



Successful treatment of enteric myxosporiosis in a collection of yellow tangs *Zebrasoma flavescens* at a public aquarium

Michael Hyatt, DVM

Randall, J.E., 1997

Randall's tank photos:

<http://fishbase.org/References/FBRefSummary.cfm?ID=28618>

Introduction

Yellow tangs *Zebrasoma flavescens* are salt water fish belonging to the surgeonfish family Acanthuridae and are one of the most common fishes in the marine aquarium hobby. They are found on shallow reefs in the Pacific and Indian Oceans between Japan and Hawaii. They feed on benthic turf algae and can grow to 20 cm¹. Although yellow tangs are the most abundant species imported from Hawaii, and third most in the world², information is lacking regarding natural parasites or disease processes that may affect fish in the hobbyist trade or public

aquaria. This report documents a pathogenic enteric myxosporean in yellow tangs and its successful treatment.

History

A large collection (approximately 160) of wild-caught yellow tangs on display at a public aquarium was evaluated for multiple daily mortalities. Numerous fish showed signs of severe emaciation, inappetance, lethargy, and sudden death. In the same system, over 1000 comingled blue devil damselfish *Chrysiptera cyanea* showed no evidence of disease and no mortality. The

5,700 gallon (21,660 liters) cylindrical display contained a crushed coral substrate with coral rubble décor. Life support consisted of rapid sand filtration and temperature regulation. Water quality parameters including total ammonia nitrogen, nitrite, nitrate, pH, and alkalinity were within acceptable limits. Fishes were fed a wide variety of food including daily romaine, chard, broccoli, zucchini, and yellow squash and a daily broadcast item, primarily gel, flake, pacifica krill *Euphausia pacifica*, or bloodworms (midge pupae) *Chironomus* spp. The system had been

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Nitrate: a rising concern in fish health management

By Heather Hamlin, MS, PhD

Once seen as a relatively innocuous end product of biological filtration, there is growing evidence that nitrate is indeed a material water quality hazard for fish health management.

Nitrogenous compounds including fish waste, uneaten feed, and decaying matter are broken down, releasing ammonia. Nitrification is the natural process by which *Nitrosomonas* and other genera of nitrifying bacteria convert ammonia to nitrite. Nitrates enter aquatic systems

through the bacterial degradation of nitrite by *Nitrobacter* and other groups of nitrifying bacteria and can also enter aquatic systems through tap water. Although 10 ppm NO₃-N is the maximum allowable limit for tap water, nitrate contamination is the leading cause of municipal water supply closures.

Ammonia and nitrite are decidedly toxic to aquatic life. Many aquarium manuals describing the nitrification cycle explain it as a process by which ammonia and nitrite are broken down into relatively harmless

nitrogenous compounds, referring primarily to nitrate.

Historically, nitrate has been viewed as non-toxic because it takes very high levels, relative to unionized ammonia and nitrite, to elicit obvious health effects. It is thought the most commonly known toxicological effects of elevated nitrate stem from its endogenous conversion to nitrite, resulting in methemoglobinemia, a condition in which the hemoglobin in the fish's blood cannot successfully carry oxygen to the tissues.

This condition, commonly

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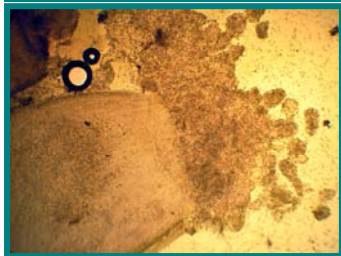


Figure 1. Wet mount of intestine with gray debris extruding. 40x magnification.

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running for 4 months prior with no medical problems reported. Also, no concerns were reported during a 45-day quarantine period.

Physical exam and necropsy

On physical examination many fish were severely emaciated and lethargic with multifocal red-splotched skin and some swam erratically. There were also more aggressive, healthy-appearing tangs in good body condition within the general population. These fish appeared to have

good appetites and were aggressively preventing some of the unhealthy emaciated fish from obtaining food. Many fish on display at public aquaria have an inherent value, financially and emotionally. Therefore, necropsies were only performed on fresh mortalities or those severely affected that were humanely euthanized due to a grave prognosis.

