Investigating the link between the dinoflagellate *Amyloodinium* sp.? and marine head and lateral line erosion (MHLLE) on *Zebrasoma scopas* (brown sailfin tangs)

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Abstract

Fish diseases are common in both commercial and personal aquariums, and captive fish are very susceptible to disease. The goal of my research was to determine if there is a link between a dinoflagellate and a serious fish disease, Marine Head and Lateral Line Erosion (MHLLE), which has been found to affect fishes in the Coral Reef Tunnel at the Newport Aquarium, Newport, Kentucky. I found a dinoflagellate, tentatively identified as *Amyloodinium* sp., which appeared to be associated with the diseased fishes. The dinoflagellate exists in association with marine sponges, protista, and other invertebrates and has proven difficult to isolate. It forms cysts and is resilient to known disease treatments. Water samples were cultured; the bacterial cultures show bacteria was an unlikely cause of MHLLE. The dinoflagellate was added to experimental tanks, which contained the dinoflagellate and healthy brown sailfin tangs, *Zebrasoma scopas*. The control tanks contained healthy *Z. scopas* only. Visual assessments using both a 35-mm and a digital camera were used to determine the progression of the disease; a compound microscope was used to determine if the dinoflagellate was present. Results thus far suggest that the dinoflagellate does cause MHLLE, either as a parasite or by producing a toxin.

Introduction

Fish diseases are common in both commercial and personal aquariums. They vary from bacterial and fungal to more serious infections with no identifiable cause. Many captive fishes are exposed each year to disease because we do not know enough about how to treat many of them. The goal of my research was to determine the relationship between a dinoflagellate and a serious fish disease, Marine Head and Lateral Line Erosion (MHLLE).

While completing an internship (BIO 397) at the Newport Aquarium, Newport, Kentucky, I discovered that some of the fishes in the aquarium's Coral Reef Tunnel showed signs of MHLLE (Figure 1). Symptoms of MHLLE include superficial erosions of the head and face that eventually progress down the lateral flank to involve the lateral line system (Varner and Lewis 1991). Research on the cause and treatment of MHLLE is rare. Blasiola (1989) and Varner and Lewis (1991) addressed this topic and gave possible causes and treatments, but both concluded that more studies were needed. It is believed MHLLE may be a result of diet or a reovirus-like agent, but the results from both studies were inconclusive. Unfortunately, the disease in captive coral reef fishes has been virtually unstudied. Several possible causes of the disease include poor water quality, vitamin A and C deficiencies, high nitrate or phosphate levels, carbon, copper, and electrical currents in the tank (Noga 1996).

While conducting an experiment based on feeding fishes from the Coral Reef Tunnel a diet high in vitamins A and C, I found that the experimental tanks and the exhibit tank (Coral Reef Tunnel) containing fishes exhibiting MHLLE also contained a white sponge that was infected with a species of dinoflagellate. Dr. Susan Carty, an International authority on dinoflagellates and from Heidelberg College in Tiffin, Ohio, classified the dinoflagellate as belonging to the genus *Amyloodinium*. Dr. Carty, however, thought that the dinoflagellate could be a new species of *Amyloodin-*

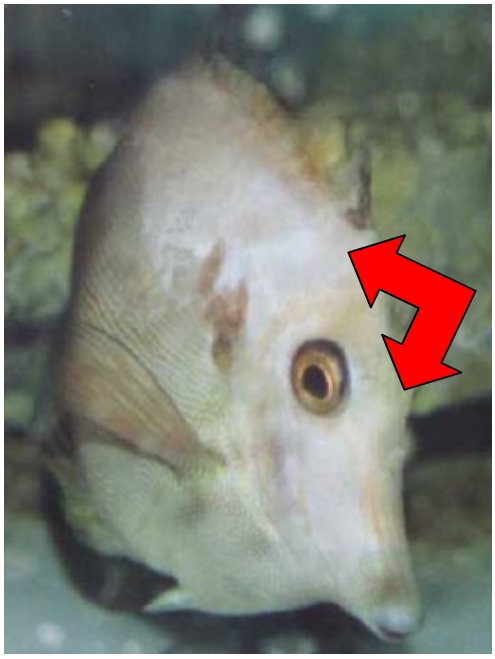

Figure 1. *Zebrasoma scopas* (brown sailfin tang) showing symptoms of marine head and lateral line erosion

ium. Because of this, I will refer to the organism as dinoflagellate and not *Amyloodinium* for the rest of the paper.

