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The potential algaecide flumioxazin has little effect on growth, survival and feed conversion of the bluegill sunfish *Lepomis macrochirus*

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ABSTRACT

An effective and benign approach to mitigating fish-killing *Prymnesium parvum* blooms in small impoundments has yet to be identified. The aquatic herbicide flumioxazin (Clipper®, Valent Corp., Walnut Creek, CA), however, shows promise as it suppresses *P. parvum* while having little effect on other plankton. Its influence on fish has yet to be evaluated. Here, we investigated potential effects of flumioxazin on growth, survival and feed conversion ratio of a non-target organism, bluegill sunfish (*Lepomis macrochirus*) in a laboratory experiment. We exposed fish to several herbicide concentrations that spanned the manufacturer's recommended dosage (0, 25, 50, 100, 200 and 400 μg L^{-1}). The duration of the experiment was six weeks with weekly re-application of flumioxazin to specified tanks. No significant differences were found in average fish weight gain ($p = 0.76$), percent survival of fish ($p = 0.83$) or the feed conversion ratio ($p = 0.89$). These findings provide further evidence that flumioxazin application may be an effective and benign approach to *P. parvum* mitigation.

1. Introduction

Prymnesium parvum is a mixotrophic, flagellated algae that occurs worldwide in both marine and inland water bodies (Brooks et al., 2011; Granéli et al., 2012). In inland water bodies, it forms blooms toxic to many organisms that spread through the entire lakes (Genitsaris et al., 2009; Michaloudi et al., 2009; Roelke et al., 2010) and propagate through watersheds spanning 100 s km (Roelke et al., 2011). During periods of high salinity and low temperature, *P. parvum* produces toxins, in part, due to these stressors. Because some of the toxins are allelopathic and grazing-inhibiting, *P. parvum* outcompetes other algae and can form near mono-specific blooms (Granéli et al., 2012). But sometimes blooms can be a mix of harmful algal taxa (Oikonomou et al., 2012). These blooms lead to mortality of gill breathing animals (Guo et al., 1996; Kaartvedt et al., 1991) and have resulted in extensive damage to fisheries worldwide, both wild and cultured (Aure and Rey, 1992; Guo et al., 1996; Lindholm et al., 1999; Otterstrom and Steelmann-Nielson, 1940; Southard et al., 2010).

In small impoundments, such as aquaculture ponds, mitigation approaches for *P. parvum* blooms have been demonstrated. For example, chemical additions of ammonium sulfate or copper-based algaecides, or application of various clay minerals are approaches that target *P. parvum* cells. They either cause mortality to *P. parvum* (Barkoh and Fries, 2005; Barkoh et al., 2010) or act to remove cells through binding and settling (Sengco et al., 2005). Other approaches involving chemical control have targeted the toxin and include addition of potassium permanganate or sulfuric acid (Barkoh et al., 2010; Prosser et al., 2012). These approaches render toxins ineffective through either oxidation or altered ionization state (Barkoh et al., 2010; Valenti et al., 2010a,b). Another chemical control approach involves fertilization. Temporarily elevating nutrient concentrations caused *P. parvum* to stop toxin production, removing its competitive edge over other phytoplankton and leaving it vulnerable to grazing (Errera et al., 2008; Kurten et al., 2007, 2010, 2011; Roelke et al., 2007).

All of these chemical control approaches were effective at mitigating *P. parvum* blooms to a degree. But all suffered from drawbacks as well. For example, addition of ammonium sulfate may produce un-ionized ammonia that is harmful to fish. Similarly, copper-based compounds indiscriminately kill other organisms along with *P. parvum*. *P. parvum* cells removed from the water column through clay flocculation are likely to quickly re-suspend in shallow water environments. Furthermore, the transport of *P. parvum* cells to the benthos may promote establishment of seedbeds, increasing the likelihood of future blooms. And fertilization with inorganic nutrients may promote elevated pH to levels lethal to some fish, and also potentially contribute to downstream eutrophication. So while great strides have been made in *P. parvum* bloom mitigation in small impoundments, there is a need for alternative approaches.

One such approach to *P. parvum* mitigation may be application of a newly registered aquatic herbicide, flumioxazin. Following initial screens of several new aquatic herbicides (which included the

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bleaching herbicides fluridone and topramazone, the acetolactate synthase inhibitors penoxsulam and bensulfuron, and the protoporphyrinogen oxidase inhibitors carfentrazone and flumioxazin), flumioxazin showed the most promise in providing effective and selective algaecidal activity on *P. parvum* (Umphres et al., 2012). Flumioxazin prevents synthesis of chlorophyll *a* and causes rapid peroxidation of lipid membranes in sensitive plant species, eventually leading to death of plant cells. In previous in-lake mesocosm experiments, addition of flumioxazin suppressed *P. parvum* bloom initiation while having little effect on other plankton (Umphres et al., 2012). In other words, the approach was effective and appeared benign to many non-target organisms of the lower food web. It is important to note that given the recent US EPA registration for aquatic use in 2010, flumioxazin could be utilized for *P. parvum* control in a variety of aquatic sites. The combination of low use concentrations, potential for algal selectivity, and rapid degradation via pH-dependent hydrolysis suggest a compound that has many favorable characteristics and therefore more detailed evaluation of this compound is warranted. The effect flumioxazin might have on higher trophic level organisms, such as fish, requires testing.

