ABSTRACT

One of the major concerns of expanding aquaculture industry is the extensive use of chemicals. For balanced and sustainable aquaculture, it is imperative to determine the optimal rate of application of chemicals and the possible effects on pond water quality, non-target species and overall fish production. To this end, we performed a series of experiments to evaluate the efficacy and effects of fertilizers, herbicides and algaecides used in percid and catfish ponds of the State Fish Hatcheries of Ohio.

To determine whether lowering of phosphorus inorganic fertilization rate will increase juvenile percid production as well as lower the risk of proliferation of inedible algal species, we conducted field experiments comparing the original phosphorus fertilization rate of 30 $\mu$g P/L with lowered fertilization rates of 20 $\mu$g P/L and 10 $\mu$g P/L and a constant inorganic nitrogen fertilization rate of 600 $\mu$g N/L. Our results showed that lowering of phosphorus fertilization rate from 30 to 10 $\mu$g P/L neither affected the larval saugeye production (in terms of survival, yield, and growth) nor the maintenance of adequate zooplankton forage base throughout the culture duration. The phytoplankton biomass and species composition remained similar among treatments; the inoculum-weighted dominance of Cyanobacteria prevented proliferation of edible algal species in the initial weeks of fish culture. Lowering the phosphorus fertilization rate in percid ponds may reduce the potential risks of elevated pH, unionized ammonia, low dissolved oxygen concentration, macrophyte infestation and the discharge of nutrient-rich effluent
into surrounding waters. Based on the results of this study, a juvenile percid pond fertilization protocol with lowered phosphorus fertilization rate of 10 μg P/L has been implemented in State Fish Hatcheries of Ohio.

To examine whether the use of herbicides and algaecides is justified in catfish ponds, we conducted field experiments to investigate the efficacy of commonly used herbicide-fluridone and algaecide-copper sulfate applications at controlling plants and algae and their possible effects on water quality, seasonal dynamics and species composition of phytoplankton and zooplankton, and overall catfish production. Our results showed that the combined application of fluridone and copper sulfate resulted in substantially less macrophyte biomass than did the fluridone-alone treatment. Fluridone and copper treatments elicited different responses within the phytoplankton community. Copper treatments reduced Cyanophyta biomass, whereas biomass of Chlorophyta and Chrysophyta was increased. Fluridone treatments reduced total phytoplankton biomass as well as Cyanophyta biomass and influenced the response of Chlorophyta and Chrysophyta to copper, demonstrating some algaecidal potential. The phytoplankton community composition shifted towards species tolerant to copper in the treated ponds which in turn affected zooplankton community composition along with direct toxic effects of copper on sensitive zooplankton species. Copper treatments significantly reduced Cladocera biomass, whereas Copepoda biomass was significantly higher in copper-treated ponds than in controls. Catfish survival and yield were not significantly different among treatments. As an aquaculture pond can be considered as a model freshwater ecosystem, this study elucidated underlying effects and responses of natural aquatic communities to environmentally realistic concentrations of fluridone and copper
sulfate, yielding meaningful results both for aquaculture ponds as well as other freshwater ecosystems.

To assess the extent and effects of carry-over copper in double-cropped ponds on juvenile percid culture, we conducted field monitoring of copper in the sediment and pond waters of double-cropped ponds at Senecaville State Fish Hatchery, where consistently low percid yield and low biomass of zooplankton forage base for planktivorous percids have been observed. To complement our field data, we conducted a laboratory experiment to assess the effect of copper concentrations encountered in Senecaville waters on the reproductive output of a resident cladoceran species (*Daphnia parvula*). We found relatively higher copper concentrations in the pond sediments from repeated applications of copper sulfate during catfish culture in summer, which remobilized into the water column during percid culture in spring. The reproductive output of *D. parvula* was significantly affected by dissolved copper concentrations as low as 6.3 and 9.9 μg/L. Further manipulative experiments need to be conducted to establish the direct and indirect effects of low concentrations of copper on plankton biomass, species composition and percid production.
Dedicated to Joanna, Pence, and all at Pamala
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To quote the words of Oliver W. Holmes, “The great thing in the world is not so much where we stand, as in what direction we are going.” Little did I know when I set foot on the foreign soil 5 years ago that I was in the “direction” of the realization of my dream of acquiring a doctoral degree from a foreign university. Had it not been for the contributions of numerous people in some measure, I would not have come thus far.

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INTRODUCTION

The aquaculture industry is growing at a phenomenal rate, doubling in product weight and value between 1986 and 1996, and the 2002 global production totaled 51.4 million metric tons with a value of US $60 billion (New 1997, FAO 2002). Declining capture fisheries and increasing world population will continue to provide impetus for further expansion of the industry in future. The unprecedented growth of aquaculture, however, has posed several risks to our natural resources and environment, including habitat modification, effluent discharge, introduction of non-indigenous species, depletion of wild seed stock, spread of diseases, etc. (Primavera 2006, Boyd 2003). Of these and other possible negative effects, a major concern to scientists, environmental managers and policy makers alike, is the extensive use of chemicals in aquaculture operations.