Even though an exact identification was not performed, I used *Amyloodinium* sp. as a model for assessing the dinoflagellate I found. *Amyloodinium* sp. infects the gills and skin of marine and brackish water fishes (Kuperman and Matey 1999). The dinoflagellate has a direct life cycle consisting of three intermittent stages (Kuperman and Matey 1999). The actively feeding parasitic trophont is attached to fish gills and skin; the reproductive encysted tomont is inserted into sediments (Figure 2); and the free-swimming infective dinospores (Figure 3) develop after the tomont divides (Kuperman and Matey 1999). Recently, in *Nature*, it was shown that *Pfiesteria piscicida* dinospores, which were originally thought to be toxin-producing, swarm toward and attach to the skin of a fish, actively feeding and rapidly denuding the fish of its epidermis (Wolfgang *et al* 2002). In addition, certain species of dinoflagellate release toxins, which may also contribute to MHLLE. Due to the parasitic action of the trophont stage on fish gills and skin, as well as its possible release of toxins, I propose that these dinoflagellates, rather than the fish's diet, could be the cause of MHLLE. Based on the life cycle of the dinoflagellate and preliminary data collected, I tested whether or not there was a relationship between the dinoflagellate and MHLLE on aquarium fishes. The results from this study will be of immediate use to the Newport Aquarium and possibly to the larger aquarium community.

Methods and Materials

Eight 10-gallon saltwater tanks and one 55-gallon tank were set up in room 102 in the Old Natural Science Building, Northern Kentucky University. The saltwater mix used was Instant Ocean Synthetic Sea Salt. Once cycled, the tanks underwent two 10% water changes per week, or as needed. .

Because *Zebrasoma scopas* tangs are susceptible to MHLLE, these fish were used in the experiment. Each tank contained one *Zebrasoma scopas*, a filter, an air pump, a heater, and a piece of PVC pipe, which served as a hiding place to decrease stress on the fish. Three 10 gallon tanks served as the controls, and each contained one *Zebrasoma scopas* without the dinoflagellate. The other five tanks were experimental, and each contained one *Zebrasoma scopas* along with the dinoflagellate. The 55-gallon tank was used as a quarantine tank. There were five experimental fish total and eight for the overall experiment. The diets

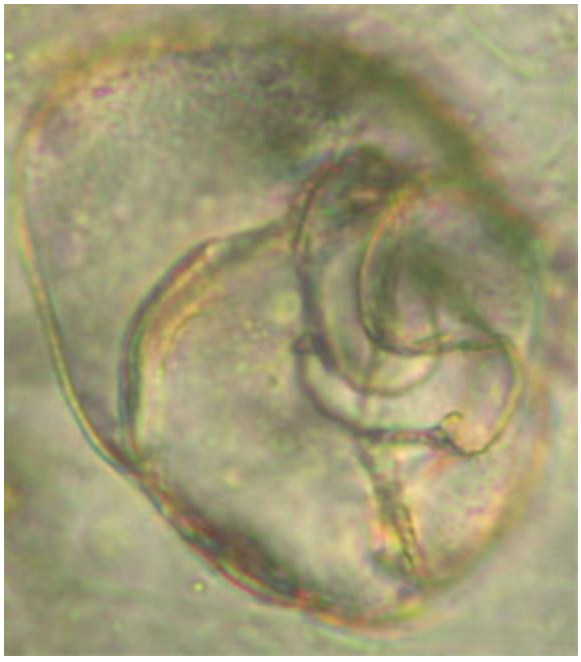

Figure 2. The encysted tumont life stage of the dinoflagellate under 400x magnification.

