etc. One must try to prevent an EM pathogen from becoming entrenched.

MB outbreaks often result from the introduction of new fish into an established tank (**Fig**). Even if the new fish is not diseased when purchased, it is often stressed and immunologically weak. At the same time, it faces a whole new set of EM for which it has no immunity. I believe this scenario fits the MB outbreak in my tanks and the one I described earlier for one Rainbowfish breeder {39}.

Disease Source? In the summer of 2004 and a few months before this photo was taken, I added four new *Melanotaenia praecox* (Neon Rainbowfish) directly to this 45 gal tank without quarantine. The *M. praecox* did unusually poorly; none survived the year. One is in the center of the photo. Next to him are previously healthy Goyder Rainbowfish that later died from MB.

The *M. praecox* might have been diseased when I added them to the tank. However, their breeder stated that he had had no problems with these fish. Perhaps the new fish, then, did not have immunity to the unique EM microflora in this tank and developed MB *after* I added them to the tank. Once they became diseased, they ramped up the numbers and virulence of the tank's EM such that no fish was safe.

For preventing MB, routine quarantining is not always a sure thing. An infected fish frequently has developed some resistance to its EM pathogen, so it may appear healthy for many months or *never* show symptoms. That said, even a short quarantine (2-3 weeks) is better than none, and it vastly reduces the risk of introducing common diseases such as Ich. To bring latent MB out into the open, one might consider adding a "sentinel fish" to the quarantine tank. (*See* footnote on page 10)

I would strongly recommend using a UV sterilizing filter in any tank that contains new fish. A UV sterilizer greatly decreases the sheer numbers of EM, thereby reducing much of the fish's exposure to *potential* EM pathogens. The new (and often stressed) fish is given precious time (~2-3 weeks) to develop antibodies and protective immunity to a new EM microflora.

UV light only kills microorganisms that are suspended in the water. It will not kill the EM in tank debris, biofilms, or in infected fish.

However, UV sterilizers worked for me. I got excellent results with 3 different brands following the manufacturers' recommendations. If the sterilizer can kill green-water algae, it should kill EM.

[Suspended algae cells are less vulnerable than bacteria to UV light, because of their larger size and protective pigments.{21}] Moreover, UV kills EM and ordinary bacteria equally {26}, so—unlike disinfection—it will not enrich the EM portion of the bacteria population.

Some fish breeding facilities emphasize disinfection and ultra-clean conditions that I believe inevitably promote MB. My tanks contain soil, plants, and are not cleaned that much, except for the regular removal of surface scum (*See* page 9). I suspect that many fish with chronic MB have passed through my tanks during the 50 plus years that I have kept fish. None caused problems until 2004. Other long-term hobbyists have also learned to manage MB successfully with a similar measured approach.

TABLE 4. Preventing and Managing MB

- Provide good conditions that won't stress the fish.
- Strictly quarantine all new fish for at least 2-3 weeks.
- Use UV sterilizing filters, especially for tanks with new fish or at the first sign of problems.
- Promptly remove dying and/or dead fish.
- Remove surface scum.
- Do not feed "feeder fish"; they are prime candidates for carrying MB.
- Recognize that routine disinfection and ultra-clean tanks can *increase* the risk of MB.
- Try to keep juvenile fish separate from older fish.
- Recognize that MB can be transmitted by aerosols from nearby tanks.

There is no practicable cure for MB.[7] Quarantining and good fish husbandry (**Table 4**) are probably more effective than trying to eradicate EM. I stopped the MB outbreak in my tanks by using UV sterilizing filters and promptly removing debilitated fish. Once the MB outbreak was under control, I believe that competition from ordinary, faster-growing bacteria kept the problematic EM in check. Thus, I managed to contain the disease without destroying all the fish and "nuking" the tanks.

In my opinion, knowing how to prevent and/or manage MB is essential for successful, long-term aquarium keeping.

An Acceptable Recovery

This 2009 picture of my 50 gal tank shows several Rainbowfish that survived the 2005 MB outbreak. Using UV sterilizing filters, I learned how to manage MB.

As of April 2017, four of the original fish are still alive—a little old but still doing okay.

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[7] The London Zoo cured MB in valuable Australian lungfish by daily oral treatment for eight months with antibiotics rifampicin, doxycycline and enrofloxacin{38}.