External cytological examination of skin scrapes, fin clips, and gill clips were unremarkable on all fish examined. Gross internal examination revealed minimal

amounts of ingesta throughout the gastrointestinal tracts. There was, however, a gray particulate matter in the mid and distal intestines. Livers appeared to be moderately atrophied and uniformly red. All other organs and structures were unremarkable. Microscopic wet mounts of different regions of the intestinal tract including, proximal, mid, and distal portions, revealed clumps of gray cellular debris (Figure 1). At higher magnification the gray debris was composed of dense

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Nitrate-Nitrogen (NO₃-N) and Nitrate Ion (NO₃⁻)

Most nitrate test kits report results as mg/L NO₃-N. Multiply mg/L NO₃-N by 4.4 to get mg/L NO₃⁻.

termed brown-blood disease, may result in brown or chocolate colored blood, although symptoms and even death could occur before the discoloration is apparent. In addition, extremely high levels of nitrate may cause death without methemoglobin levels reaching fatal thresholds, indicating another mode of toxicity is present, but not well understood.

These symptoms of toxicity have become the threshold of safety, below which most fish appear healthy and grow as well as fish housed in low

nitrate environments. There is growing evidence, however, that even relatively low concentrations of nitrate are capable of causing significant damage to the endocrine system¹. Therefore, the most commonly harmful effects of nitrate are the least obvious and least likely to be treated.

In commercial aquaculture, nitrate concentrations in recirculating systems with limited water exchange often exceed 60 mg/l NO₃-N. In these types of systems, nitrate is the limiting factor for water exchange. Although

most commercial systems currently use pond or flow through culture systems which keeps nitrate levels low due to heavy water exchange, the value of water is increasing at an alarming rate, and the indiscriminate use of water is becoming severely restricted.

Aquaculture, by definition, requires water to cultivate its products. As water becomes increasingly limited, nitrate contamination will become a significant concern.

Denitrification filters capable

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Michael Hyatt received a bachelor's degree in Marine Science and Biology from the University of Miami in 2000, and a DVM from the University of Florida (UF) in 2006. After completing a small animal internship at Affiliated Veterinary Specialists in Orlando, followed by a year in private practice, he completed a veterinary internship at the

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Heather Hamlin has a Bachelor of Science in Biology from the University of Maine, a Masters in Marine Bio-Resources from U-Maine and a PhD in Fisheries and Aquatic Science, UF. Currently she is a

postdoc in the Biology Department at UF and is also the Associate Director of the HHMI GATOR mentorship program. Prior to and while receiving a PhD, she worked as a Senior Biologist in the Center for Aquaculture Research and Development at Mote Marine Laboratory in Sarasota, Florida, for 8 years. ➡

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populations of spore-forming parasites, believed at the time to be a coccidian (Apicomplexa), although later identified as a myxosporean (Myxozoa) (Figure 2). Depending on developmental maturity, the spores were round with a definite sutural line with bilaterally symmetric portions, each containing two elliptical polar capsules at each end (Figure 3), or they were semicircular with polar capsules at each end as if the former had divided (Figure 4). The semicircular stage was predominant. A moderate *Spironucleus*-like flagellate population was also noted. Intestinal tissue samples for histology were collected in 10% neutral buffered formalin. There was no evidence of extraintestinal myxosporeans in other tissue wet mounts.

Differentials and Ante-mortem Diagnostics

Differential diagnoses for emaciation include poor nutrition, inadequate nutrition secondary to chronic stress, tank-mate aggression or other causes of anorexia, parasitism, enteritis, or sepsis. Differential diagnoses for red skin lesions include trauma, dermatitis, or sepsis. Depending on animal size, diagnostics may include blood work, blood cultures, radiography, ultrasonography, or even surgical biopsy. In this case, an appropriate diagnostic could include a cloacal wash and direct fecal examination under sedation. For most myxosporeans, if a species identification is requested, advanced diagnostics such as electron

microscopy or polymerase chain reaction (PCR) may be pursued.