Successful fish culture often requires addition of many chemicals such as therapeutants, disinfectants, soil and water treatment compounds, algaecides, herbicides, feed additives, fertilizers, minerals, etc. (Boyd and Tucker 1998). The use of these chemicals has certainly improved fish production world-wide and has become an indispensable part of the standard management protocol for culture of many fish species. For example, fertilization of larval percid ponds to stimulate phytoplankton growth, and addition of herbicides and algaecides to control algal and macrophyte infestation in catfish ponds are widely practiced in North America.
While chemicals may ensure increased fish production, their use in fish ponds should be based on ecologically sound and cost-effective rates and methods of application. The achievement of desired results from chemical application in fish ponds depends on several factors such as geographic location, climate, weather patterns, source water quality, sediment properties, etc., suggesting the adoption of site-specific best management practices for the use of chemicals. Additionally, different fish management practices followed by hatchery managers could produce undesirable consequences of application of chemicals in ponds. Many aquaculture ponds are double-cropped, i.e., the same pond is used to raise two species of fish at different times of the year. Chemicals applied during culture of one species can negatively impact culture of the other species in the same pond. And most importantly, the use of chemicals in fish ponds could result in changes in water chemistry, changes in plankton community structure and abundance, release of endotoxins from toxic algae, bioaccumulation of chemicals in fish and plankton, accumulation in sediments and the discharge of potentially harmful effluents to surrounding water bodies (Boyd and Massaut 1999).

For balanced and sustainable aquaculture, it is therefore imperative to determine the optimal rate of application of chemicals in the pond and the possible effects on pond water quality, non-target species and overall fish production. Any chemical addition to fish ponds requires site-specific monitoring to know whether its use is justified. Understanding pond system responses to chemicals can help formulate best management practices (BMPs) for chemical use that can be adopted by fish hatchery managers. Considering the enormous risk of potentially harmful effluent polluting the surrounding natural waters, monitoring and assessment of the efficacy and effects of chemicals
applied in fish ponds will not only benefit aquaculture industry, but also contribute to protecting our natural resources and environment.

In the ensuing dissertation, I have focused on the pertinent issues associated with the use of chemicals in culture ponds of Ohio State Fish Hatcheries, to aid hatchery managers in implementing optimal and cost-effective fish management protocols for the use of chemicals. Using sound ecological approaches and a combination of field and laboratory experiments, I have sought to identify the optimal application rate of fertilizers, ecological impacts of the use of herbicides, algaecides and the extent of carry-over effects of chemicals from culture of one species to another. Each of these aspects is dealt with in separate chapters.

Chapter 1 presents the fertilization experiments comparing three different inorganic phosphorus fertilization rates in larval percid rearing ponds and treatment success was evaluated on the basis of fish survival, yield, availability of zooplankton forage base, phytoplankton composition and biomass and water quality. We found that the ponds fertilized at lower rates (10 or 20 μg P/L) than the original target concentration (30 μg P/L) yielded comparable fish production and biomass of zooplankton forage base. We found similar phytoplankton biomass and species among phosphorus treatments with high inocula of Cyanobacteria from source water. Lowered phosphorus fertilization rates may reduce the risk of deteriorated water quality, potential macrophyte infestation and the potential risk of discharge of phosphorus-rich effluent into surrounding waters. Based on our encouraging results, Hebron State Fish Hatchery, Ohio, has implemented a fertilization protocol with lowered phosphorus fertilization rate (10 μg P/L) beginning in 2005.
Chapter 2 presents the experimental evaluation of the efficacy and ecological effects of two widely used chemicals, the herbicide fluridone and the algaecide copper sulfate, in catfish ponds. Using a randomized complete block design, we investigated the effectiveness of fluridone and copper sulfate on macrophyte biomass and their possible effects on water quality parameters, phyto-/zooplankton biomass, plankton community structure and overall fish survival and yield in sixteen ponds across three different treatments (fluridone alone, copper sulfate alone, fluridone plus copper) and controls. The estimated half-life of fluridone in pond waters ranged from 10.8 to 1.6 days. Free copper ion activity expressed as pCu ranged from 7.7 to 8.9 whereas in controls, it ranged from 12.3 to 13.4. The combined application of fluridone and copper sulfate resulted in substantially less macrophyte biomass than did the fluridone-only treatment. Copper treatments elicited different responses within the phytoplankton community, which shifted towards species tolerant of copper in the treated ponds. Though the total zooplankton biomass remained unaffected by copper additions, differences in sensitivity of zooplankton groups were observed. Fluridone treatments reduced total phytoplankton and Cyanophyta biomass, which suggests its algaecidal potential. Catfish survival and yield were not significantly different among treatments. As the use of copper and fluridone is not limited to aquaculture ponds, this study has yielded meaningful results not only for aquaculture but also for other natural freshwater ecosystems and has aided in filling the gap in knowledge of ecosystem toxicology of copper and fluridone compared to species specific toxicological data available in literature.

