An Investigation of Fin Erosion in Channel Catfish, *Ictalurus punctatus*

by

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Abstract

An ongoing case of fin erosion affecting *Ictalurid* catfishes at an aquaculture research facility in the southeastern United States was investigated. Circumstantial evidence collected at this facility has provided several clues as to the cause of the fin lesions. The appearance of the insult is isolated to a heated and dechlorinated municipal water supply when used for flow-through culture systems. Three species of catfish have been affected while species representing five other families are not affected when exposed to the same conditions. The severity of fin erosion increases with temperature and water exchange rate. A pilot study demonstrated that treatment of the water supply with activated alumina protected channel catfish against fin erosion, while increasing hardness or alkalinity did not. A larger study investigated the possibility that chronic exposure to zinc could cause fin erosion in channel catfish. The results of this study showed that fin erosion occurred but was not significantly influenced by zinc exposure. This study also revealed that blood calcium concentration declines over time under these conditions. The cause of fin erosion at this facility remains unidentified.

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Chapter 1

A Case of Fin Erosion at an Aquaculture Research Laboratory

Introduction

Fin erosion in cultured fish is a common and widespread problem affecting many different species and industries. This issue is a growing concern in aquaculture and fisheries management with regards to the appearance of product sold to market and with the survival of stocked fish (Latremouille, 2003). Fin erosion is generally considered to be a clinical sign shared by several diseases and insults. Known causes include abrasion, aggression (i.e., fin nipping), bacterial infection, nutritional deficiency, and poor water quality. Additionally, fin erosion does not appear to occur under natural conditions, being observed predominantly in intensive culture (Klima *et al*., 2013) and in polluted coastal ecosystems (Sherwood and McCain, 1976). Due to its broad etiology and absence in the natural environment, the degree of fin erosion, or rather fin condition, has been suggested as a useful indicator for animal welfare status (Ellis *et al*., 2008). While erosion of fins is certainly a deviation from normal and clearly indicates a problem with the culture environment, the multifactorial nature of the lesion makes it a poor diagnostic tool. Since the cause of the manifestation can be a pathogen, toxin, mechanical injury, inadequate diet, or some other stressor; uncovering the source of fin erosion can be quite challenging.

The potential difficulty in identifying the source of a fin erosion problem has been well demonstrated by an aquaculture research facility in the southeastern USA. This laboratory has been investigating an ongoing fin erosion case at the facility for several years. Early work on this problem has attributed the issue to the municipal water supply. Screening for pathogenic organisms consistently yielded negative results, ruling out disease as a possible causative agent. The problem is also not related to nutrition, as the facility feeds the highest quality species-specific diets and fin erosion has been observed in fish receiving diets from multiple suppliers. Simple abrasion on rough surfaces, such as concrete tanks, has been shown to cause fin erosion (Bosakowski and Wagner, 1995). This is not likely to apply here since the lesions are observed on fish held in non-abrasive tanks made of gel-coated fiberglass, polyethylene, or glass. This facility relies heavily on the municipal water supply because the research conducted requires flow-through rearing systems with pathogen-free source water, and groundwater in the area is not available in sufficient quantity. However, fin erosion is repeatedly observed when fish are held on this water source. Recent observations at the facility may shed more light on the water quality deficiency or toxicant that is affecting the fish.

Background

This research facility has dealt primarily with Channel Catfish, but in recent years work has expanded to include a variety of other cultured fishes. The facility began recording fin conditions on a monthly basis in June 2017, and so far fin erosion has been observed exclusively in catfishes. During this time three species from the family *Ictaluridae*; channel catfish, *Ictalurus punctatus*; blue catfish, *Ictalurus furcatus*; and flathead catfish, *Pylodictis olivaris*; were present with all three developing fin erosion after 3-5 weeks of holding on flow-through municipal water using standard culture practices. The incidence of fin erosion with catfish under these conditions is 100%. Seven other species from the families *Centrarchidae, Percidae, Cyprinidae, Cichlidae*, and *Moronidae* have been held under the same conditions for 5 weeks or longer without developing lesions (Table 1). While these observations have not been formally investigated in a replicated scientific study, these observations suggest that scaled fishes are tolerant of the insult that causes fin erosion in catfishes.

Table 1. Fish species held on flow-through municipal water under standard culture conditions since regular monitoring of fish fin conditions began. Shown is the longest duration that each species had been held under those conditions and, if fin erosion was observed, the time until eroded fins were first reported.

Species	**Time on Flow-Through (weeks)**	**Time to Fin Erosion (weeks)**
Flathead Catfish, *Pylodictis olivaris*	4	3
Blue Catfish, *Ictalurus furcatus*	6	4
Channel Catfish, *Ictalurus punctatus*	11	5
Largemouth Bass, *Micropterus salmoides*	5	Did Not Occur
Walleye, *Sander vitreus*	6	Did Not Occur
Goldfish, *Carassius auratus*	7	Did Not Occur
Grass Carp, *Ctenopharyngodon idella*	7	Did Not Occur
Striped Bass, *Morone saxatilis*	7	Did Not Occur
Golden Shiner, *Notemigonus crysoleucas*	12	Did Not Occur
Nile Tilapia, *Oreochromis niloticus*	22	Did Not Occur

A recirculating aquaculture system (RAS) was installed at this facility for long-term holding of fish. As this system was being brought online four tanks were stocked with channel catfish fingerlings representing two different strains (Marion and Stuttgart). Those fish had moderate degrees of fin erosion after being held for eight weeks or longer on flow-through systems. Fin conditions were monitored closely during this time and rapid recovery was observed following stocking into the RAS (Figure 1). Multiple studies have established a positive correlation between stocking density and fin erosion in other fish species, presumably as a result of increased aggression (Person-Le Ruyet and Le Bayon, 2009; North *et al*., 2006;

Rafatnezhad *et al*., 2008). Stocking density does not appear to be a factor in this case since one of those tanks was stocked six weeks before the RAS was operational, and while it received flow-through municipal water fin conditions declined. Fin conditions improved once the RAS was provided without moving any fish. During three years of operation no fin erosion has developed in this RAS, even though municipal water is used to fill it. Water treatment of the RAS is limited to biological filtration, ultraviolet disinfection, and increased alkalinity by addition of sodium bicarbonate. The reason why municipal water would cause fin erosion in flow-through systems but not RAS is unknown.

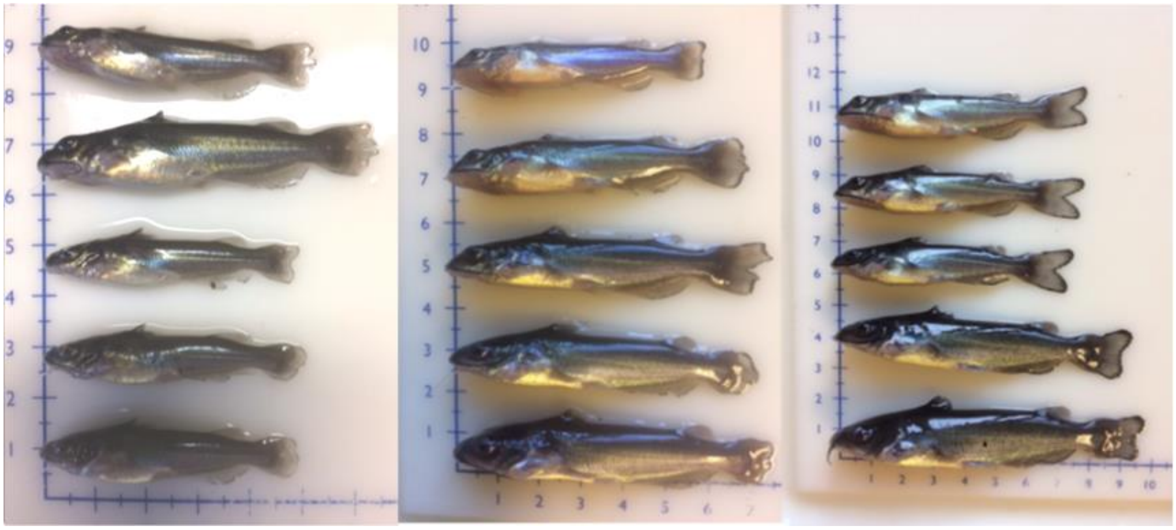

Figure 1. Photographs of a sample of channel catfish, *Ictalurus punctatus*, two weeks before being placed on the RAS (left), two weeks after the RAS (center), and six weeks after the RAS (right). In the left photograph active fin erosion is seen with blunted caudal fins lacking lobes and a black trailing edge. The center photograph shows a margin of new fin growth with black edge present but no lobes. The right photograph shows nearly full restoration of caudal fins.

Fish held at this facility on flow-through systems are generally provided an exchange rate of one tank volume per hour, as recommended by Piper *et al.* (1982). At one point a small number of channel catfish with eroded fins were split from a larger cohort and stocked into another tank. Two months after they were split, the subunit of fish showed dramatic improvement in fin condition while the source population continued to decline (Figure 2). The only notable environmental differences between these two groups were the tank's physical characteristics, stocking density, and water exchange rate. A tank's physical characteristics have not been observed to influence fin erosion at this facility, and the RAS has demonstrated that stocking density is not a factor, so it was presumed that the difference in exchange rate provided the best explanation. Another researcher at the same

Figure 2. A sample from a subunit of channel catfish, *Ictalurus punctatus,* held at a lower water exchange rate for eight weeks (left) and a sample from the source population (right). Caudal fins of the fish pictured at left have recovered from mild fin erosion while the caudal fins of the fish pictured at right have declined to a moderate state of erosion.

facility repeated these results by holding 200 healthy channel catfish on flow-through municipal water at 6 LPM, which was one tank volume per hour, to induce fin erosion. After 7.5 weeks 25 affected fish were moved to an adjacent identical tank and held with a flow rate of 1 LPM. Two weeks later fin regeneration was clearly noticeable (John Shelley, DVM, personal communication, May 27, 2020). Regeneration of lost fin tissue was observed in the RAS as well, but this case is interesting in that the water chemistry is not being altered by filtration; simply increasing the retention time in the tank prevents fin erosion. Unfortunately, this does not provide a suitable solution for this facility since an exchange rate of one tank volume every six hours cannot support high stocking densities. Perhaps the most obvious difference in water quality between high turnover and low turnover would be an accumulation of suspended solids and dissolved organics in the low flow system.

As stated previously, this facility relies heavily on a municipal water supply. Additionally, this water supply is heated to 28°C to support the type of research that is conducted. Only recently, a supply line of unheated municipal water was provided to the wet laboratories. To determine if heating the water had any effect on fin erosion two identical tanks were each stocked with 100 channel catfish with intact fins. One tank was provided with heated municipal water at 25°C while the other was provided with unheated municipal water at 16°C, both with an exchange rate of one tank volume per hour. Five weeks later their fin conditions were assessed and showed that the fish held on heated water exhibited fin erosion as expected while the fish on unheated water remained unaffected (Figure 3). The water heaters used are commercial-grade gas boilers intended for potable water supplies. The same chemical parameters reported in Table 2 were measured in heated and unheated water with no differences detected. It appears that the causative agent of fin erosion at this facility is influenced by water temperature.

Figure 3. Photographs of a sample of channel catfish, *Ictalurus punctatus*, held at 16°C (left) and 25°C (right). Fish held at cooler temperature did not develop fin erosion while those at warmer temperature show frayed, reddened, and bleeding caudal fins.

In an effort to reveal a potential toxicant, a complete water chemistry analysis was conducted (Table 2). Following procedures for an aquaculture source water quality assessment described by Zweig *et al*. (1999), screening begins with phase I basic water quality criteria. Relevant parameters for this assessment are alkalinity, pH, hardness, hydrogen sulfide, and total gas pressure. Optimal alkalinity, pH, hardness, and sulfide levels for channel catfish aquaculture are 20-400 ppm, pH 6-9, 20-400 ppm as CaCO3, and <0.01 ppm respectively (Tucker and Robinson, 1990). Total gas pressure below 100% saturation is ideal for general aquaculture (Zweig *et al.*, 1999). This facility's basic water quality criteria are within recommended ranges. Phase II of the assessment addresses anthropogenic water quality parameters such as metals,

Table 2. Results of a comprehensive water quality analysis of municipal water being supplied to wet laboratories for fish holding. An asterisk by a value indicates that the value is below the limit of detection and should be viewed as an estimate.

