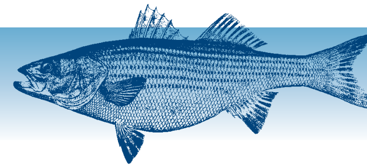

Prevention and Disinfection of *Mycobacterium* sp. in Aquaculture

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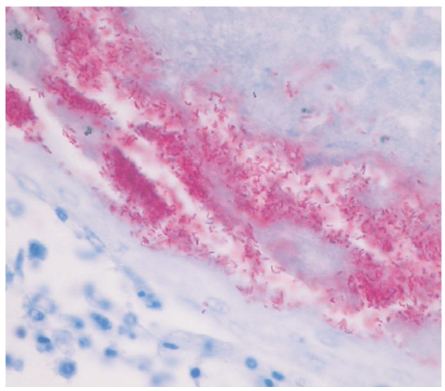
Introduction

Mycobacteria are Gram positive, aerobic, rod-shaped bacteria that are free living saphrophytes in the environment. There are over 54 members of the genus *Mycobacterium*, most of which are harmless to fish and humans. However, several are serious fish pathogens and have a long history of causing significant losses in food, pet (ornamental/tropical) and baitfish culture. The purpose of this fact sheet is to provide basic guidelines for the prevention of mycobacteriosis in aquaculture facilities, control of the spread of disease, and disinfection of tanks and equipment when necessary.

Why Treat *Myco* Differently from Other Bacteria?

While many of the mechanisms by which pathogenic mycobacteria function are still being discovered, there are several properties that make these bacteria extremely difficult to eradicate. First, several species have shown resistance to the fish immune system. They infect and live freely in the very cells responsible for their destruction. Second, many *Mycobacterium* are extremely hardy and have a dense cell wall that prevents the penetration of many common disinfectants. Thus, agents such as chlorine require higher doses and extended contact time. Finally, some



species of aquatic *Mycobacterium* can cause disease in humans, especially in immuno-compromised individuals. While primarily manifesting as skin infections of appendages, treatment with antibiotics can be lengthy.

Control and Prevention

Control of mycobacteriosis in culture settings is extremely difficult once an epizootic has occurred. In most cases, eradication or depopulation of infected animals followed by disinfection of the culture system is the only acceptable means. While treatment with antibiotics has been attempted with certain species of *Mycobacterium*, limited examples of success have been reported (Conroy and Conroy 1999). Currently, there are no FDA approved drugs for the treatment of mycobacteriosis, and experimentation in culture settings is highly discouraged. Without knowledge of the efficacy of the drug and dose being used, there is a great risk of compounding the problem by producing highly resistant strains. With limited treatment options, the best mechanism of control is prevention. As in all disease prevention, good husbandry practices are essential. This means minimizing stressors such as poor water quality, improper nutrition, excessive handling, and crowding. Just as important is preventing the transfer of pathogens through some common sense practices:

• ***Know the Source and History of New Animals.*** Knowledge of the facility of origin, its sanitation practices, and disease history can prepare you for potential problems. In addition, strain history may provide clues to disease susceptibility.

• ***Quarantine All Sick or Newly Arriving Animals.*** Having a separate, physically isolated, system for new arrivals can greatly reduce the risk of disease outbreaks affecting brood stock and production animals. Maintain animals in quarantine for a minimum of several weeks to ensure proper feeding and health of the population. Fish showing clinical sign of disease should be immediately removed and quarantined to reduce the risk of pathogen transfer.

• ***Practice Good Sanitation.*** Good sanitation practices will alleviate potential sources of contamination and minimize pathogen transfer. This includes general cleanliness of the facility and floors, control of organic buildup, disinfection of nets and brushes after each use and when used from tank to tank, foot baths by entrances, and proper personal hygiene.

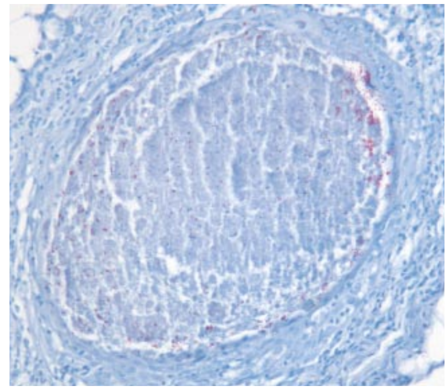
General disinfection can be accomplished by soaking nets and brushes for 5 minutes in 200 mg/L available chlorine, followed by freshwater rinse. To ensure neutralization of chlorine, a sodium thiosulfate solution can be used. For each gallon of 200 mg/L chlorine solution, 5.6 grams of sodium thiosulfate is required. Disinfectant for foot baths can be prepared similarly and should be used by all personnel upon entering or exiting the building. Finally, all personnel should regularly wash their hands after working in the facility, and wear gloves when in contact with fish or water. One of the most common routes of infection in humans is through cuts or scrapes on hands or feet. A detailed description of these practices is available in the article "Sanitation Practices for Aquaculture Facilities" provided by the University of Florida's Cooperative Extension Service at http://edis.ifas.ufl.edu/AE081.

• ***Use Ultraviolet Sterilization.*** The effectiveness of UV sterilization on fish pathogens has been well documented, and increases dramatically with pre-filtration to remove solids (Bullock and Stuckey 1977, Liltved and Cripps 1999). UV lighting is currently used in many hospitals to control airborne pathogens, including *M. tuberculosis*. It is important, however, that the appropriate system is chosen for your facility's water flow rate, and that bulbs are cleaned and replaced at regular intervals to ensure effectiveness. Exposure intensities of over 45 mW/cm^2 will control the more resistant aquatic *Mycobacterium* (Miyamoto et al. 2000).