REFERENCES

1. Adekambi T *et al*. 2006. Survival of environmental mycobacteria in *Acanthamoeba polyphaga*. *Appl Environ Microbiol* 72: 5974-5981.
2. 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.
3. Angenent, Lars. Personal communication in 2006.
4. Astrofsky, KM *et al*. 2000. Diagnosis and management of atypical *Mycobacterium* spp. Infections in established laboratory Zebrafish (*Brachydanio rerio*) facilities. *Comp Med* 50: 666-72.
5. Bardouniotis E, Ceri H. and Olson ME. 2003. Biofilm formation and biocide susceptibility testing of *Mycobacterium fortuitum* and *Mycobacterium marinum*. *Cur Microbiol* 46: 28-32.
6. Beran V *et al*. 2006. Distribution of mycobacteria in clinically healthy ornamental fish and their aquarium environment. *J Fish Dis* 29: 383-393.
7. Cangelosi J *et al*. 2004. Biology of waterborne pathogenic mycobacteria. In: Pedley S *et al* (eds). *Pathogenic Mycobacteria in Water*. IWA Publishing (London) for the World Health Organization, pp. 39-54.
8. Cirillo JD *et al*. 1997. Interaction of *Mycobacterium avium* with environmental amoebae enhances virulence. *Infect Immun* 65: 3759-67.
9. Dailloux M, Laurain C, Weber M and Haretmann PH. 1999. Water and Nontuberculous Mycobacteria. *Water Research* 33: 2219-28.
10. Davis JM, Clay H, Lewis JL, Ghori N, Herbomel P and Ramakrishnan L. 2002. Real-time visualization of *Mycobacterium*-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. *Immunity* 17: 693-702.
11. Falkinham JO *et al*. 2001. Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and other mycobacteria in drinking water distribution systems. *Appl Environ Microbiol* 67: 1225-31.
12. Falkinham JO, III. 2009. Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment (Review). *J Appl Microbiol* 107: 356-367.
13. Francis-Floyd, R and Yanong R. 1999. Mycobacteriosis in Fish. University of Florida. http://edis.ifas.ufl.edu/VM055
14. Gauthier, DT *et al*. 2003. Experimental mycobacteriosis in striped bass *Morone saxatilis*. *Dis Aquat Organisms* 54: 105-17.
15. Gomez S. 2008. Prevalence of microscopic tubercular lesions in freshwater ornamental fish exhibiting clinical signs of non-specific chronic disease. *Dis Aquat Organisms* 80: 167-171.
16. Griffith DE. 2010. Nontuberculous mycobacterial lung disease. *Curr Opin Infect Dis* 23: 185-190.
17. 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.
18. Hongslo T and E Jansson. 2016 Mycobacteria occurrence in aquarium fish from Swedish wholesalers. *Bull. Eur. Assoc. Fish Pathol*. 36: pp. --.
19. Kazda J, Pavlik I, Falkinham III JO, Hruska K (editors). 2009. *The Ecology of Mycobacteria: Impact on Animal's and Human's Health.* Springer Publishers (New York), p. 9.
20. Kazda (2009), p. 236.
21. Kaiser E and Herndl GJ. 1997. Rapid recovery of marine bacterioplankton activity after inhibition by UV radiation in coastal waters. *Appl Environ Microbiol* 63: 4026-4031.
22. Kent ML *et al*. 2004. Mycobacteriosis in zebrafish (*Danio rerio*) research facilities. *Comp Biochem Physiol* 138: 383-90.
23. Kent ML, Whipps CM *et al*. 2009. Recommendations for control of pathogens and infectious diseases in fish research facilities. *Comp Biochem Physiol, Part C* 149: 240-248.
24. Kirschner RA Jr *et al*. 1992. Epidemiology of infection by nontuberculous mycobacteria. *Am Rev Respir Dis* 145: 271-275.

25. Kušar D… M Pate. 2016. Mycobacteria in aquarium fish: results of a 3-year survey indicate caution required in handling petshop fish. *J Fish Diseases* DOI: 10.1111/jfd.12558
26. 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), pp. 143-168.
27. Lescenko P *et al*. 2003. Mycobacterial infection in aquarium fish. *Vet Med* 48: 71-78.
28. Marsollier L *et al*. 2004. Aquatic snails, passive hosts of *Mycobacterium ulcerans*. *Appl Environ Microbiol* 70: 6296-98.
29. Mason T,…Kent ML, Whipps CM, and Peirce J. 2016. Strategies to mitigate a *Mycobacterium marinum* outbreak in a zebrafish research facility. *Zebrafish* 13: S77-S87. {Available for free download at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4931754/}
30. Mura M *et al*. 2006. Replication and long-term persistence of bovine and human strains of *Mycobacterium avium* subsp. *paratuberculosis* within *Acanthamoeba polyphaga*. *Appl Envir Microbiol* 72: 854-859.
31. 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.
32. 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.
33. Primm TP *et al*. 2004. Health impacts of environmental mycobacteria. *Clin Microbiol Rev* 17: 98-106.
34. Rawls JF *et al*. 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci* 101: 4596- 4601.
35. Rom, WN and SM Garay (Eds.). 2004. *Tuberculosis* (2nd Ed). Lippincott Williams & Wilkins (New York), p. 17 (of 944 pp).
36. Sakai M *et al*. 2005. Characterization of a *Mycobacterium* sp isolated from guppy *Poecilia reticulate,* using 16S ribosomal RNA and its internal transcribed spacer sequences. *Bull Eur Assoc Fish Path* 25: 64-69.
37. 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.
38. Strike, TB *et al*. 2017. Investigation and management of an outbreak of multispecies mycobacteriosis in Australian lungfish (*Neoceratodus fosteri*) including the use of triple antibiotic treatment. *J Fish Dis* 40: 557-70.
39. Tappin, Adrian R. *Rainbowfishes. Their Care and Keeping in Captivity*. 2010. Art Publications, pp. 450-551. Book of 493 pages available at: rainbowfishes@optusnet.com.au
40. Watral V and Kent ML. 2007. Pathogenesis of *Mycobacterium spp*. in zebrafish (*Danio rerio*) from research facilities. *Comp Biochem Physiol, Part C* 145: 55-60.
41. Wetzel RG. 2001. *Limnology. Lake and River Ecosystems* (Third Ed.). Academic Press (New York), p. 493.
42. Whipps CM… Kent ML. 2008. Distribution and genetic characterization of *Mycobacterium chelonae* in laboratory zebrafish *Danio rerio*. *Dis Aquatic Organisms* 82: 45-54.
43. Whipps CM… Wagner R. 2012. Mycobacteriosis in zebrafish colonies. *ILAR Journal* 53: 95-105.
44. 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 844-69.
45. Zanoni RG *et al*. 2008. Occurrence of *Mycobacterium* spp. in ornamental fish in Italy. *J Fish Dis* 31: 433-441.

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