Parameter	Value	Units	Parameter	Value	Units
Arsenic	0.000*	mg/L	Total Nitrogen	0.14	mg/L as N
Cadmium	0.000*	mg/L	Soluble Reactive Phosphorus	0.174	mg/L as P
Chromium	0.000*	mg/L	Total Phosphorus	0.195	mg/L as P
Cobalt	0.000*	mg/L	Total Suspended Solids	0.3*	mg/L
Iron	0.000*	mg/L	Fluoride	0.641	mg/L
Lead	0.000*	mg/L	Total Organic Carbon	1.65	mg/L
Manganese	0.000*	mg/L	Potassium	2.07	mg/L
Molybdenum	0.000*	mg/L	Magnesium	4.173	mg/L
Nickel	0.000*	mg/L	Chloride	8.127	mg/L
Selenium	0.00*	mg/L	Sodium	6.447	mg/L
Sulfide	0.000*	mg/L	Calcium	13.328	mg/L
Titanium	0.001*	mg/L	Sulfate	16.518	mg/L
Vanadium	0.001*	mg/L	Hardness	50.5	mg/L as $CaCO_3$
Copper	0.002*	mg/L	Alkalinity	35	mg/L
Aluminum	0.003*	mg/L	Conductivity	145.1	uS/cm
Chlorine	0.004	mg/L	pH	7.3	
Boron	0.033*	mg/L	Total Gas Pressure	99.6	% Saturation
Nitrate-N	0.045	mg/L as N	Oxygen Partial Pressure	92.7	% Saturation
Barium	0.062*	mg/L	Nitrogen Partial Pressure	100.5	% Saturation
Zinc	0.09	mg/L			

metalloids, and organic compounds. Since this is a municipal water source many of these compounds are already limited by the municipality according to Environmental Protection Agency primary drinking water standards (USEPA 2018). Elements identified by Zweig *et al.* (1999) as problematic to aquaculture at levels below those established by the drinking water standards and detected at this facility are chlorine and zinc. This facility utilizes catalytic activated carbon filtration for chlorine adsorption and ultraviolet irradiation for destruction of residual chlorine resulting in an average chlorine level of 4 ppb; this is below the limit of 20 ppb recommended by Buttner *et al*. (1993) and close to the 3 ppb suggested by Zweig *et al.* (1999). Zinc concentrations detected at this facility range from 0.05 to 0.16 ppm, averaging at 0.09 ppm.

This level is well above the limits of 0.05 ppm recommended by Zweig *et al.* (1999) and 0.005 ppm proposed by Noga (2010). Based on these data, zinc toxicosis could be the agent behind this case of fin erosion.

Chapter 2

Zinc Toxicosis in Aquaculture: A Review

Introduction

Heavy metal toxicity is a relatively uncommon ailment in today's aquaculture environment compared to pathogenic and other environmental diseases thanks to an early understanding of the risks associated with contaminated water supplies. When selecting a site for a fish hatchery, farm, or other aquaculture operation the quantity and quality of water is of primary importance and construction materials that can introduce heavy metals are avoided (Piper *et al*., 1986). However, there remains a significant number of cases where waters have been inadvertently or unavoidably contaminated with heavy metals resulting in clinical disease of fish, particularly with the elements copper and zinc (Noga, 2010). The hazards associated with copper tend to be better understood than zinc, due to the widespread use of copper compounds as chemotherapeutants and algaecides. Zinc toxicity, on the other hand, remains important but comparatively less studied and reported.

Zinc is naturally occurring in soils and pond sediments, where it is tightly bound to clays and minerals and does not readily dissolve under normal aerobic conditions (Silapajarn *et al.*, 2004). Zinc is also a major component of many metal construction materials (Noga, 2010). When these zinc-containing sources are in contact with soft, acidic water or in the presence of anaerobic biological processes zinc is released into solution as a bioavailable divalent cation (Zn^{2+}), the form most toxic to aquatic life. Once in the water, zinc ions readily form complexes with dissolved organic compounds rendering them harmless, but in waters with low organic content the ions will remain in the free state (Zweig *et al.,* 1999). The toxicity of zinc to aquatic animals is influenced by the presence of other divalent metals, decreasing with the presence of alkaline-earth metals such as calcium and magnesium which act as antagonists, and increasing with the presence of heavy metals such as copper, cadmium, and cobalt which have toxicities synergistic with that of zinc (Skidmore, 1964).

Sources of Contamination

The concentration of zinc in surface waters is generally low (Silapajarn *et al.,* 2004) but can be high locally due to discharge from mining and industrial operations (Zweig *et al.,* 1999) or released into the hypolimnion of lakes and reservoirs under anoxic conditions (Noga, 2010). Ground water has a greater potential for natural zinc contamination if it is soft, acidic, and deficient in oxygen (Silapajarn *et al.,* 2004). Anthropogenic sources of zinc contamination are primarily from galvanized metals, brass pipe fittings, and paints containing zinc oxide (Piper *et al.,* 1986). Most aquaculture facilities are built using non-metallic plumbing and tanks, but zinc contamination can be especially problematic in municipal water sources where galvanized pipes and brass fittings are widely used. A common practice in municipal water treatment is the addition of zinc orthophosphate to inhibit corrosion. Zinc concentrations will become higher over time if water is allowed to rest in a galvanized pipe. Metallic pipes may be necessary in some applications, such as for deep wells where plastic pipes may not support the weight of the pump and water column. If water is used intermittently the lines should be flushed before delivering water to fish (Noga, 2010). Extra care must be taken in RAS that all metallic components are stainless steel or titanium and only fish-safe paints and coatings are used. The presence of a zinc source in RAS, similar to allowing water to rest in a galvanized pipe, causes the concentration of dissolved zinc to rise over time. Although uncommon, there is new evidence linking activated carbon filtration to disease signs consistent with heavy metal poisoning (Stamper *et al.,* 2011). Activated carbon is most frequently used to improve water clarity in RAS and to remove chlorine and chloramines, both strongly associated with municipal water supplies. It is possible, but so far untested, that as activated carbon removes dissolved organics from the water any zinc that was complexed is then released in the toxic free state.

Pollutants in industrial and wastewater effluent streams are regulated by the EPA and by each state. The amount of zinc that is permitted to be discharged varies by industry and location, but is generally based on the change in zinc concentration of the receiving water body caused by the effluent stream in relation to its effect on aquatic life (USEPA, 1991). Typical surface waters have a zinc concentration around 0.02 ppm, but waters high in zinc associated with drainage from mining operations and industrial effluents contain 0.1 – 1.0 ppm zinc (Irwin, 1997). Drinking water limits recommended by the EPA and enforced by each state cap zinc concentrations at 5 ppm, although actual levels rarely approach that limit due to the metallic flavor caused by zinc (USEPA, 2017). Aqueous zinc is relatively non-toxic to mammals compared to aquatic organisms, resulting in drinking water limits higher than what is generally allowed for effluent waters. Drinking water tends to have high concentrations of zinc compared to natural waters due to leaching from plumbing and tank structures, with levels greater than 1.0 ppm not uncommon (Irwin, 1997).

Toxicity in Fish

In fish, zinc is an essential element for a range of physiological processes involving the immune system, neurotransmission, and cell signaling (Authman *et al*., 2015). Fish can acquire bioavailable zinc from feed as well as directly from water via the gills. However, fish appear to have little control over how much zinc is taken through the gills. In trout, it has been shown that there is a dietary requirement of 15-30 ppb of zinc in feed, but as the concentration of water-borne zinc increases so does their uptake of the nutrient regardless of the quantity of zinc in the diet. Additionally, a surplus of dietary zinc appears to be harmless while a surplus of water-borne zinc will cause toxicosis. The two pathways are complex and closely related, but not equal. Dietary zinc appears to be preferred by fish, as an increase in dietary zinc can replace a lack of water-borne zinc but increased water-borne zinc will only partially alleviate a deficiency in dietary zinc (Spry *et al*., 1988). Sauer and Watabe (1989) revealed that zinc-exposed mummichogs accumulated zinc in the calcified regions within the scales, suggesting that this could be a mechanism for detoxification of excess heavy metals. Additionally, Coello and Khan (1996) found that with the addition of fish scales goldfish, *Carassius auratus;* green sunfish, *Lepomis cyanellus;* and largemouth bass, *Micropterus salmoides* became tolerant of concentrations of lead and mercury that would otherwise be lethal. They suggested that the scales functioned to buffer pH and chelate heavy metals.

The toxicity of zinc is strongly influenced by other water quality parameters. Calcium hardness and pH have been shown to have a powerful effect on zinc toxicity. Everall *et al.* (1989) demonstrated that a reduction in hardness and/or pH increased the toxicity of zinc in brown trout, *Salmo trutta,* and Bradley and Sprague (2011) saw similar results with rainbow trout, *Oncorhynchus mykiss*. Hardness, alkalinity, and pH appear to have a complex relationship with zinc toxicity. Reduced pH and hardness together amplify zinc toxicity, but below pH 5 hardness no longer plays a role. Conversely, increased pH will cause zinc to precipitate into a non-toxic form but increases the toxicity of the zinc remaining in solution, while increased alkalinity reduces zinc toxicity only when pH is greater than 7 (Everall *et al.*, 1989). Additionally, using channel catfish, Wurts and Perschbacher (1994) showed that copper toxicity, which has a similar activity to zinc, is reduced by increased Ca^{2+} when alkalinity was 75 ppm but not when alkalinity was 20 ppm.

Temperature also appears to have an interesting relationship with zinc toxicity. Hodson and Sprague (1975) and Perschbacher (2005) exposed Atlantic salmon, *Salmo salar,* to zinc and channel catfish to copper, respectively, at varying temperatures. Both studies observed that survival time is negatively correlated with temperature while LC50 is positively correlated with temperature. In other words, as temperature increases a higher concentration of zinc is required to kill fish but the fish die faster. Zinc readily complexes with organic solids and dissolved organic carbon (DOC) (Zweig *et al.,* 1999). The toxicity of complexed zinc has not been investigated, but the related metal copper has been the subject of studies. The presence of at least 4.8 ppm DOC has been shown to prevent copper from adhering to the gills of fathead minnows, *Pimephales promelas,* (Playle *et al.*, 1993) and removal of DOC increases the toxicity of copper to fathead minnows (Welsh *et al.*, 1993).

The concentration of zinc which is toxic to fish is typically described as either acute or chronic. Acutely toxic concentrations are usually determined by a 96-hr LC50 and have been investigated extensively, while chronic doses are relatively understudied with available reports using a range of endpoints. Of the available reported chronically toxic zinc concentrations, the

Lowest Observable Effect Limit (LOEL) is the most useful measure for aquaculture, but it should be noted that within these reports the physiological process being measured plays an important role in the accuracy of the LOEL. Additionally, the concentration of zinc that causes adverse effects varies between species (Table 3) and within species by population and life stage.

Table 3. Comparison of 96hr LC50 (the concentration at which 50% of fish die after 96 hours of exposure) and Lowest Observable Effect Limit (the lowest concentration at which physiological changes can be detected) in species of freshwater fish for which those values have been determined.

Common Name	**Scientific Name**	**Acute 96hr LC50**	**Chronic LOEL**	**Source**
Rainbow Trout	*Oncorhynchus mykiss*	1.010	0.010	USEPA 2018
Mottled Sculpin	*Cottus bairdi*	0.156	0.027	Woodling *et al.*, 2002
Mozambique Tilapia	*Oreochromis mossambicus*	1.600	0.032	USEPA 2018
Flagfish	*Jordanella floridae*	1.500	0.051	Spehar 1976
Fathead Minnow	*Pimephales promelas*	3.830	0.106	USEPA 1987
Sockeye Salmon	*Oncorhynchus nerka*	1.502	0.242	USEPA 1987
Chinook Salmon	*Oncorhynchus tshawytscha*	0.446	0.371	USEPA 1987
Mummichog	*Fundulus heteroclitus*	0.975	0.784	USEPA 2018
Brook Trout	*Salvelinus fontinalis*	2.100	0.854	USEPA 1987

As described earlier, the toxicity of zinc is strongly influenced by accompanying water parameters. The research reflected in Table 1 used a standard hardness for zinc toxicity studies of 50 mg/L as CaCO3, but temperature, pH, and organic content varies between studies. Given the difficulty in comparing toxicity studies and the great diversity in water quality and chemistry between aquaculture facilities, there are very few general guidelines reported for the maximum safe zinc concentration in aquaculture. The few guidelines that are available range from 0.005 ppm (Noga, 2010) to 0.1 ppm (UKY, 2000). The most complete assessment of normally safe levels of zinc comes from European Union guidelines which considered average aquaculture water quality criteria and individual species sensitivities. This report recommended a maximum zinc concentration for warmwater fish hatcheries of 0.05 ppm (Zweig *et al.*, 1999).