Treatment

Initially the organism was believed to be a coccidian based on morphology and enteric origin. As there were no published treatment recommendations for piscine coccidiosis, an empirical treatment of sulfadimethoxine (Albon®) was selected. The 5% oral solution was added into a gel-based diet and fed at a dose of 50 mg/kg. Unfortunately, mortalities persisted during the 31 days of treatment. There was also no change in clinical signs. Treatment was switched to a different coccidiostat consisting of a combination of amprolium and salinomycin. Twice daily, fish were fed approximately 100 mg/kg of

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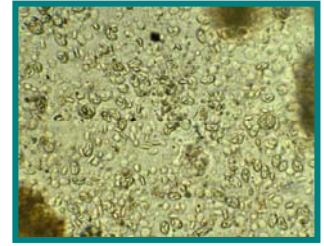


Figure 2. Wet mount of intestinal gray debris revealing heavy population of a myxosporean in different stages of development. 400x magnification.



Figures 3 above and 4 below: Disporoblastic and unisporoblastic myxospore stages, respectively, with divergent terminal polar capsules. Wet mount photos at 400X magnification, enlarged with Adobe Photoshop.

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of reducing nitrate are generally costly to operate, and as such, aquatic managers will need to understand exactly what concentrations of nitrate are to be targeted in these systems. New research has shown that previous limits designed to prevent traditional toxic effects of nitrate, such as brown-blood disease and mortality, may not be adequate to prevent considerable sublethal health effects.

Issues concerning water use and discharge will likely be similarly problematic for aquariums, and aquarists will also need to be aware of the possibilities of nitrate induced illnesses.

Captive female Siberian sturgeon *Acipenser baerii* exposed to 57 mg/l

NO₃-N have been shown to have significantly elevated plasma testosterone and estradiol concentrations². Researchers speculated that the elevations may not have been caused by increased steroid synthesis, but by reduced hormone clearance by the liver. If liver function is compromised, the fish will not be able to adequately remove toxins and therapeutants from circulation as well.

Whitetip reef sharks *Triaenodon obesus* housed in aquariums with elevated nitrate have been shown to develop hypothyroidism and goiter³. Nitrate has been shown in terrestrial animals to compete with iodine in the thyroid leading to similar conditions.

Mosquitofish *Gambusia affinis*

in Florida springs exposed to nitrate concentrations as low as 4-5 mg/L NO₃-N are less likely to be pregnant during the reproductive season, and males have a significantly shortened gonopodium, a modified fin which transfers sperm to the female^{4,5}. Those fish that are pregnant have significantly smaller offspring.

Sensitivity to nitrate varies widely among fish species and lethal concentrations can vary considerably. Planehead filefish *Stephanolepis hispidus* have a 96-hr LC₅₀ of 577 ppm NO₃-N whereas the 96-hr LC₅₀ of beaugregory *Stegastes leucostictus* is over 3000 ppm NO₃-N^{reviewed in 6}. Mortality of larval cutthroat trout *Oncorhynchus clarki*, Chinook salmon *Oncorhynchus tshawytscha*, and rainbow trout *Oncorhynchus mykiss* has been shown to occur at

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gel diet at 2% body weight for 16 days. After approximately 3 days on the new treatment regime, mortalities stopped. By the end of treatment clinical signs had dissipated and body condition was improving. One mortality did occur after 2 weeks, but no myxosporeans were observed at necropsy. One yellow tang was sedated with tricaine methane sulfonate (MS-222) and examined for parasites by direct examination of feces obtained from a cloacal wash. Once again, no organisms were observed. The treatment was discontinued and a cloacal wash was performed once weekly on a random fish for 3 consecutive weeks; no parasites were seen at any time.

