

Bacterial infections in otocinclus sp catfish

clinicopathologic conference

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## **Introduction**

Treatment of bacterial infections in aquatic species presents a unique problem compared to mammals and other non-aquatic based animals. Not only does the animals health have to be considered but also the type of system it is in such as recirculating or flow through aquariums. There are three major routes in which fish are treated; oral, injectable, and water-borne. Water-borne is the most common method of drug administration, but the treatment water must be shut off from any chemical and biological filtration to not reduce the efficacy of the treatment or to kill the essential bacteria for life support [3,6]. This invariably causes some stress on the fish, which can further exasperate the problem.

## **History**

A group of approximately 1,000 otocinclus species catfish were imported wild caught from South America to be used in a breeding program. The otocinclus species are popular with the aquarium trade, but at this time are all imported from wild stocks. A selection of four fish were euthanized and processed for an initial health screen on 6/13/16. This included external wet mounts of sections from the fin and gills as well as a skin scrape, bacterial cultures of the posterior kidney and brain on TSA + Blood agar, and squash preparations of internal organs including stomach, liver, anterior and posterior kidneys, spleen, intestines, and gonadal tissues. No abnormal findings were found during the initial health screen and bacterial cultures came back negative.

On 6/23/16 nine fish were brought in with a history of 1% loss over the past week. They had been placed in a recirculating freshwater system (0 ppt salt) with source water that was a mixture of city water and water treated with reverse osmosis. This mixture is aerated for 24hrs.

before being used in the system. Life support systems include air stones, UV sterilizers, and sponge trickle filters.

Water quality tested by the facility measured at a total ammonia of 0.104 mg/L, nitrite of 0.0484 mg/L, 4 degrees' general hardness, pH of 7, and a total alkalinity of 29 mg/L. Reference ranges for water quality depend on the fish species and system type, but for a freshwater recirculating aquariums a general guideline is less than <1.00 ppm (mg/L) for total ammonia, <0.5 ppm for nitrite, a pH of 6.5-7.5. Alkalinity and hardness affect the buffering capacity of the water [1,5]. This water would be considered soft with a poor buffering capacity. While the nitrite was under the reference range any detectable nitrite may indicate a problem or insufficiency of the filter. Water quality was also tested using the bag water that came in with the fish using a Hach test kit. Total ammonia measured at 0.3-0.4 ppm, nitrite- 0.33 ppm, pH-7.25, total hardness 68.4 ppm, and alkalinity at 51.3 ppm. The discrepancy between values may be due to the differing sensitivities of the test kits used as well as the fish continuing to add waste to the bag water with no filtration before being tested.

There were also problems prior to the current incident with this group of fish with a *Ichthyophthirius multifiliis* infection. The infection was resolved before the current deaths started occurring. They had also been treated using a 5 ppt saltwater dip on arrival at the facility for 30 minutes for biosecurity purposes of external parasites. They were feed a diet of solient green and repashy gel until saturation twice a day.

### **Diagnostic Approach**

Of the nine fish that were submitted six were alive in the bag while the other three had died in transit according to the owner. Two of the live fish were euthanized in MS-222 (tricaine

methanesulfonate) and saved for histopathology. The other live fish euthanized and processed in the same manner as described above for the initial health screen. The fish measured between 2.5 and 3.8 cm in length and weighed between 0.1 and 0.5 grams. All of them were moderately emaciated with two of them being endophthalmic bilaterally. One of the fish had a light gyrodactalus species burden of the gills. On internal wet mounts all four livers were noted to be pale and there was a light balantidium ciliate burden in one of the intestines. Bacterial cultures of two of the fish's brain and kidneys came back positive with two different colony types. These were isolated and re-plated for sensitivity and identification. Only one was successfully identified on first attempt using a Biolog ID database, and due to monetary concerns the other was not pursued. The identified bacteria keyed out as *Aeromonas salmonicida ss pectinolytica*. On sensitivity the unidentified bacteria were found to be resistant to nalidixic acid and nitrofuratoin. The aeromonas species did not have any significant resistances.

### **Treatment and Management**

The infections with two bacterial isolates and the lack of other obvious clinical signs led to the decision to place the fish on an antibiotic regime. *Aeromonas salmonicida* species have been associated with disease in fish most notably *Aeromonas salmonicida ss salmonicida* as the causative agent of furunculosis and atypical ulcerative dermatitis [2]. Consideration also had to be given for the large number of fish and the type of system they were in. In general, it is not recommended to use a bath antibiotic treatment in a recirculating system [7]. While the bacterial identifications and sensitivity were still pending they were started on a gel feed mixed with oxylinic acid at 150mg/Lb. Due to the resistance found to naladixic acid and the availability of medications they were switched to a kanamycin bath at 200 mg/gal for 6-8 hours for three doses every three days [4]. The fish were moved to a separate holding tanks that were aerated for

treatments so as not to kill the biofilters. The ammonia and nitrite were kept in check with frequent large water changes and dosing with Amquel, a water conditioner used to help remove nitrogen compounds. According to the owner at this point the mortality rate seemed to slow down, but did not stop. In response to this and taking into account that *Aeromonas salmonicida* can sometimes be difficult to eradicate they were placed on an enrofloxacin bath at 95 mg/10 gallons for at least five hours every other day [4]. They were originally being moved to a different tank for treatments, but it was decided that the stress of moving as their spines would become caught in the net was causing significant mortalities on its own. Beginning with the third treatment their system would be turned off and drained to 50 gallons instead of moving to a different tank. The water would then be siphoned out as much as possible and refilled before turning the system back on. A few of the fish found freshly dead at this point were reported to have hemorrhage around the head and coelomic cavity. It was considered to add salt to their system to help with osmoregulation and reduce stress, but previous experience with these fish seemed to indicate that they do not do well with a low ppt salt addition for extended periods of time. At this point the fish that were saved for histology were submitted, but there were no significant findings. An acid fast stain to look for mycobacterium was also performed after the owner indicated that the UV sterilizer being used had previously been in a system infected with it. The UV sterilizer had been thoroughly disinfected with bleach and left to dry though and no mycobacterium was seen with staining. After eight treatments of enrofloxacin it was decided to end the treatment even though mortalities were still occurring. It was suspected that daily manipulation and stress was causing the mortalities at this point, roughly 1-2 per day. They were moved to a brood stock tank with a working bio-filter and allowed to settle for several days.

## **Outcome**

The mortalities still continued though at a decreased rate. It was suspected that this was continued damage from the stress and hopefully not a reinfection with *A. salmonicida*. Afterwards this group was lost to follow up, but another batch of otocinclus catfish were eventually imported to continue with the program.

## References

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