In a case of acute zinc toxicity high levels of mortality are likely to be observed. At high concentrations, the primary mode of action by zinc is damage to the gills by detachment and sloughing of epithelial cells (Matthiesen and Brafield, 1973). The breakdown of the epithelial layer increases the distance between capillaries and oxygen-rich water, impeding gas exchange and resulting in death by hypoxia (Burton *et al*., 1972). Clinical signs of acute zinc poisoning may include excess mucus and hemorrhage at the gills, gathering around aerators and influents, piping at the water surface, and death. At a chronic level, the most important effect of zinc is disruption of calcium uptake of the fish by binding to calcium channels in gills, skin, and lateral line, ultimately inducing hypocalcemia (Authman *et al*., 2015). There is strong evidence that this calcium interference results in bone deformities, especially spinal deformities (Salvaggio *et al.*, 2016; Sfakianakis *et al.*, 2015). The lateral line may also be susceptible to damage by zinc. The function of the lateral line relies heavily on calcium channels (McGlone *et al.*, 1979), can be experimentally ablated using cadmium (Faucher *et al.*, 2006), and lateral line lesions have been induced by copper (Gardner and LaRoche, 1973). Even in the absence of visual indications of zinc poisoning, the metal has been shown to reduce growth, survival, and reproduction (Authman *et al*., 2015; Brungs, 1969). Diagnosis of zinc toxicosis is generally presumptive by observation of the above-mentioned clinical signs with absence of other causes of disease, and definitive by histology and testing of water and tissues for zinc (Noga, 2010).

Treatment

Fish suffering from acute or chronic zinc intoxication have an exceptional ability to recover when moved to contaminant-free water (Matthiesen and Brafield, 1973). There are few “treatment” options available for fish in zinc-contaminated water, but one study using three-spined stickleback, *Gasterosteus aculeatus*, showed that the addition of 50 ppm calcium using calcium nitrate or calcium chloride will neutralize the toxicity of 2 ppm zinc (Jones, 1938). The potential for added calcium to render zinc harmless has been documented with other species by showing that increased calcium reduces zinc uptake (Barron and Albeke, 2000), presumably by reducing the probability that a zinc ion will contact a receptive calcium channel. However, the amount of calcium ions required to detoxify zinc will vary between species. Another option may be to increase carbonate alkalinity with the addition of sodium bicarbonate, which will induce precipitation of zinc at pH levels greater than 7.0, thereby reducing toxicity to some extent (Bradley and Sprague, 2011).

As discussed, it may be possible to temporarily mitigate the effects of zinc, but ultimately the contamination of source water must be addressed to prevent problems from continuing or recurring. If the source of contamination is within the aquaculture facility it should be fairly straightforward to locate and resolve the issue. As mentioned earlier, zinc is often released by galvanized and brass plumbing structures, so replacing such components may be an adequate remedy. Unfortunately, it is not always so simple, such as in cases where groundwater is contaminated or municipal water is used. Under these circumstances it may be necessary to install a treatment system for zinc removal, for which there are a few options used primarily in other industries.

Ion exchange filters are very effective in removing zinc ions from water and are widely available for commercial and industrial applications. These filters work by using a resin media to remove divalent cations from the water, and in exchange release sodium ions. These filters are inexpensive to operate, and when saturated are easily recharged without media replacement (Alyuz and Veli, 2009). However, ion exchange systems will remove all divalent cations indiscriminately, including calcium and magnesium. For aquaculture applications the calcium and magnesium hardness would need to be restored before treated water could be used in culture systems.

Another simple filtration option is the use of a standard bed filter with an adsorbent media having a strong affinity for zinc. The most widely used media for zinc adsorption at this time is activated alumina (Al_2O_3). This media is readily available but is primarily marketed as a fluoride adsorbent for use in drinking water applications. Activated alumina readily adsorbs fluoride, heavy metals, and phosphates without affecting important water quality parameters such as hardness, pH, and alkalinity. When saturated, this media can be chemically regenerated without media replacement (USEPA, 2014). Possible drawbacks of activated alumina include a relatively long contact time and low adsorption capacity, requiring frequent regeneration. Other adsorbent materials being investigated include clarified sludge, rice husk ash (Bhattacharya *et al.*, 2006), and bentonite clay (Kaya and Oren, 2005). Activated carbon has a low affinity for heavy metals, including zinc (Ferro-Garcia *et al.*, 1988).

Flocculation and precipitation are commonly used in the wastewater industry for removal of heavy metals. In this process, pH is adjusted to 9-10 and hydrated lime is used as a flocculant, causing precipitation of zinc complexes with calcium oxide. The precipitant is removed as a sludge by filtration or sedimentation. This process is inexpensive and can remove up to 99% of zinc in highly polluted water (Fu and Wang, 2011). However, this method of zinc removal has not previously been utilized in aquaculture, requires significant space, would require adjustment to pH post-treatment, and results in quantities of sludge that must be disposed of.

Possibly of high interest to aquaculture is the potential to remove heavy metals by oxidation with ozone. Ozone has been used extensively in aquaculture for disinfection and clarification, and its use and safety in treating fish culture water is well understood. A single study has been published on the efficacy of ozone in zinc removal, in which zinc was reduced to below detection limits with 10 minutes contact time of 2 ppm ozone (Nieminski and Evans, 2008). More research needs to be done in this area to understand how variable water quality parameters may impact the success of an ozone treatment system. The presence of organic matter has been shown to protect other metals from oxidation (Reckhow *et al*., 1991).

Conclusions

Zinc is the second most abundant trace element after iron, present in most water sources, and necessary for life (Authman *et al.*, 2015). Zinc is also used extensively in construction and manufacturing processes and released into the environment by mining operations (Irwin, 1997). The best course of action for aquaculture operations is to avoid water sources and construction materials high in zinc. However, it is not always possible to avert such circumstances, or the fish culturist may be unaware that zinc is a problem at his/her facility. In cases of unavoidable zinc exposure, it is possible to alleviate the effects on the fish by adjusting certain water quality parameters, particularly calcium hardness. In a facility where zinc contamination is a problem it will be necessary to address the issue head-on. Water treatment options currently available for aquaculture facilities are filtration by ion exchange resins or activated alumina. Areas where further research is needed are the evaluation of superior adsorbents for zinc removal by filtration and verification of the efficacy of detoxification of zinc by oxidation with ozone.

Chapter 3

Evaluation of Zinc Toxicity and Fin Erosion in Channel Catfish

Introduction

An aquaculture research facility in the southeastern United States has been investigating a case of fin erosion in channel catfish that occurs when a municipal water supply is used in flow-through culture units. It has been observed that fin erosion occurs only in catfishes and does not occur in families of scaled fishes, that fin erosion does not occur in RAS or low-exchange systems where organic solids are more prevalent, that fin erosion occurs in warmer waters but not in cooler waters, and that zinc is present at levels that may cause chronic toxicity. The literature supports the idea that heavy metal poisoning, such as with zinc, could explain such observations. Zinc forms complexes with organic solids and dissolved organic compounds (Zweig *et al*., 1999), in which state other heavy metals are rendered harmless (Playle *et al*., 1993; Welsh *et al*., 1993). This could explain the observations in RAS and low-flow systems. The toxic effects of zinc appear to have a delayed effect at cooler water temperatures (Hodson and Sprague, 1975; Perschbacher, 2005), which may explain why fin erosion did not occur in unheated water. It has been proposed that scales provide protection from sub-lethal levels of heavy metals (Sauer and Watabe, 1989; Coello and Khan, 1996). It is possible that because catfish are scaleless they are more susceptible to chronic zinc exposure than other families. Several investigations into the acute effects of zinc toxicosis have been conducted with channel catfish (Table 4), but they did not determine a 96-hour LC50 that could be compared to other species. There has not been any work published on the chronic effects of zinc to North American catfishes. Zinc has never been linked to fin erosion, but it has been shown to cause hypocalcemia (Authman *et al*., 2015) and bone deformities (Salvaggio *et al.*, 2016; Sfakianakis *et al.*, 2015). It is possible that chronic zinc poisoning could manifest differently in scaleless fish such as catfish, and cause decalcification of fin rays that would be prone to damage and erosion.

Table 4: A summary of available literature on acute concentrations of zinc to channel catfish, *Ictalurus punctatus*.

Concentration	Duration	Survival	Notes	Source
12 ppm	40 hours	0%	Time to 100% mortality	Lewis 1971
30 ppm	6 hours	0%	Time to 100% mortality	Lewis 1971
8.2 ppm	14 days	50%	14-day median tolerance limit	Reed 1980
6.5 ppm	24-30 hours	0%	Calcium 0.1 mM	Bentley 1992
6.5 ppm	6 days	100%	Calcium 3 mM	Bentley 1992
0.65 ppm	7 days	100%	Calcium 0.1 mM	Bentley 1992

To show that zinc exposure results in fin erosion would be a novel discovery requiring scientific investigation. Since the water supply at this facility already contains levels of zinc suspected of causing fin erosion, a logical next step for investigation is to determine if removal or detoxification of zinc would prevent fin erosion. To accomplish this a pilot study was designed to test three methods of remediation. The first method selected was removal of zinc by filtration with activated alumina. Activated alumina is the most readily available filter media with good affinity for heavy metals, including zinc. The second method was addition of calcium chloride to raise water hardness, which should counteract the toxic effects of zinc (Jones, 1938; Barron and Albeke, 2000). The final method selected was addition of sodium bicarbonate to raise alkalinity and induce precipitation of non-toxic zinc carbonate as suggested by Bradley and Sprague (2011).

Materials and Methods

Fish used for this study were 2018 year-class Stuttgart strain channel catfish. Average fish weight was 6.1 grams per fish. These fish were removed from an outdoor pond on 23 August 2018 with intact fins and transferred to a 100-gallon holding tank supplied with well water to preserve fin condition. On 20 September 2018 four 50 L glass aquaria were each stocked with 20 fish, one tank per treatment and control. Due to the scale of this investigation as a pilot study, numbers of fish were not available for replication. Each tank was provided with an inflow rate of 0.8 L/min to provide approximately one tank volume per hour, which is the exchange rate that has induced fin erosion in past observations.

The control tank received heated dechlorinated municipal water that has been associated with fin erosion at this facility. The activated alumina treatment group received heated dechlorinated municipal water that had been prefiltered through activated alumina media. The supply line to this tank was fitted with a Hayward FLV Series Simplex Double Length bag filter vessel that had been filled with approximately 0.75 cubic feet of AA-400G Fluorograde 14 x 28 mesh activated alumina for zinc removal. The hardness treatment group utilized an LMI A-series Chemical Metering Pump to inject a stock solution of calcium chloride into the water supply line to raise hardness from 50 ppm as $CaCO_3$ to 150-300 ppm as $CaCO_3$. The alkalinity treatment group used an identical setup to the calcium group to deliver a solution of sodium bicarbonate to raise alkalinity from 35 ppm to 100-200 ppm.

Fish were fed a standard catfish diet at 3 grams per tank per day, 2.5% body weight per day based on initial average weight. At least twice per week water samples were collected from the influent to each treatment and analyzed for pH, alkalinity, hardness, and zinc concentration.

pH was measured using a Fisherbrand accumet AP110 Portable pH Meter. Alkalinity was determined by acid titration to fixed pH endpoint as described by Nollet (2000). Hardness was analyzed using a Hach DR2800 Spectrophotometer with test method 8030. Once per week 5 fish from each treatment were anesthetized by immersion in 140-270 ppm buffered MS-222 and photographed to monitor fin conditions. The study continued for 42 days until moderate fin erosion was experienced, at which point the study was terminated and fish were humanely euthanized by immersion in >300 ppm buffered MS-222 for 10 min according to Institutional Animal Care and Use Committee requirements.

Results

Water quality parameters were fairly stable overall and mostly within the ranges determined for each treatment (Table 5). Alkalinity in the alkalinity treatment was maintained well above the other treatments, except for a low reading on day 30 due to the metering pump malfunctioning, and mostly within the desired range of 100-200 ppm. The other treatment groups had steady alkalinity readings of 29-47 ppm (Figure 4). Hardness measurements fluctuated widely in all treatment groups, possibly a result of error or contamination. The test method used for hardness was very sensitive, with a measuring range of 0.05-4.00 mg/L as $CaCO_3$, requiring samples to be diluted by 100×. Even so, the hardness of the hardness treatment group was maintained largely within the 150-300 mg/L as $CaCO_3$ range with a mean of 239 mg/L as $CaCO_3$, while the other three treatments had mean hardness of 91-106.5 mg/L as $CaCO_3$ (Figure 5). The activated alumina filter demonstrated an acceptable ability to remove zinc from the supply water. While the other three treatments had a mean zinc concentration of 0.08 ppm, the alumina treatment had a mean of 0.03 ppm. There was no overlap in measured zinc values between the alumina treatment and the other three treatments (Figure 6). There is no evidence that the alkalinity treatment effectively removed zinc from solution by precipitation.