Discussion

To the best of our knowledge, this case documents the first report of an enteric myxosporean in yellow tangs. Myxosporeans, once believed to be protozoal parasites of fish and aquatic invertebrates, are now classified as metazoa³. They are characterized by multicellular spores with polar capsules containing polar

filaments. They are morphologically diverse, varying in both shape and size. For most genera the polar capsules are in close proximity, but in some genera the polar capsules are found at opposite ends of the spore. Some myxosporeans show high host specificity while others may infect a wide host range. Myxosporeans have been found in fresh, brackish, and marine bodies of water and at all temperature ranges⁴.

Many myxosporeans have a complex life cycle involving indirect or direct transmission depending on genera. For those with an indirect life cycle, final hosts are believed to be oligochaete and polychaete worms or bryozoans with fish as an intermediate host. The most common oligochaete host is the tubificid worm, *Tubifex tubifex*. The life cycle involves three stages of development: schizogony, or proliferative stage, gametogony, and sporogony. For those with indirect life cycles, all three stages occur within the invertebrate host producing an actinospore infective to the intermediate fish host. Once in fish, schizogony occurs to

produce myxospores. Genera with a direct life cycle do not produce an actinospore. However, for many myxosporeans, life cycles have not been elucidated⁴.

Myxosporeans infect a wide range of tissues and organs including skin, muscle, gills, cartilage and bone, gonads, brain and spinal cord, kidney and urinary tract, gall bladder and liver, swim bladder, and intestines, although rarely. Details on extraintestinal infections are out of the scope of this paper, but some well studied diseases include proliferative gill disease (*Henneguya ictalur*) of channel catfish *Ictalurus punctatus*, proliferative kidney disease (*Tetracapsuloides bryosalmonae*) of salmonids, and whirling disease (*Myxobolus cerebralis*) of salmonids^{4,5}.

Enteric myxosporeans usually have a direct life cycle with infections transmitted via coprophagy, feeding on infected tissues, cohabitation with infected fish, and exposure to contaminated water. In salmonids, *Ceratomyxa shasta* causes losses in hatchery stocks, wild juveniles, and pre-spawning adults^{6,7}. Clinical signs include

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nitrate levels as low as 2.3-7.6 ppm NO₃-N⁷. These concentrations are well below the drinking water standard of 10 ppm NO₃-N.

Additionally, nitrate tolerance can change dramatically for different life stages. Siberian sturgeon are far more tolerant to nitrate as fry, but become increasingly susceptible to nitrate toxicosis as they age⁶. Therefore, maximum allowable nitrate concentrations will vary and depend not only on species,

but life stage as well.

Although the sublethal effects of nitrate are only beginning to be understood, it is clear the potential for significant reproductive and thyroid effects. Although more work is necessary to determine the mechanisms involved, as well as long term consequences of exposure, no longer should nitrate be considered inconsequential in aquatic fish health management.

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anorexia, lethargy, emaciation, and abdominal distension secondary to severe hemorrhagic enteritis. *Enteromyxum scophthalmi* is highly pathogenic causing enteromyxosis in cultured turbot *Scophthalmus maximus*^{8,9}. *E. leei* is a pathogen of gilthead sea bream *Sparus aurata* and other Mediterranean marine cultured species causing severe enteritis and mortalities^{10,11}. In Japan, emaciation disease of tiger puffers *Takifuga rubripes* caused by *E. leei*, is characterized by general emaciation, appearance of bony ridges along the head, and mortalities as high as 60%^{4,12}. In Florida a *Fabespora* spp. was identified in the intestines of common snook *Centropomus undecimalis*¹³; however, clinical signs were not reported.

Morphologically, the myxosporean in this case closely resembled *Fabespora*

spp. with its semicircular spore and divergent terminal polar capsules. The major difference between the tang myxosporean and *Fabespora* spp. is the shape of the polar capsules. Like most polar capsules, *Fabespora* spp. polar capsules are round¹³. The tang myxosporean polar capsules were elliptical, similar in appearance to coccidian sporozoites, hence the initial misidentification. Other myxosporeans with divergent terminal polar capsules include *Myxidium* spp., *Sphaeromyxa* spp.⁴, and *Zschokkella* spp.⁵, but differ from the present myxosporean in shape and site of infection.