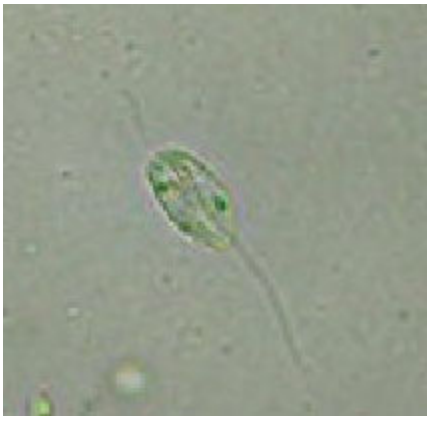

Figure 3. The free-swimming infective dinospore life stage of the dinoflagellate under 400x magnification.

for all of the fish were the same. Based upon my experience and recommendations in other studies (Skomal 1997), the fish in each tank were fed a combination of flake food and red marine algae. The dinoflagellates added to the experimental tanks were cultured from water samples that were collected from the Newport Aquarium using sterile 200 ml and 30 ml plastic containers. The water samples were collected during the fall semester 2001 and contained the dinoflagellate. The containers were left sealed and were put under a light to facilitate growth. Within weeks, growth was seen in these containers. Late in the spring semester 2002, a 10 gallon tank was set up that contained a *Zebrasoma scopas* and dinoflagellates obtained from the sponges collected at the aquarium. There was no way to separate the dinoflagellate cysts from the sponge, so whole sponges were added to the tank.

The next step was to "wake up" the cysts and add the actively swimming dinoflagellates to the experimental tanks. Several test tubes were set up on an orbit shaker under a light to see if the cysts could be "awakened." Adding swimming dinoflagellates to encysted dinoflagellates was the best procedure found to "awaken" the dinoflagellates. Even after the dinoflagellates were "awakened," however, it was still very hard to keep them "awake"; they would encyst again seemingly without reason. As soon as the dinoflagellates were in the swimming stage, however, I added as many cysts and swimming dinoflagellates as possible to each experimental tank. The amounts ranged from 200 to 400+ per tank. However, in one of the tanks, the day the dinoflagellate cysts were added, I found that a fish had died. The cause of death in this instance was due to a hairline fracture in the heater. Over time enough water had entered the heater, and the cause of death was determined to be an electrical shock.

Each remaining fish was assessed visually with both a digital and a 35 mm camera to document whether the dinoflagellates would erode the lateral line area. Also, once a week, a sample from the side of each tank was obtained to document the existence of the dinoflagellate. The tanks were also tested weekly using standard water quality test procedures for changes in temperature (° C), salinity (ppt), pH, NH_3, NH_4, NO_3, and NO_2. Light and filtration were also controlled on a set schedule. Black construction paper was placed on the back and sides of the tank to further control the amount of light each fish received, which also reduced stress. Information from both the digital and 35 mm photographs was used to determine the degree of infection (i.e., size of the erosion area) on each fish. A skin scrape of the eroded area on each infected fish was taken to determine if the dinoflagellate was parasitic or toxin producing.

Using water collected from The Coral Reef Tunnel where MHLLE was found, I made bacteriological cultures. A streak of the sample was placed on suitable growth medium (R2A agar), and two sets of the cultures were incubated, one in the light and the other in the dark. Once colonies developed, each different colony was isolated and placed on a TSA slant. Once growth on the TSA slants was visible, a gram stain was made of the bacteria colony. Standard gram staining techniques were used.

Results/Discussion

After I initially obtained the fish, several problems developed, including water quality issues: NKU's tap water has a copper level of over 0.225 ppm, which is toxic for fish. An infection with Cryptocaryon, a marine fish disease, also developed. As a result most of the fish were lost, and the experiment was set back by nearly 1½ months

Despite these setbacks the results so far look promising. One tank that I set up in spring 2002 shows signs of MHLLE. Figure 4 shows what the fish looked like before the dinoflagellates were added, and Figure

Figure 4. *Zebrasoma scopas* before exposure to the dinoflagellate.

View A

View B

Figure 5. *Zebrasoma scopas* after exposure to the dinoflagellate exhibiting MHLLE.

5 shows what the fish looked like in August 2002. From Figure 5 it can be seen that the area around the eyes eroded first, and then erosion continued down the lateral line to base of the tail. Some of the fish exposed to the dinoflagellate show early signs of the disease. Other tanks that have been reestablished show signs of growth on the tank walls, but it will be several weeks before any clear results are visible. We will also be conducting skin scrapes to determine whether the dinoflagellate is parasitic or if it produces a toxin.

The results of the gram stain showed gram positive, spore forming rods. Most likely there are bacteria in the genus *Bacillus*. These bacteria are an unlikely cause of MHLLE.

Due to these unforeseen setbacks I do not expect to have this project completed until the end of October 2003. At that time I will submit an addendum to this report with my final results.

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