Table 5. Mean, minimum, and maximum water parameter values recorded from 13 sampling points over 42 days.

	pH				**Alkalinity (ppm)**			
	Control	**Alumina**	**Alkalinity**	**Hardness**	**Control**	**Alumina**	**Alkalinity**	**Hardness**
Mean	7.42	7.31	8.37	7.48	35	35	141	36
Low	7.01	6.89	7.61	6.73	29	29	41	28
High	7.92	7.63	9.43	8.11	45	47	220	47
	Hardness (mg/L as CaCO3)				**Zinc (ppm)**			
	Control	**Alumina**	**Alkalinity**	**Hardness**	**Control**	**Alumina**	**Alkalinity**	**Hardness**
Mean	107	93	91	239	0.08	0.03	0.08	0.08
Low	30	50	30	180	0.07	0.01	0.06	0.06
High	320	205	190	320	0.09	0.06	0.11	0.16

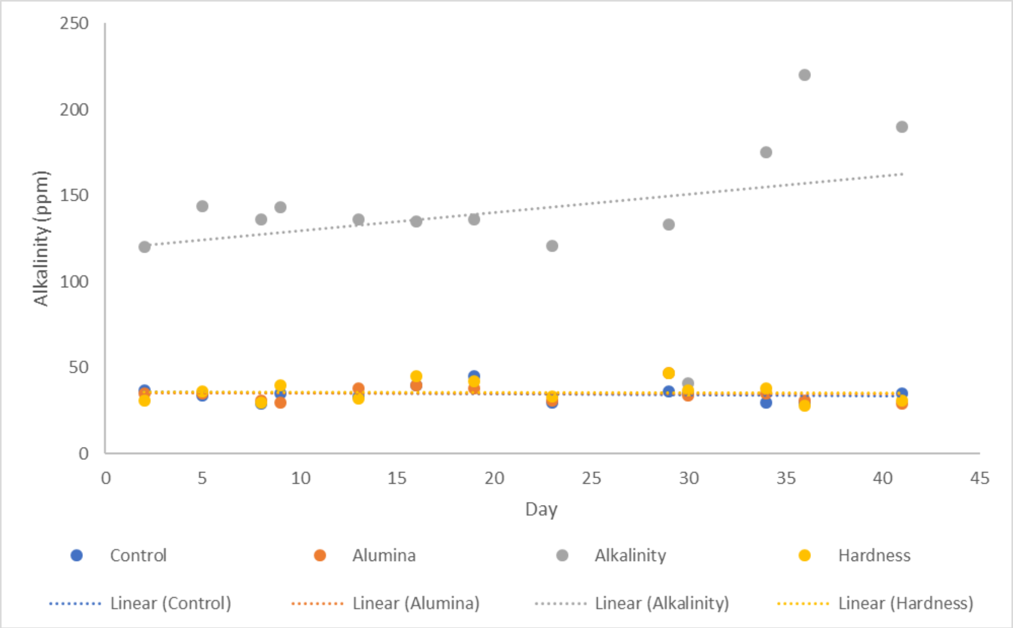

Figure 4. Alkalinity measurements plotted against time for three treatments and control representing 13 sampling points over 42 days.

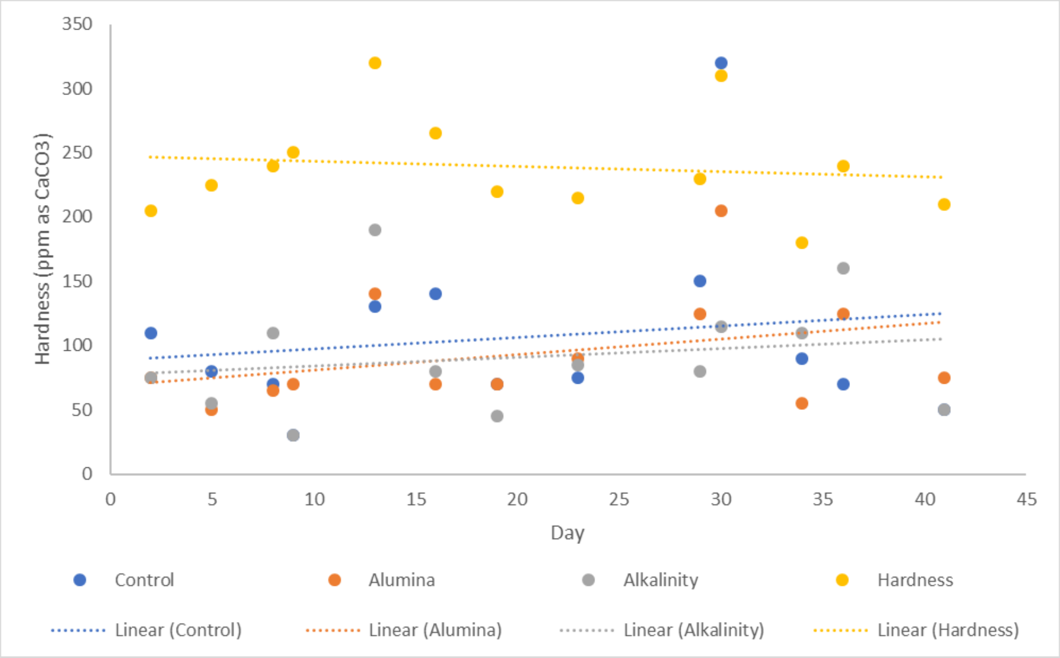

Figure 5. Hardness measurements plotted against time for three treatments and control representing 13 sampling points over 42 days.

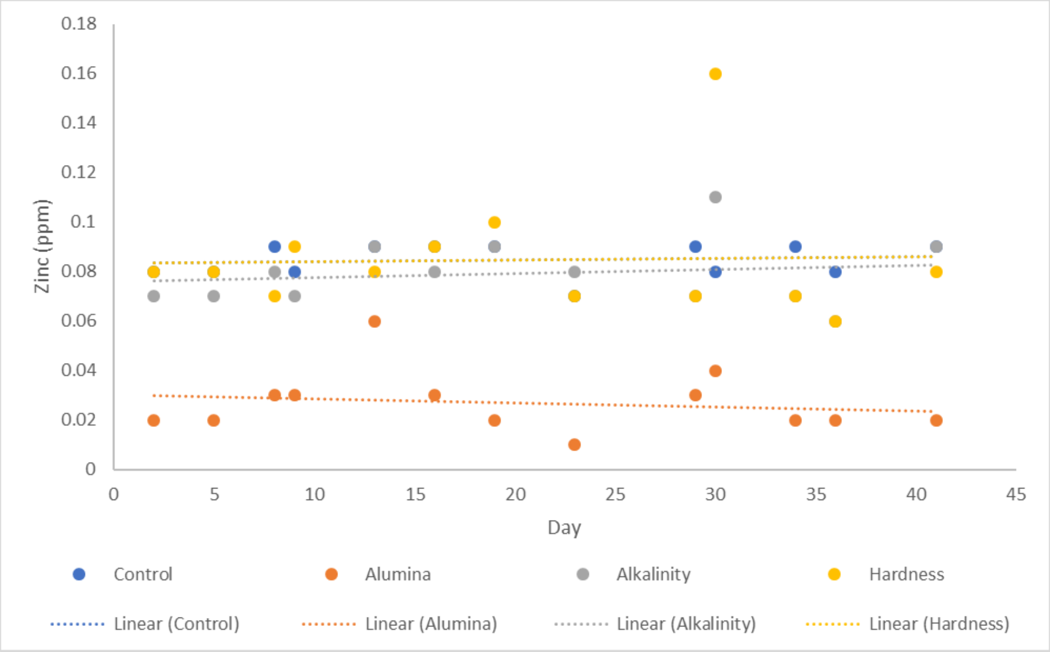

Figure 6. Zinc concentration measurements plotted against time for three treatments and control representing 13 sampling points over 42 days.

Visual interpretation of fin conditions from the final day of the study showed that fin erosion progressed as expected in the control group (Figure 7). Fin erosion also occurred in the hardness and alkalinity groups, without any discernable difference from the control. Those two treatments, as well as the control, exhibited severely shortened caudal and anal fins with active hemorrhaging and exposed fin rays. The alumina treatment, on the other hand, showed markedly superior fin conditions. The caudal fins of most fish in that group appeared to be fully intact. Some fish suffered from minor fraying of those fins without hemorrhage. Primarily the larger fish were affected in this way while the smaller fish were unaffected, so it is thought that this may be caused by aggressive fin-nipping rather than toxicant-induced fin erosion.

Figure 7. Photographs of a sample of 5 channel catfish, *Ictalurus punctatus*, from each treatment group. Shown clockwise from top left: control, alumina, hardness, alkalinity.

Without replication it is not possible to statistically analyze these results, but for proof of concept an attempt was made to place a continuous numerical value on the fin conditions. It would not be possible to simply measure the fins because doing so would not account for size differences between fish or growth during the study. Two publications reported the use of a formula for monitoring fin condition. Bosakowski and Wagner (1994) determined that "relative fin length" = fin length/total body length × 100 using rainbow, cutthroat, and brown trout; and Kindschi (1987) proposed that fin factor (%) = (fin length × 100)/total length for any population of fish. Both equations are the same and provide the percent of the total length measurement that is attributed to the caudal fin. A serious limitation in applying that formula to channel catfish is that channel catfish have a deeply forked caudal fin and the lobes do not necessarily erode equally. A second possible drawback of this formula is that it assumes a simple linear relationship between fish size and caudal fin length even though Ellis *et al*., (2009) found that relative caudal fin size decreases with increasing fish size in rainbow trout. It is proposed here that measurement of fin area rather than fin length can account for irregular erosion of fin lobes. A relationship will need to be determined between caudal fin area and body size using channel catfish with intact caudal fins. If a strong relationship exists, then it would be possible to predict the area of caudal fin that should be present based on fish size. Therefore, fin condition can be assessed using the equation "fin condition (%) = measured caudal fin area/predicted caudal fin area × 100".

To establish the relationship of caudal fin area to fish size the photographs of fish from day 1 of the study were used, with the assumption that fins were intact at that time (Figure 8). Fish size was determined by measuring body area to avoid influence by fin condition. Measurements were made using ImageJ software calibrated by the scale, in cm, present in each photograph. This method, with n=20, provided a linear relationship with equation fin area = body area × 0.18 – 0.10. The equation was used to predict the caudal fin area that should be present on each sampling day and determine the percent of caudal fin remaining at that time (Figure 9). Without replication a statistical analysis cannot be conducted to determine if a significant difference exists between treatments, but the results suggest that fin erosion did not occur in the alumina treatment. A positive slope is present in this treatment even though no slope would be expected if fin erosion did not occur. This could be a result of extrapolation of the fin condition formula. The formula was determined using fish with body area of 5.1-15.1 cm² but was applied to fish with body area as high as 25.1 cm². It also appears that the hardness and alkalinity treatments did not differ from the control.

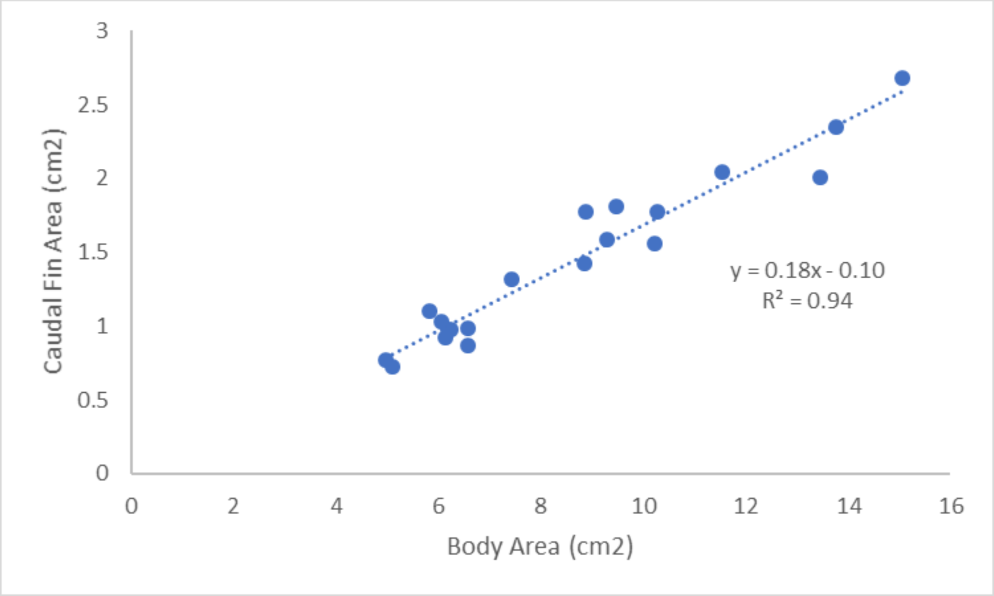

Figure 8. Linear relationship between caudal fin area and body area in channel catfish, *Ictalurus punctatus,* using measurements from 20 individuals on day 1 of the study with intact caudal fins.