In addition to enteric infections in snook, *Fabespora* spp. has been described in the gall bladders of knout goby *Mesogobius batrachacephalus* and *F. nana* in the small scale scorpionfish *Scorpaena porcus* in the Black Sea¹³. *F. vermicola* infects the parenchymal cells and

integument of the digenean parasite *Crassicutis archosargi* that infects sheepshead *Archosargus probatocephalus*¹⁴. There is little published information on *Fabespora* spp; regardless, more work is needed to determine the exact identity (genus and species) of this parasite.

The high infection rate in a closed system demonstrates the likely direct life cycle of this enteric myxosporean. Fish were densely populated and often were observed in coprophagy. It is unknown how the organism was introduced, but is believed to have been brought in from the wild within an infected yellow tang. Mortalities probably did not occur until myxospore levels were at a severe level. However, we cannot rule out a possible invertebrate host. One possible source of infection could have been invertebrate-based food. As part of their diet the tangs are offered pacifica krill and midge pupae. To prevent the *Continued on page 6...*

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⁴ Edwards, T.M., Guillette, L.J.Jr., 2007. Reproductive characteristics of male mosquitofish (*Gambusia holbrooki*) from nitrate-contaminated springs in Florida. *Aquat. Toxicol.* 85:40-47.

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springs. *Environ. Health Perspect.* 114: 69-75.

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For More Information:

Ammonia in Aquatic Systems
<http://edis.ifas.ufl.edu/pdf/FA/FA03100.pdf>

Nitrite in Fish Ponds
<http://srac.tamu.edu/tmppdfs/6167142-462fs.pdf?CFID=6167142&CFTOKEN=3152022&jsessionid=9030e7baa736d4b826b76e261f73b2a43761>

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transmission of parasites and disease, most krill and bloodworms are freeze-dried. Mendez-Hermida et al. demonstrated that live *Artemia* spp. could serve as a possible vector for transmission of cryptosporidiosis in cultured fish¹⁶.

It is of interest to note that the more aggressive and larger yellow tangs that constantly chased, nipped, and prevented the submissive from feeding did not appear to be affected. They did not show any clinical signs and there were no mortalities in their tank. This likely caused chronic stress leading to a suppressed immune system and development of disease in subordinate fish. In retrospect, we could have performed cloacal washes and direct fecal examinations on clinically healthy yellow tangs to determine if they, too, were infected with the myxosporeans.

One clue that this myxosporean may show host specificity is the fact that the blue devil damselfish inhabiting the same system showed no clinical signs of disease, and no myxosporeans were found during necropsy of the few mortalities.

Historically, treatments for myxosporeans have been unsuccessful, however, some chemotherapeutics, such as fumagillin or toltrazuril, may be effective against only some myxosporeans⁴. More recently, new anti-coccidial drugs have been evaluated for their effectiveness on myxosporeans^{16,17}. Both studies found the combination of amprolium and salinomycin mixed in the feed was the most efficacious and had no pathological evidence of toxicity in the treatment of *Myxobolus* sp. and *E. leei*, respectively, in short snout sea bream *Puntazzo puntazzo*. We used the same dosing as in the above studies (100mg/kg of feed for each)

but mixed into a gel-based diet. The fish readily ate the gel.

In conclusion, this case reports an apparent, clinically successful treatment, using a combination of the coccidiostats amprolium and salinomycin, of an enteric infection by myxosporeans similar morphologically to *Fabespora* spp., in a population of yellow tangs. Another commonly used coccidiostat, sulfadimethoxine, was not effective against this myxosporean. This paper also demonstrates how quarantine, though important, will not prevent all pathogens from causing disease.

Acknowledgements

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