Foot baths (top) and net bins (bottom) of 200 mg/L available chlorine can greatly reduce pathogen transfer. Solutions need to be changed weekly to maintain effectiveness.

The use of ozone has also gained popularity for the enhancement of water quality. Unfortunately, the efficacy of ozone for mycobacterial fish pathogens has not been well established (see Taylor et al. 2000 for related information).

• ***Clinical Signs.*** Mycobacteriosis, like many bacterial diseases, has no unique external clinical signs. Emaciation, external lesions, bulging of the eyes, and reduced growth have all been associated with this disease. Internally, grayish-white nodules (granulomas) may be visible in the spleen, kidney, or liver of severely affected fish. However, the presence of granulomas does not confirm the presence of mycobacteria. Diagnosis requires the detection of acid fast (retains stain after acid-



Top: Developing granuloma in tissue section; bacteria appear pink in this photo. Bottom: Grossly visible granulomas in fish spleen.

alcohol wash), rod-shaped, non-branching bacteria in histological sections. To be certain, confirmatory diagnosis should be sought by either culture of the bacteria or through genetic identification.

• ***Disinfection/Decontamination.*** Complete disinfection of an aquaculture facility can be a daunting task, and will severely limit production during the decontamination process. If a severe outbreak of mycobacteriosis, or other zoonotic disease occurs, decontamination is a necessity. It is also wise to plan for facility disinfection on a regular schedule to reduce the risk of disease transmission. The first step in decontamination is a thorough cleaning of the facility to remove organic material. Organic material can harbor bacteria and greatly reduce the effectiveness of most chemical disinfectants. Additionally, all readily replaceable equipment and materials likely to have been exposed should be either discarded or cleaned in preparation for decontamination.

As mentioned previously, many mycobacteria are highly resistant to low levels of chlorine commonly used in waste-water treatment and swimming pools. However, higher concentrations can be quite effective. Best et al. (1990) demonstrated that 10,000 ppm available chlorine was effective against *M. tuberculosis* (considered the most resistant of the *Mycobacterium*), in both direct contact and on surfaces with organic material within one minute of application. While most of the data on tuberculocidal agents comes from medical applications where longer contact times are not practical, others have documented efficacy in practice with lower concentrations. Astrofsky et al. (2000) effectively managed an outbreak of several species of aquatic *Mycobacterium* by using both quarantine practices and system disinfection in two zebrafish (*Brachydanio rerio*) culture facilities. The authors circulated 800 ppm available chlorine (¼ cup of bleach per gallon) through the system for 3 days, followed by draining, refilling, and aeration for an additional 3 days. Other commercial disinfecting compounds containing phenols or glutaraldehyde have been shown to be effective and are available through scientific or laboratory supply vendors. An example of common disinfectants and doses is available online at http://www.oie.int/eng/normes/fcode/A_00069.htm.

Many of these disinfectants, however, can leave residues, have offensive fumes, and in the case of glutaraldehyde, require longer contact times. Alcohol (70% isopropyl alcohol) can also be used for disinfection and is available in non-aerosol con-

Table 1. Agents available for general disinfection of facilities and equipment and efficacy against *Mycobacterium* sp. Always consult manufacturer for use and safety requirements.

Compound	Use	Pros	Cons
Sodium hypochlorite	Foot baths, spills, floors, walls, counters, nets, brushes, and whole tanks	Broad spectrum, cheap, readily available	Inactivated by organic matter, corrosive, irritant at high doses required for *Mycobacterium* sp.
Glutaraldehyde	Instruments, small materials	Highly effective	Toxic, requires fume hood and protective equipment
Formaldehyde	Large and small equipment	Highly effective	Potential carcinogen, fume hood and protective equipment required
Iodophores	Metal surfaces and equipment	Effective	Inactivated by organic matter
Phenolics	Alternative to bleach	Effective, less corrosive than bleach	Residuals, can be an irritant
Alcohols	Equipment and surfaces	Effective, readily available	Evaporates quickly which can reduce contact time, flammable, organic matter reduces effectiveness
Quaternary ammonia	Foot and equipment baths, surfaces	Broad spectrum, readily available	Low activity against *Mycobacterium* sp.
Heat	Autoclave of tools, 120° and higher	Effective	Expensive initial equipment cost, only works for smaller items
UV	Source and effluent waters	Effective reduction and prevention tool, broad spectrum if high rates are used	Effectiveness decreases with bulb life and organic load

tainers for use on small areas and surfaces such as table tops, as well as equipment which may be sensitive to other chemicals. In all cases, be sure to follow product labels closely for dosage, contact times, and safety of personnel.

Summary

Mycobacterium causes a chronic disease in freshwater and marine fish often resulting in significant losses. Infected fish have signs similar to that of other diseases requiring confirmation by histological analysis, bacterial culture, or genetic identification. Since there is no known treatment for infected fish, depopulation is often required to reduce the risk of spreading the disease to other culture systems or facilities. Preventative measures including quarantining new fish shipments and sick animals, using good culture management practices, and dedication to sanitation are especially important for culture facilities. Mycobacteria in general have a thick, waxy-like cell wall which makes them more resistant to many common disinfecting agents. Several commercial disinfectants are effective and should be used regularly to sterilize harvesting equipment and culture systems. Product labels should be closely followed and safety precautions taken prior to use. UV light can be an effective tool in all systems and can be used to treat incoming water and facility effluent. Another important consideration with certain aquatic mycobacteria is their ability to cause persistent skin infections in humans. It is important to keep open wounds away from infected water and fish, to use gloves and to wash with antibacterial soap. Attention to proper sanitation practices can be the best defense to this troublesome and persistent disease.

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