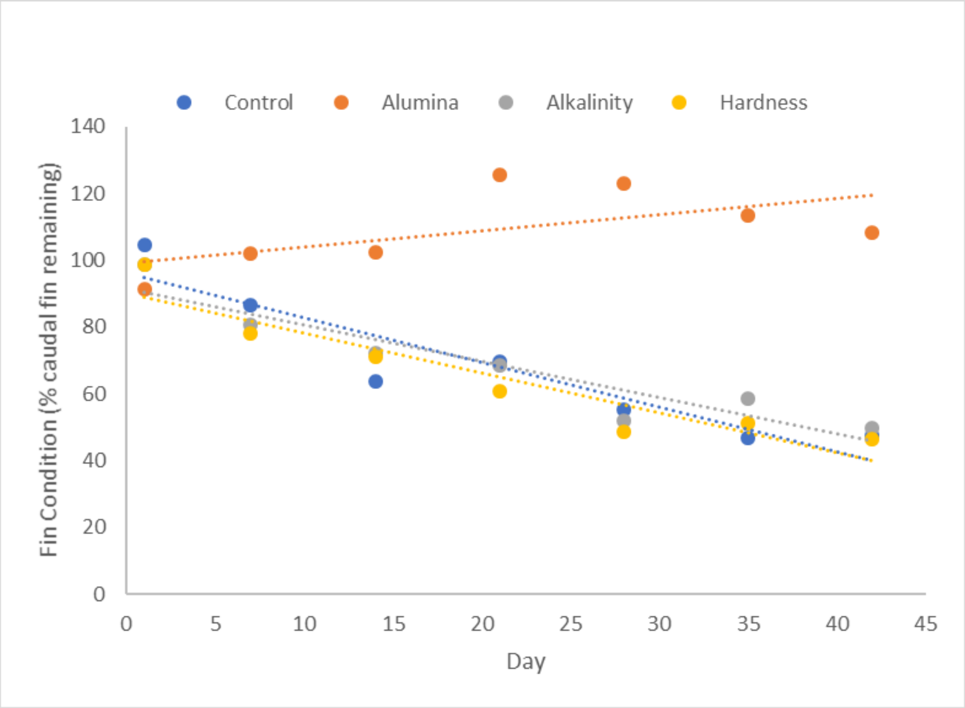

Figure 9. Calculated fin conditions of channel catfish, *Ictalurus punctatus*, over time. Three treatments to remediate chronic zinc exposure and a control without remediation. Control R^2 = 0.87, alumina R^2 = 0.35, alkalinity R^2 = 0.88, and hardness R^2 = 0.89.

Discussion

It is suspected that zinc toxicosis is the etiology behind fin erosion in catfishes at this research facility. This study was not designed to confirm that zinc is the causative agent, but to provide more evidence for the argument and to evaluate methods for remediation. Two treatments failed to protect channel catfish from fin erosion. The alkalinity treatment was thought to remove zinc from solution by precipitation, but the concentration of zinc in this study may be too low for solubility to be reduced by the pH and alkalinity that was provided. After all, the study that showed this could be achieved was investigating acute effects at higher concentrations

(Bradley and Sprague, 2011). Substantial fin erosion also occurred in the hardness treatment even though the ability of calcium to alleviate zinc poisoning has been well documented (Jones, 1938; Barron and Albeke, 2000). It is possible that such protection did not occur because the alkalinity was quite low, as was observed by Wurts and Perschbacher (1994) in their investigation of copper toxicity to channel catfish. A different result may have been observed if the hardness and alkalinity treatments had been combined.

The alumina treatment, on the other hand, showed promising results. It could be presumed that removal of zinc by activated alumina protected fish from fin erosion, but whether the media affected any other important water quality parameters is not known. A sample of water was collected from immediately upstream of the alumina filter and a second sample was collected from immediately downstream of the filter. Those samples were sent to the Arkansas Water Resources Center Water Quality Laboratory located in Fayetteville, AR for complete analysis (Table 6). Pre-filter and post-filter values were compared and only four parameters were influenced by more than 10%. Nitrate concentration more than doubled but remained very low. Activated alumina does not contain any nitrogen so could not be releasing nitrate into the water. This effect would be best explained by the potential for nitrifying bacteria to grow in the media. Phosphorus was reduced by 92-93%, but it is highly unlikely that phosphorus is the toxicant causing fin erosion at this facility. While phosphorus is considered to be an environmental pollutant as a factor in algae blooms, it is generally considered non-toxic to fish. Indeed, Kim *et al*. (2013) determined that the LC-50 for phosphate compounds with Japanese rice fish, *Oryzias latipes*, was >100 ppm, concluding that those compounds did not pose a toxicity risk. Fluoride was reduced by only 12%. A greater reduction was expected since activated alumina is marketed primarily as a fluoride adsorbent, but it is possible that the media was nearing saturation by the

Table 6. Comparison of water quality parameters measured before and after filtration with activated alumina. Parameters that showed a change greater than 10% are highlighted. Percent change was not calculated for parameters below detection levels, and the values shown should be considered estimates only.

Parameter	Before Alumina	After Alumina	% Change	Below Detection Limit	Units
Arsenic	0	0.001	N/A	Yes	mg/L
Cadmium	0	0	N/A	Yes	mg/L
Chromium	0	0	N/A	Yes	mg/L
Cobalt	0	0	N/A	Yes	mg/L
Iron	0	0	N/A	Yes	mg/L
Lead	0	0	N/A	Yes	mg/L
Manganese	0	0	N/A	Yes	mg/L
Molybdenum	0	0	N/A	Yes	mg/L
Nickel	0	0	N/A	Yes	mg/L
Selenium	0	0	N/A	Yes	mg/L
Titanium	0.001	0.001	N/A	Yes	mg/L
Vanadium	0.001	0	N/A	Yes	mg/L
Copper	0.002	0.001	N/A	Yes	mg/L
Aluminum	0.003	0.01	N/A	Yes	mg/L
Boron	0.033	0.032	N/A	Yes	mg/L
Nitrate-N	0.045	0.097	115.56	No	mg/L as N
Barium	0.062	0.053	N/A	Yes	mg/L
Zinc	0.067	0.018	-73.13	Yes on second measure	mg/L
Total Nitrogen	0.14	0.15	7.14	No	mg/L as N
Soluble Reactive Phosphorus	0.174	0.011	-93.68	No	mg/L as P
Total Phosphorus	0.195	0.015	-92.31	Yes on second measure	mg/L as P
Total Suspended Solids	0.3	0.7	N/A	Yes	mg/L
Fluoride	0.641	0.563	-12.17	No	mg/L
Total Organic Carbon	1.65	1.56	-5.45	No	mg/L
Potassium	2.07	2.1	1.45	No	mg/L
Magnesium	4.173	4.065	-2.59	No	mg/L
Chloride	8.127	8.225	1.21	No	mg/L
Sodium	6.447	6.499	0.81	No	mg/L
Calcium	13.328	12.847	-3.61	No	mg/L
Sulfate	16.518	17.41	5.40	No	mg/L
Hardness	50.5	48.8	-3.37	No	mg/L as $CaCO_3$
Conductivity	145.1	141.3	-2.62	No	uS/cm
pH	7.3	7.3	0.00	No	

end of this study. Fluoride was not identified by Zweig *et al*. (1999) as a concern in aquaculture source water assessments. Finally, zinc was reduced by 73%, and the values determined by the Water Quality Laboratory agree with values measured during the study.

The results of this pilot study provide more evidence that zinc is causing fin erosion in catfish at this facility. Zinc toxicity aligns with earlier observations that fin erosion does not seem to occur in water with high organic content, has not been observed in scaled fishes, and may be influenced by water temperature. Now the results of this study suggest that removal of zinc prevents fin erosion. Further investigation is required to determine definitively that zinc can cause fin erosion.

Chapter 4

Chronic Effects of Zinc Exposure in Channel Catfish with an Emphasis on Fin Erosion

Introduction

An aquaculture research laboratory in the southeastern United States has seen strong circumstantial evidence that chronic exposure to zinc results in fin erosion in several Ictalurid catfish species. To definitively link zinc with fin erosion it will be necessary to establish that the severity of fin erosion is positively correlated with zinc concentration and that fin erosion does not occur in the absence of zinc, without any variability in other water quality parameters that could offer an alternative explanation. The previous pilot study revealed that pre-treatment with activated alumina reduces zinc and prevents fin erosion. A basic treatment design for a follow-up study could be to use activated alumina for zinc removal of all treatment groups, and then add varying levels of zinc back in for each treatment. A study such as this would require alumina treatment on a larger scale than was used in the pilot study, so a system was set up to test this and to repeat the observation from the pilot study.

A Pentair Arias 6000 60-AQ sand filter was loaded with 5.5 ft^3 of AA-400G Fluorograde 14 x 28 mesh activated alumina and fitted to the supply line for a 300-liter circular fiberglass tank. A second identical tank was used for a control group and supplied with the same water without alumina filtration. Both tanks were provided a flow rate of 5 LPM for an exchange rate of one tank volume per hour. Each tank was stocked with 100 Stuttgart strain channel catfish, 2019 year-class with an average weight of 5.97 grams/fish. Five weeks later 10 fish from each tank were anesthetized and photographed for comparison of fin condition (Figure 10). The control tank exhibited typical fin erosion while the fish receiving alumina-filtered water had excellent fin condition. Zinc concentration of the control water and filtered water was 0.07 ppm and 0.02 ppm, respectively. It appears that a similar setup would be suitable for a larger study.

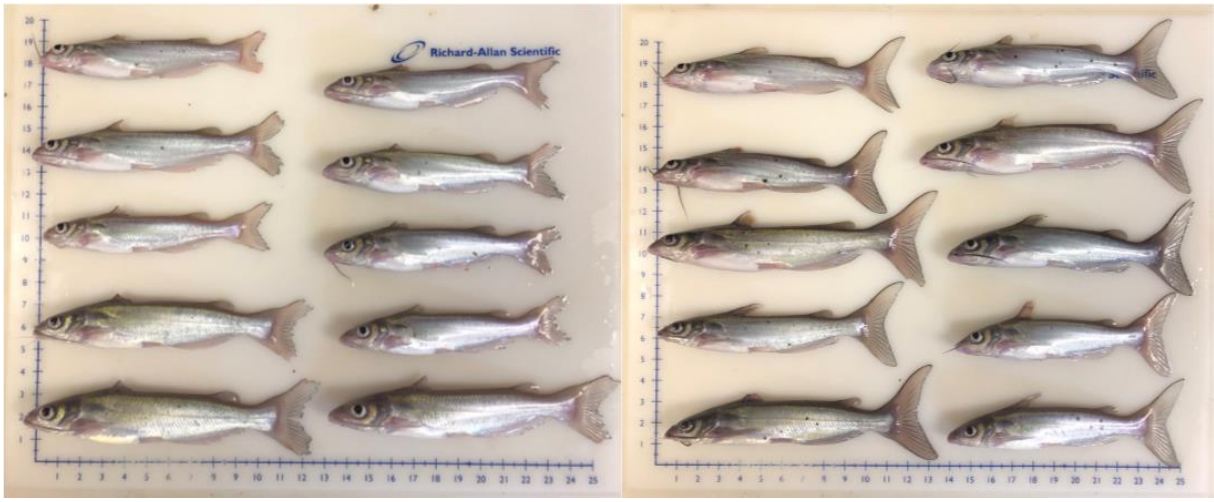

Figure 10. Results of a test system using a filter with activated alumina media. Channel catfish, *Ictalurus punctatus,* pictured left received dechlorinated heated municipal water without alumina filtration and experienced fin erosion. The fish pictured right received the same water with alumina filtration and did not develop fin erosion.

The present study will determine if zinc is responsible for the fin erosion that has been observed at this facility by exposing fish to different concentrations of zinc and comparing the condition of the caudal fin. It is expected that fish will not develop fin erosion in the absence of zinc and that the extent of erosion will increase with zinc concentration. Additional sampling will monitor growth and blood chemistry to explore if other effects of zinc exposure can be found.

Materials and Methods

The wet laboratory utilized for this study was fitted with a Hayward S270T2 sand filter loaded with 6 ft^3 of AA-400G Fluorograde 14 x 28 mesh activated alumina on the heated dechlorinated municipal water supply line for zinc removal by adsorption. Two PVC lines from this filter were configured for water delivery to 16 50-liter glass aquaria, providing two supplies to each tank. One of the supply lines was fitted for injection of a zinc chloride stock solution using an LMI A-series Chemical Metering Pump. Treatment rates were delivered by manually controlling the flow rate to each tank with Asahi ¼” labcock valves. Total combined water flow rate to each aquarium was 800 mL/min to exchange one tank volume per hour, which is the standard exchange rate at this facility. Adequate oxygen levels were maintained by providing light aeration with a single airstone in each aquarium.