Here, we investigate the effects of flumioxazin on bluegill sunfish (*Lepomis macrochirus*) growth, survival and feed conversion ratio. This is an important species in recreational fisheries. Originally native to the Mississippi River drainage, *L. macrochirus* now inhabits the entire United States and can be found worldwide (Welcomme, 1988). In the USA, *L. macrochirus* is considered a valuable primary forage fish for larger game species such as largemouth and smallmouth bass, the species is also sought after for angling (Thomas et al., 2007).

2. Methods

2.1. Acclimation period

Our experiment was carried out at the Texas A&M Aquatic Research and Teaching Facility in College Station, TX. Prior to the initiation of the experiment, a fourteen-day period was allowed for disease free *L. macrochirus* (The Bait Barn Fisheries, Bryan TX) to acclimate to new 23 L tank conditions. During this period water temperature remained ~22 °C ± 1° with a pH of ~8.3 ± .1 while dissolved oxygen remained ~8.0 mg L^{-1} ± 1 mg L^{-1}. Our ambient lighting conditions were set on a 12:12 day/night cycle. To allow adequate removal of wastes, water was recirculated at a rate of ~1 L min^{-1} with a residence time of 23 min and filtered through mechanical/biological media. Low-pressure electrical blowers provided adequate aeration through the use of air stones. To reach salinites typical of *P. parvum* bloom conditions, mixed Stock Salt (United Salt Corp) was added to reach a final salinity of 4 psu. Feeding was done once daily at 15:00 to apparent satiation with 1.6 mm floating pellets comprised of 44% protein (Rangen EXTR 450).

2.2. Screening experiment

Twenty-one 23 L aquaria were utilized in our experiment to evaluate the effect of flumioxazin on the growth *L. macrochirus*. For this study we randomly selected 3 tanks for each treatment concentration of 25, 50, 100, 200 and 400 μg L^{-1}. Six tanks were randomly selected for our control treatments. Upon initiation, fish were graded by size and weighed 12 at a time. Fish of similar size were then added to each tank.

Our experimental design was limited. Operation of a single pump and filtration system fitted to 21 aquaria would have resulted in complete mixing of all treatment concentrations of flumioxazin. To prevent this, at the initiation of the experiment the recirculating pump was turned off and each aquarium became static except for air stones providing aeration. After an additional 0.5 h acclimation period, we added packets of pre-weighed flumioxazin granules to achieve the designated concentrations. A subsequent 3 h period was allowed for the flumioxazin granules to fully dissolve before feeding to apparent satiation. Our feeding rate was adjusted daily to minimize waste, and after each feeding the weight in grams fed was recorded.

To minimize stress caused by ammonia buildup and declining water quality, every seventh day a 100% water exchange was performed. To accomplish this, we oxygenated (~12 mg L^{-1}) pre-mixed salt water (4 mg L^{-1}) in a secondary 950 L tank with pure liquid O_2 (Airgas Inc.). Water from aquaria was then pumped out and replaced with new water. During this period of handling, fish from their respective tanks were weighed and recounted before being replaced into their aquaria. Then, flumioxazin was again added following the methods previously described. Thus the treatment levels represent a pulsed experimental design with weekly flumioxazin dosages.

Total ammonia nitrogen and dissolved oxygen were measured bi-weekly. Alkalinity, hardness, salinity and pH were measured weekly. Additionally, during our daily feeding period, fish were counted, and general swimming and feeding behavior was observed. Dead fish were removed and weighed, and additional ammonia levels were measured and recorded for those tanks each day throughout the experiment.

2.3. Statistical analysis

Average fish weight per treatment were analyzed using a general linear model (GLM) repeated measures ANOVA (JMP Pro 9) where treatment differences were considered significant at $p<0.05$. Additionally, overall percent survival, percent weight gain and feed conversion ratios were analyzed using One-Way ANOVAs (JMP Pro 9).

3. Results

Previous monitoring of total ammonia nitrogen in densely stocked tanks with *L. macrochirus* indicated acute toxicity at levels of 1.3 mg L^{-1} (Umphres personal observation). Similar results were seen in Mayes et al. (1986) and Ruffier et al. (1981). For the duration of this experiment, total ammonia nitrogen remained at relatively non-toxic levels for *L. macrochirus* (mean = 0.17 mg L^{-1} SD ± 0.06). Moderately high levels of alkalinity and hardness were observed throughout the experiment (190.5 SD ± 12.6, 691.5 SD ± 23.3) due to the addition of salts prior to experiment initiation. Salinity remained at approximately 4.5 mg L^{-1} SD ± 0.08. The pH also remained stable at 8.31 SD ± 0.04. During our experiment, dissolved oxygen was high at the start of each week due to the 100% water exchange with highly oxygenated water (12.0 ± 1 mg L^{-1}) but steadily decreased until reaching a steady state at ~8.0 ± 1 mg L^{-1} by the second or third day.

L. macrochirus average weights increased throughout the experiment for all treatments (Fig. 1). Repeated measures GLM showed no significant differences between treatments ($p = 0.11$). In this study, fish growth and feeding was not affected by the flumioxazin dosage. Table 1 summarizes performance indicators for *L. marcrochirus* for this experiment. Total survival was relatively high at 92% and no significant difference was observed between any treatment concentrations ($p = 0.83$). Similarly, no statistically significant differences in percentage of initial-weight gained ($p = 0.76$) or feed conversion ratios ($p = 0.89$) were observed between treatments. By visual observation, some *L. macrochirus* seemed more aggressive than others, resulting in some mortality from constant fighting within tanks.

4. Discussion

Visual observations through the duration of the experiment revealed no apparent changes in behavior (e.g., erratic swimming, gill movement) between aquaria exposed to flumioxazin and the controls. Additionally, the lack of statistical difference between treatments for weight gain, percent survival and feed conversion ratios