Treatment rates selected were 0×, 0.5×, 1×, and 2× based on the average zinc concentration that has been associated with fin erosion at this facility. The pilot study showed an average zinc concentration of the municipal water supply was 0.08 ppm; this was rounded to 0.1 ppm in this study for simplicity so that treatments would be 0.5× = 0.05 ppm zinc, 1× = 0.1 ppm zinc, and 2× = 0.2 ppm zinc. The 0× treatment was not expected to be a true 0.00 ppm since activated alumina has not been 100% effective for zinc removal in previous trials. The pilot study showed good fin conditions at 0.03 ppm and a trial using a larger filter resulted in a zinc concentration of 0.02 ppm. For this study the 0x treatment was expected to be ≤0.02 ppm zinc. The 0x treatment receives 800 mL/min alumina-filtered water with ≤0.02 ppm zinc, the 2x treatment receives 800 mL/min zinc-injected alumina-filtered water with 0.2 ppm zinc, and the 0.5x and 1x treatments receive a combination of both water supplies to provide 0.05 ppm and 0.1

ppm zinc, respectively (Figure 11). Four tanks were provided to each treatment for replication, and treatments were randomly assigned to 16 aquaria.

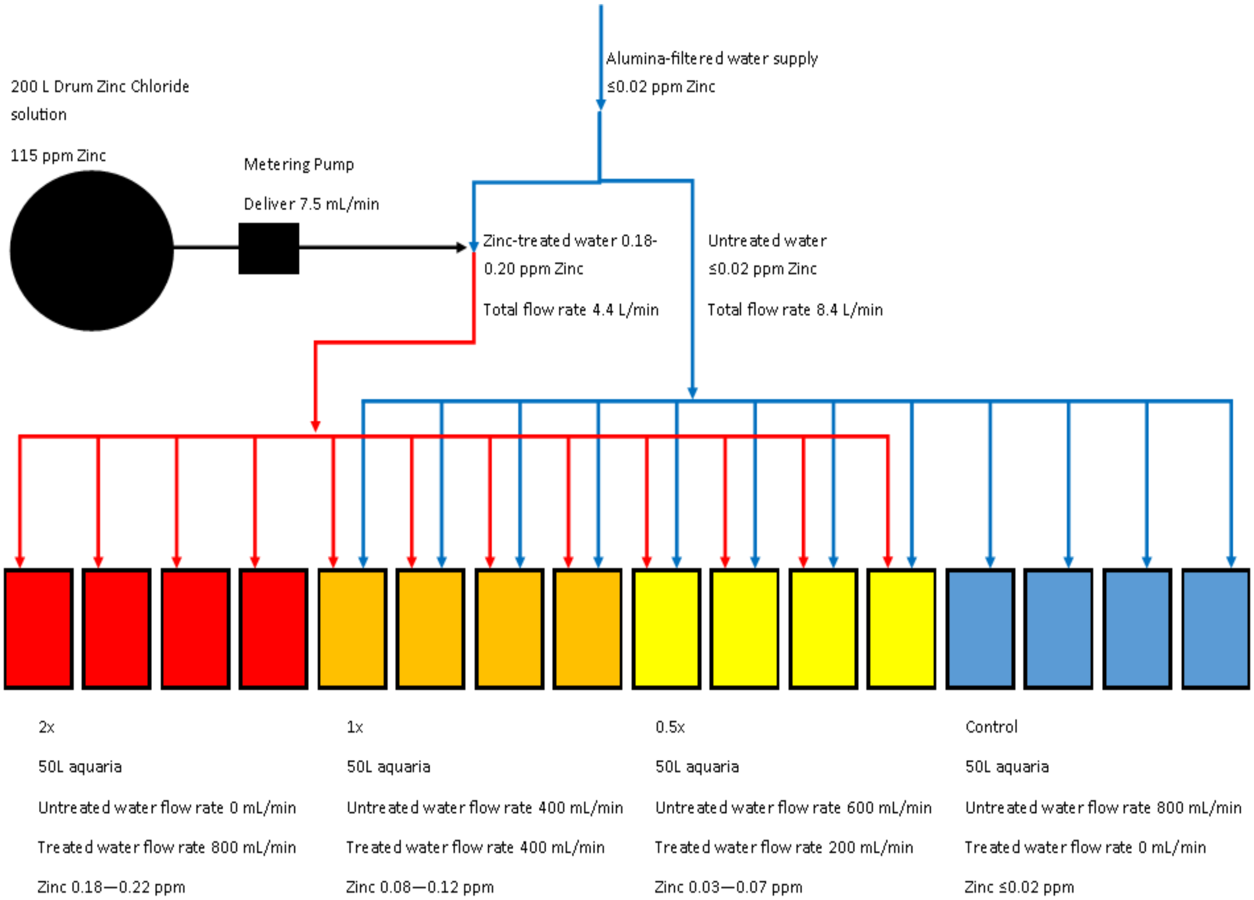

Figure 11. Treatment design showing removal of zinc from water supply, then addition of zinc to a treated water line. Delivery of untreated and treated water is controlled to each tank to provide the intended zinc exposure. In practice the treatments were randomly assigned to each tank rather than the grouping showed here.

For animal welfare, dissolved oxygen and temperature were measured using a YSI ProDO meter in four random tanks each day. Water samples were drawn from the zinc-treated supply line and the untreated line 2-3 times per week for determination of zinc concentration using a Hach DR2800 Spectrophotometer with test method 8009. The spectrophotometer was calibrated, and an accuracy check performed, before the study began using dilution of a 25 mg/L zinc standard solution, Hach product number 1424610. The metering pump and/or the stock solution concentration was adjusted as needed to maintain a treated supply of 0.18-0.22 ppm zinc. Since zinc exposure in each aquarium was dependent on water flow rate, the flow rates of all tanks were measured twice per day using a 1000 mL graduated cylinder and stopwatch. Flows were adjusted as needed to be within 10 mL/min of the target flow.

Fish used in this study were 2019 year-class Stuttgart strain channel catfish that were produced at this facility and held in a recirculating aquaculture system (RAS). Three days before the study began 400 fish were transferred from the RAS to the wet lab and stocked into 50 L glass aquaria at 25 fish per tank and provided alumina-filtered water to acclimate to study conditions. On day 1 of the study fish were randomly distributed to the experimental units at 25 fish per tank with water supplied according to treatment group. Each tank was fed a 2% body weight ration once per day in the AM; the ration was recalculated on days 14, 28, and 42 based on total fish mass in each tank. Feed used was Skretting Classic Fry 1.5 mm floating pellet until day 26 when all tanks were switched to Skretting Pond LE 1.5 mm floating pellet for the remainder of the study. The change in diet was a result of product availability but is not expected to interfere with any treatment comparisons since all fish were affected equally.

The study continued for 56 days. Fish samples were collected on days 1, 14, 28, 42, and 56 of the study. Five fish were randomly collected from each tank, anesthetized by immersion in 140-270 mg/L buffered MS-222, and photographed to document fin condition. Photographs were analyzed using ImageJ computer software. Total mass of fish was measured in each tank to monitor growth and adjust feeding schedule. On days 1, 14, 28, and 42 five fish from one tank in each treatment were anesthetized and blood samples collected from the caudal vasculature using tuberculin syringes. Immediately following blood collection those fish were euthanized by immersion in >300 ppm MS-222 for 10 min. Blood samples from each fish were pooled within treatments to provide a large enough sample for analysis. Whole blood was transferred into microcentrifuge tubes and stored refrigerated until spun in a centrifuge at 5000 rpm for 5 minutes. Serum was decanted into a new microcentrifuge tube and stored at -20°C until analysis. Serum analysis was conducted using an Abaxis VetScan VS2 with Comprehensive Diagnostic rotors. Since blood collection results in the loss of the fish the same tanks were used for this sample so that density would not be impacted in the other three tanks of each treatment. This was done so that the other three replicates would remain useful for analysis of growth data, since growth in channel catfish is known to be negatively correlated with fish density (Engle and Valderrama, 2001; Refaey *et al.*, 2018). On day 56 blood was collected from five fish in every tank by the same procedures.

Results

Water quality parameters were quite steady throughout the 8-week study (Table 7). Temperature fluctuated by only 2.4°C with a mean of 26.2°C, and dissolved oxygen (DO) ranged from 5.32-8.13 mg/L. DO measurements from day 14 and 15 were erroneously low due to a meter malfunction and not included. Zinc concentrations were measured 22 times during the 56-day study on the zinc-treated supply and the untreated supply. Concentrations delivered to each tank were calculated based on flow rates. The calculated zinc concentrations showed mean zinc

Table 7. Mean, high, and low values for water quality parameters that were monitored over 56 days.

	Control Zinc (ppm)	0.5× Zinc (ppm)	1× Zinc (ppm)	2× Zinc (ppm)	DO (mg/L)	Temperature (°C)
Mean	0.01	0.05	0.10	0.20	7.35	26.2
High	0.02	0.06	0.12	0.22	8.13	27.3
Low	0.00	0.05	0.09	0.16	5.32	24.9

exposure to all treatments was equal to the target exposure, and there was no overlap between treatments (Figure 12). Zinc concentration of the untreated water supply decreased to undetectable on day 26 and remained undetectable for the remainder of the study. This occurred because the zinc concentration of the municipal water supply to the facility decreased from a typical range of 0.06-0.16 ppm to 0.03 ppm, which allowed the alumina filter used for the study to reduce that concentration to below detection.

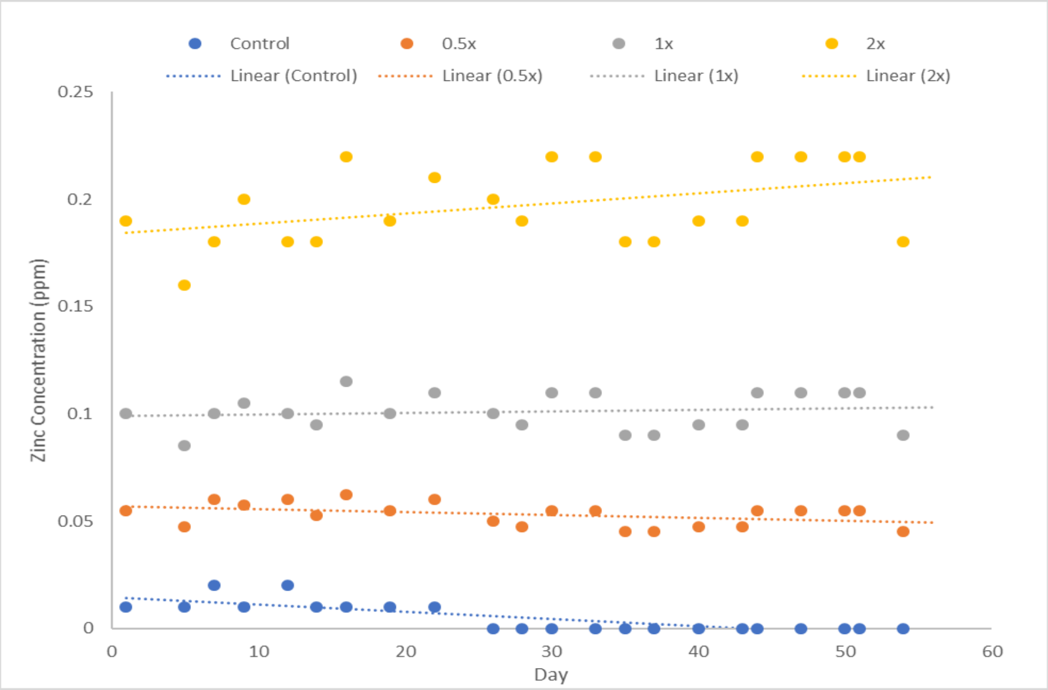

Figure 12. A plot of calculated zinc concentrations for each treatment based on measured zinc concentration in both water supplies. 22 measurements over the course of 56 days.

A previous pilot study demonstrated the possibility to predict caudal fin area by measuring body area. For this study caudal fin area was compared to both body area and standard length (Figure 13). Fish used for this comparison were 80 study fish photographed on day 1 that had intact fins, as well as 25 larger fish from the population the study fish were sourced from to cover the size range measured during the study. A non-linear model best fit the data and showed very strong correlation in both comparisons. The function provided by standard length (SL) was selected for fin condition assessment in this study because SL is a less time-consuming measurement than body area. The equation "caudal fin area" = $(0.04 \times SL^2) - (0.01 \times SL) - 0.09$, from Figure 13, was used to predict the caudal fin area that would be expected if fins were intact. The fin condition of each fish was then reported as a percent of caudal fin remaining by the equation "measured caudal fin area/predicted caudal fin area × 100". With this method it is possible to quantify the degree of fin erosion as a continuous numerical variable.

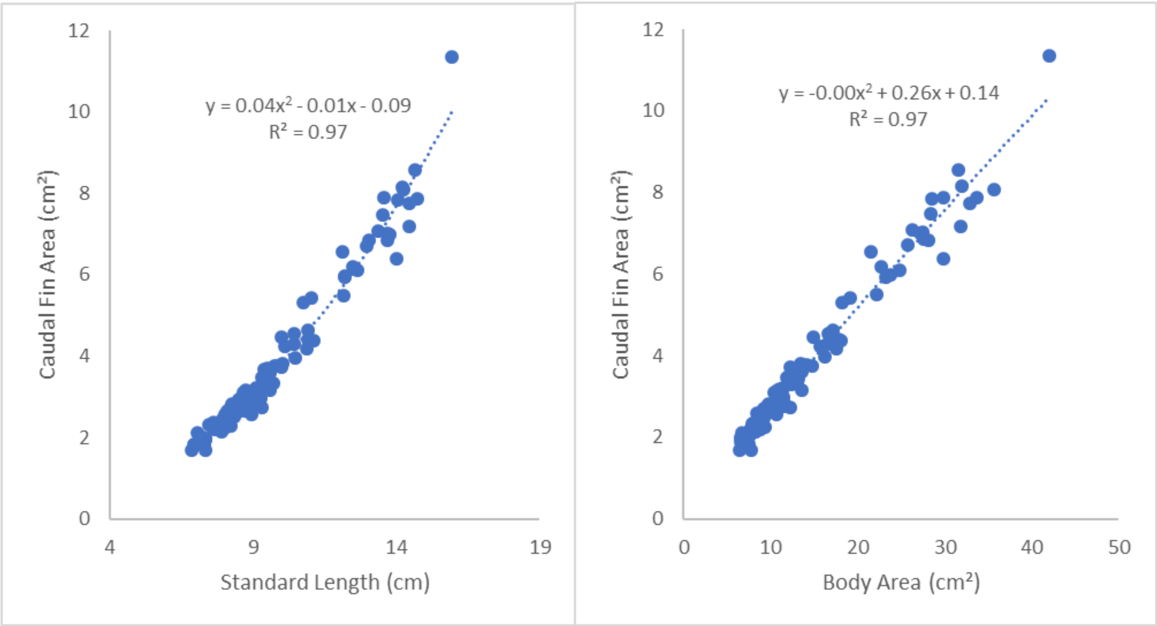

Figure 13. The non-linear relationship between standard length and caudal fin area (left) and body area and caudal fin area (right) in juvenile channel catfish, *Ictalurus punctatus*, with intact fins.

Fin condition declined over time for all treatment groups, including the control. Linear regression does not show a difference between treatments over time. In fact, the degree of fin erosion and rate of fin loss are remarkably similar in all treatments (Figure 14). Fin condition data was tested for normality using the Shapiro-Wilke test and for homogeneity of variances using Levene's test; the data was then log-transformed for normality. Analysis using generalized linear model revealed a significant difference in fin condition by day ($P<0.0001$). Tukey-Kramer LSD test showed that days 1 and 14 were not different ($P=0.740$), but there were significant differences between days 14 and 28 ($P=0.0007$), 28 and 42 ($P<0.0001$), and 42 and 56 ($P<0.0001$) (Figure 15). Analysis using generalized linear model showed no significant difference in fin condition by treatment ($P=0.798$). On day 56, when the greatest treatment differences were expected to be observed, mean fin conditions of treatments 0×, 0.5×, 1×, and 2× were 71.0%, 71.2%, 68.4%, and 69.7%, respectively (Figure 16). Fin erosion occurred during this study but was not influenced by treatment.

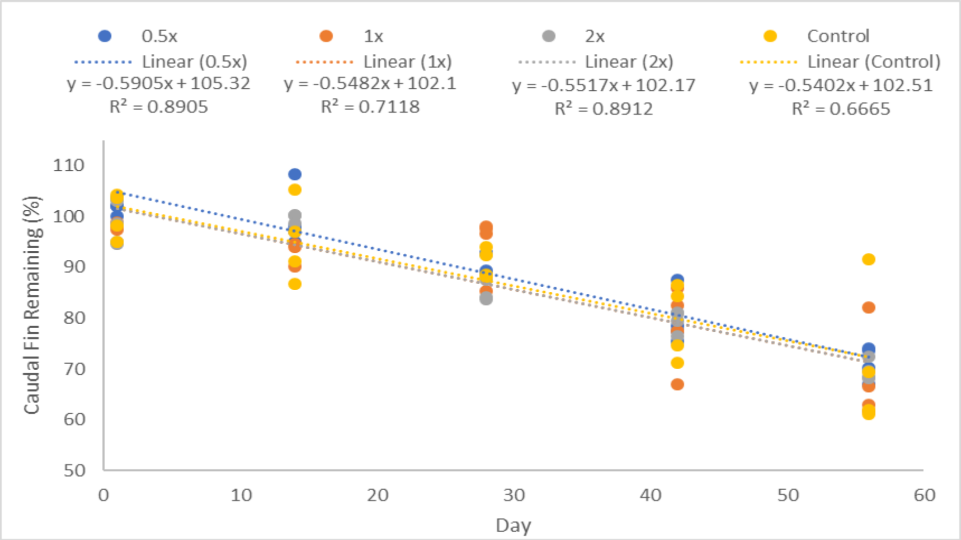

Figure 14. Caudal fin conditions of channel catfish, *Ictalurus punctatus*, over time with exposure to different concentrations of Zn^{2+}. Fin condition deteriorated in all treatments with no difference between treatment on day 56.

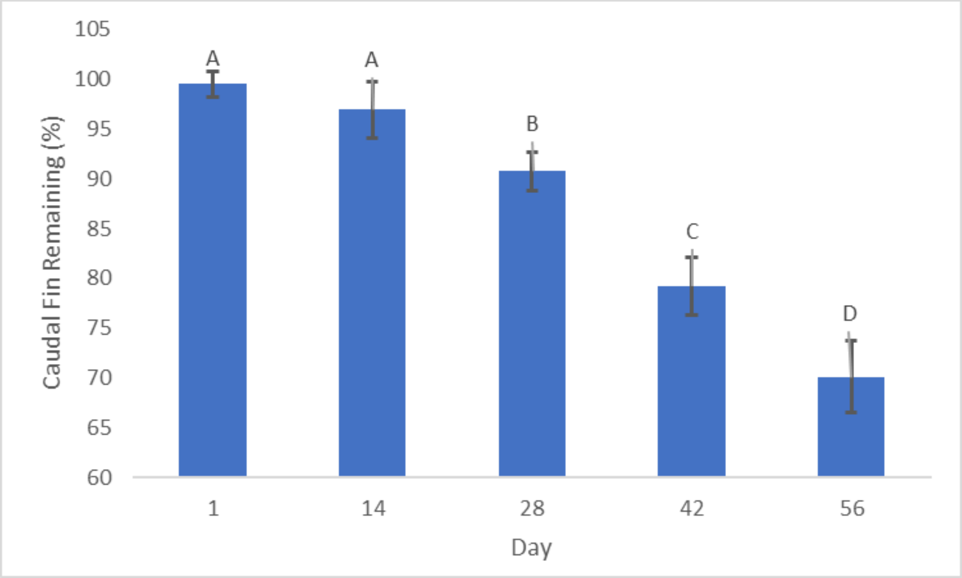

Figure 15. Comparison of fin condition by day, all treatments combined. Significant differences were found between A, B, C, and D. Error bars represent ± standard error.

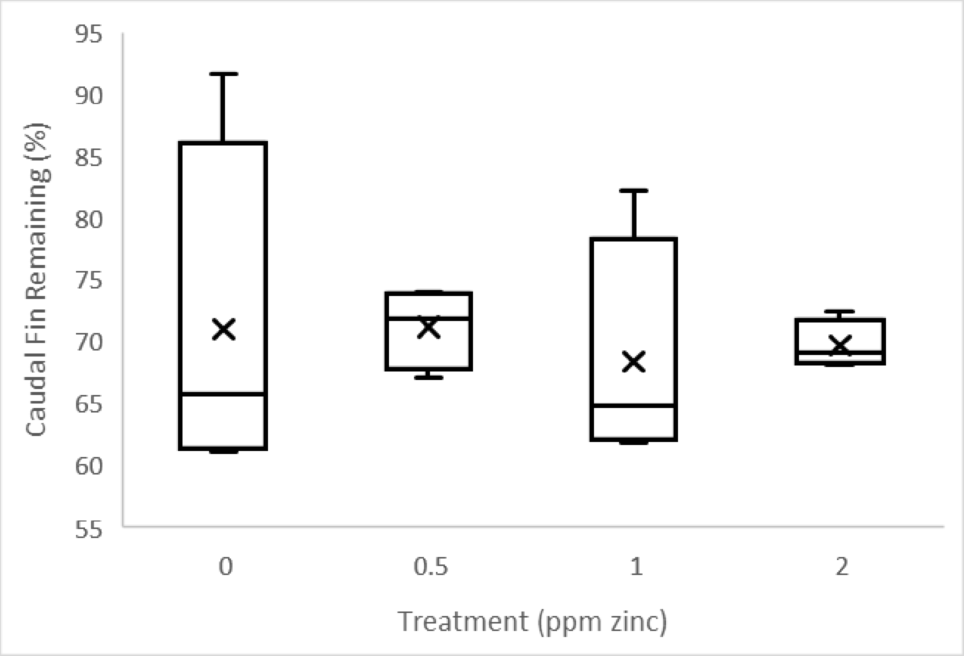

Figure 16. Comparison of fin conditions by treatment on day 56. X = the mean. Middle line in each box = the median. Top and bottom of box = first quartile and third quartile, respectively. Whiskers mark maximum and minimum values. No outliers are present.

Total mass of fish in each tank was determined on days 1, 14, 28, 42, and 56 to compare growth between treatments. Since the fish were randomly assigned to experimental units, the units did not have equal total mass on day 1, but instead ranged from 154.8-202.5 g per tank. Because of the variation in initial weight, weight over time could not be directly compared. Instead, feed conversion ratio (FCR) and growth rate (g/fish/day) were calculated for comparison (Table 8). These data were tested for normality using the Shapiro-Wilke test and for homogeneity of variances using Levene's test; the data was then log-transformed for homogeneity of variances. Analysis with generalized linear model did not detect a significant difference in FCR (P=0.9781) or growth rate (P=0.1680) between treatments. Chronic exposure to zinc at concentrations of 0.01-0.2 ppm did not impact FCR or growth rate in juvenile channel catfish.

Table 8. Mean feed conversion ratios (FCR) and growth rates (g/fish/day) ± standard error of channel catfish, *Ictalurus punctatus*, after 56 days of zinc exposure. Treatments of 0×, 0.5×, 1×, and 2× were exposed to 0.01, 0.05, 0.10, and 0.20 mg/L zinc, respectively.

Treatment	Mean Final FCR	Mean Growth Rate
0×	0.935 ± 0.003	0.268 ± 0.005
0.5×	0.920 ± 0.016	0.249 ± 0.007
1×	0.941 ± 0.060	0.236 ± 0.015
2×	0.938 ± 0.035	0.261 ± 0.009

Blood serum samples were analyzed using the Abaxis VetScan Comprehensive Diagnostic Profile. This test measures 14 blood parameters simultaneously, but it is designed primarily for testing mammalian blood samples. Five of the parameters tend to measure out-of-range using fish blood and were not included in analysis. On days 1, 14, 28, and 42 only a single sample was collected from each treatment group, so statistical analyses cannot be conducted to compare treatments on those days. On day 56 samples were collected from all tanks and concentrations of albumin, alkaline phosphatase, amylase, blood urea nitrogen, calcium, glucose, sodium, total protein, and globulin were measured. All data was log-transformed for normality and homogeneity of variances. Analyses with generalized linear model did not show a significant difference between treatment groups for any blood parameter (Table 9).

Table 9. Final mean concentrations of 9 blood parameters ± standard error for juvenile channel catfish, *Ictalurus punctatus,* exposed to different concentrations of zinc for 56 days. Treatments of 0×, 0.5×, 1×, and 2× were exposed to 0.01, 0.05, 0.10, and 0.20 mg/L zinc, respectively. ALB = albumin, ALP = alkaline phosphatase, AMY = amylase, BUN = blood urea nitrogen, CA = calcium, GLU = glucose, NA = sodium, TP = total protein, GLOB = globulin.

	ALB (g/dL)	**ALP (U/L)**	**AMY (U/L)**	**BUN (mg/dL)**	**CA (mg/dL)**	**GLU (mg/dL)**	**NA (mmol/L)**	**TP (g/dL)**	**GLOB (g/dL)**
0×	3.0 ± 0.97	56 ± 14	36 ± 10	5 ± 0.0	10.1 ± 0.912	15 ± 2.5	141 ± 5.44	4.3 ± 0.45	1.4 ± 0.14
0.5×	2.8 ± 0.52	54 ± 4.2	28 ± 5.8	4 ± 0.0	9.6 ± 0.67	16 ± 0.65	135 ± 1.11	4.0 ± 0.13	1.2 ± 0.12
1×	2.8 ± 0.74	58 ± 9.0	29 ± 6.6	4 ± 0.4	10.2 ± 0.431	18 ± 1.7	134 ± 1.16	4.2 ± 0.39	1.4 ± 0.13
2×	3.1 ± 0.30	74 ± 19	37 ± 7.0	4 ± 0.4	9.8 ± 0.32	20 ± 2.5	143 ± 3.97	4.4 ± 0.25	1.3 ± 0.23
P=	0.883	0.362	0.803	0.108	0.916	0.241	0.306	0.860	0.716

Since there were no treatment differences on the final day of the study, the treatments were pooled and all blood parameters compared against time. Doing so provides replication at each sampling point and may show a trend that corresponds with the observed fin erosion. Linear regression of each blood parameter was performed with time as the explanatory variable rather than treatment (Figure 17). Two parameters showed a strong relationship with time, calcium ($y = -0.06x + 13.62$, $r^2=0.60$) and glucose ($y = -1.07x + 83.67$, $r^2=0.62$). Despite apparent correlation, mean glucose levels do not seem to follow a downward trend; with mean values of 64.8, 85.8, 45.5, 73.8, and 17.1 mg/dL on day 1, 14, 28, 42, and 56, respectively. On the other hand, mean calcium levels of 13.2, 12.5, 12.5, 12.4, and 9.9 mg/dL on day 1, 14, 28, 42, and 56, respectively, show a clear reduction of blood calcium over time. It seems that an unknown variable caused a reduction of blood calcium over the course of this study regardless of treatment group.

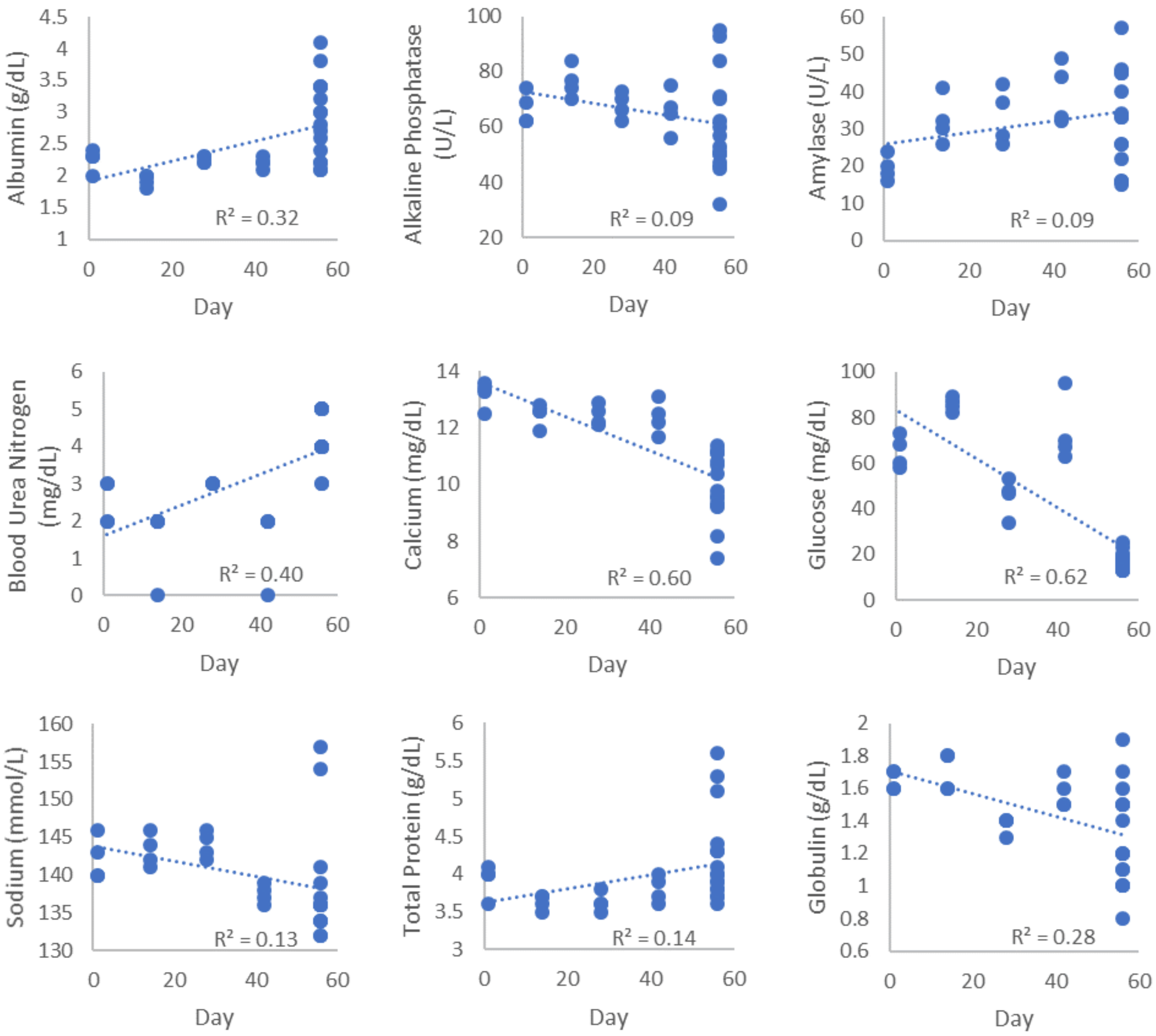

Figure 17. Measured blood parameters over time for juvenile channel catfish, *Ictalurus punctatus,* developing fin erosion. Treatments of different levels of chronic zinc exposure were eliminated as a factor and samples from all treatments pooled with day as a factor.

Statistical analysis of the pooled blood samples was performed using repeated measures ANOVA. Kenward-Rogers approximation for standard errors was used since the data were unbalanced, with 4 samples from each of days 1, 14, 28, and 42, and 16 samples from day 56. These data violated both assumptions of normality and of homogeneity of variance, requiring the data to be log-transformed. No significant differences were detected for alkaline phosphatase, amylase, sodium, or total protein ($P = 0.360$, 0.132, 0.216, and 0.075, respectively). Significant differences were found for albumin ($P = 0.0025$) and globulin ($P = 0.0104$). In both cases the difference lies only between days 14 and 56. Differences in these protein concentrations are probably not related to fin erosion without a clear upward or downward trend over time, but instead may have been influenced by some other stressor or environmental stimuli.

Significant differences were also detected for glucose ($P <0.0001$) and blood urea nitrogen ($P <0.0001$). Glucose was significantly different between day 56 and all earlier time points ($P <0.0001$). Glucose had additional differences between days 14 and 28 ($P = 0.0002$) and days 28 and 42 ($P = 0.005$), but not between days 14 and 42 ($P = 0.686$). Blood urea nitrogen was significantly different between day 56 and day 1 ($P = 0.0035$), day 14 ($P <0.0001$), and day 42 ($P <0.0001$), but not day 28 ($P = 0.0665$). Differences were also detected between days 14 and 28 ($P = 0.0372$) and days 28 and 42 ($P = 0.0372$), but not between days 14 and 42 ($P = 1.000$). While differences were detected with glucose and blood urea nitrogen, those differences do not make sense chronologically. These two parameters may be related to each other, since they showed differences in mostly the same places. Additionally, Figure 17 appears to show an inverse relationship for these parameters. It is suspected that metabolic activity occurred while blood samples were in refrigerated storage, resulting in the consumption of glucose and production of urea. These two parameters are therefore confounded since the duration of refrigeration was inconsistent between sampling days.

Finally, significant differences were detected for blood calcium (P <0.0001). Differences were between days 1 and 56 (P <0.0001), 14 and 56 (P = 0.0013), 28 and 56 (P = 0.0014), and 42 and 56 (P = 0.002). Calcium on day 56 was different from all earlier time points. Although the mean values stated earlier decreased over time, there were no significant differences between days 1, 14, 28, or 42. Perhaps with more replication on those earlier sampling days some differences would have been revealed. Even so, calcium is the only blood parameter measured where the statistical differences align chronologically with the observed fin erosion. The apparent relationship between calcium and time presented in Figure 17 is made stronger by this statistical analysis.

Discussion

The goal of this study was to determine if chronic exposure to zinc causes fin erosion in juvenile channel catfish. Fin erosion developed equally in all treatments, including the control where zinc concentrations were below detection for much of the study and below levels that had been associated with good fin conditions in past observations. Therefore, zinc is not the etiology behind the fin erosion at this facility, but the causative agent was present in this study. Water used for this study was treated by activated alumina filtration, which had been shown in two previous trials to protect against fin erosion, so it would have been expected that erosion should not have occurred since zinc was not the cause. Fin erosion did develop, so some other toxicant could be present that was adsorbed by activated alumina in previous trials but not in this one.

Adsorption by activated alumina is dependent on contact time, and the adsorption efficiency and capacity differ between contaminants (Naiya *et al.*, 2009; Singh and Pant, 2004; Ghorai and Pant, 2005). The flow rate in relation to media volume of the previous trials where fin erosion was prevented were 1.07 LPM/ft^3 and 0.91 LPM/ft^3, compared to 2.13 LPM/ft^3 for the present study. Although this contact time was sufficient for zinc removal, it may have interfered with adsorption or resulted in rapid media saturation and breakthrough of some other toxicant.

Pierson (1981) demonstrated that chronic exposure to 0.607 mg/L zinc for 134 days negatively affected wet weight of the guppy, *Poecilia reticulata*. Zinc is known to interfere with calcium uptake in fish, leading to hypocalcemia (Authman *et al.*, 2015), so a reduction in blood calcium would have been expected in the present study. This study did not detect any treatment differences in growth or blood chemistry. The results of this investigation indicate that channel catfish are tolerant of zinc concentrations at least as high as 0.20 mg/L. The fin condition data showed that caudal fins lost area over time, and blood chemistry analyses revealed that blood calcium levels declined over time. Neither observation was associated with zinc treatment. There is a strong possibility that some other toxicant is responsible for both observations.

Besides zinc, activated alumina was shown to adsorb phosphates and fluoride in the previous pilot study. Phosphates are considered non-toxic to fish (Kim *et al.*, 2013), but fluoride, on the other hand, may not be so innocuous. Although Zweig *et al.* (1999) did not identify fluoride as a concern when assessing aquaculture source water, Sigler and Neuhold (1972) describe serious aquatic toxicity in their review. Even so, as was the case with zinc, literature has not been published that directly links fluoride exposure with fin erosion. Cao *et el.* (2013) noted inhibition of several enzymes when common carp, *Cyprinus carpio*, were exposed to 35-124 mg/L F^{-} for 90 days. Bajpai and Tripathia (2006) reported alterations of protein and lipid content in multiple tissues following exposure of stinging catfish, *Heteropneustes fossilis,* to 77.2 mg/L F^- for 90 days. Singh *et al.* (2017) saw changes in gene expression in zebrafish, *Danio rerio,* after a 30 day exposure to 71.12 mg/L F^-. Yadav *et al.* (2014) found changes in enzyme activity and liver tissue damage when stinging catfish were exposed to 35-70 mg/L F^- for 90 days. Considering the high doses delivered and relatively minor effects observed in these chronic toxicity studies, it seems unlikely that the 0.64 mg/L F^- present in this facility's water supply could cause the kind of fin damage seen in this study. Perhaps the best explanation for the fin erosion at this time is that another toxicant that has not been tested for is present in the municipal water supply.

Conclusions

An aquaculture research facility in the southeast United States has been investigating a case of fin erosion occurring at that laboratory. Circumstantial evidence suggests that only catfishes are affected, fin erosion is inhibited by dissolved organic carbon and low water temperature, and activated alumina removes the toxicant at 1 LPM water flow rate per cubic foot of media but not at 2 LPM/ft^3. Experimental investigation has eliminated zinc as a possible cause and shown that blood calcium decreases over time. This facility will continue to struggle with fin erosion until the etiology is revealed and remediation can be implemented. Continued work should include more extensive water analyses and a more thorough assessment of the protection provided by activated alumina filtration. When one or more contenders are uncovered a small pilot study should be conducted to provide more evidence that the suspected cause results in fin erosion before starting a larger trial. When a promising candidate is found, the study design presented here could be used as a format for investigation of another toxicant.

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