Chapter 3 presents the evaluation of the extent and effects of carry-over copper in double-cropped aquaculture ponds using a combination of field and laboratory
experiments. We hypothesized that repeated applications of copper during summer catfish culture will result in the build up of high concentrations in the sediment, which in turn will get remobilized during juvenile saugeye culture. Our field investigation of double-cropped ponds at Senecaville State Fish Hatchery revealed a relatively higher concentration of copper in the pond sediments. Pond waters in Senecaville ponds had higher total and dissolved copper concentrations than did the source water, which suggests the remobilization of copper from the sediment into the water column. We observed no response of phytoplankton populations to repeated applications to liquid inorganic fertilizers, relatively lower zooplankton biomass and fish percent survival and yield in Senecaville ponds compared to previous results from Hebron State Fish Hatchery. The laboratory experiment conducted to assess the chronic toxic effect of copper on a native cladoceran species showed that the low dissolved copper concentrations (6-9 μg/L) and low free copper ion activities (10^{-10}M-10^{-11}M) encountered in Senecaville waters can negatively affect the reproductive output of cladocerans. We recommend conducting further manipulative field experiments to establish the direct and indirect effect of low concentrations of copper on plankton biomass, species composition and fish production.
References


CHAPTER 1

EXPERIMENTAL EVALUATION OF THE IMPACTS OF REDUCED INORGANIC PHOSPHORUS FERTILIZATION RATES ON JUVENILE SAUGEYE PRODUCTION

INTRODUCTION

Walleye, a popular sport and food fish, is among the most heavily exploited species in North America (Kendall 1978). To maintain the wild populations, one of the popular methods adopted is the stocking of farm-raised walleye fingerlings in natural waters (Schweigert et al. 1977, LaJeone et al. 1992, Mathias et al. 1992). Earlier research has shown that hybrid walleye (saugeye, walleye ♀ [Sander vitreus] × sauger ♂ [Sander canadensis; formerly Stizostedion canadense; Nelson et al. 2003]) has desirable characteristics similar to those of walleye and is better suited to culture conditions than purebred walleye (Malison et al. 1990, Summerfelt et al. 1996b). Hence saugeye are routinely raised to fingerling stage in fish hatcheries for subsequent stocking in natural waters in many states of North America. In the state of Ohio alone, 5-10 million saugeyes are stocked annually in reservoirs and lakes (Garcia-Abiado et al. 2002).

The traditional method of pond culture of saugeye includes the capture of wild brood stock during the spawning season (early to mid-March), the fertilization,
incubation and hatching of eggs in hatchery jars and subsequent stocking of viable fry into hatchery ponds for rearing to fingerling size. The culture of saugeye fingerlings in rearing ponds presents many challenges, including identifying and providing ideal physical, chemical and biological conditions for saugeye fry. The consistent production of saugeye fingerlings from fish hatcheries each year is important for fisheries managers for successful implementation of stocking plans. Though many years of research have been dedicated to perfect the methods of saugeye culture, large annual variations in saugeye survival and yield have been observed in many fish hatcheries (Tew et al. 2006).

Factors possibly responsible for the variation in larval saugeye growth and survival in rearing ponds are 1) cannibalism (Cuff 1977, Loadman et al. 1986); 2) availability of forage base (Li and Mathias 1982, Colestante et al. 1986); 3) poor water quality parameters such as low dissolved oxygen concentration, high pH, unionized ammonia, etc., (Loadman et al. 1989, Tew 2003); 4) over-fertilization leading to high biomass of inedible phytoplankton species and poor water quality (Tew 2003); 5) varying fish stocking densities; 6) pond age; 7) double-cropping with chemical treatments (Tew 2003) and 8) the quality of source water used to fill the ponds.

Though cannibalism is considered as an inherent characteristic of walleye (Dobie 1956) and saugeye and is a major concern in intensive fish culture, it has not been encountered in extensive fish culture at typical stocking densities of 100,000 to 600,000 fish/ha (Culver et al. 1993, Tew 2003). Tew (2003) examined a 12-year data set from Hebron Hatchery, Ohio, USA, and found that pond age and stocking density do not significantly affect juvenile saugeye growth and survival. However, in the same hatchery, he found that double-cropping of ponds (i.e., culturing more than one species of
fish in the same pond at different times of the year) along with copper sulfate treatments affected saugeye survival and growth negatively (Tew 2003), so this aspect will be examined in detail in Chapter 3. Studies on the effects of quality of source water on saugeye growth and survival are limited, but are outside the scope of this study. Instead, in this chapter, I will examine the role of phosphorus fertilization on maintaining a plankton forage base for juvenile saugeyes in earthen ponds.

A major challenge of pond culture of saugeye is the adequate and consistent supply of forage base throughout the saugeye growing season (Culver et al. 1996). Larval saugeye feed on zooplankton such as copepods (especially cyclopoid copepods) and cladocerans such as *Daphnia*, *Bosmina*, etc. Many experimental studies have noted the decline in zooplankton abundance about 4-5 weeks after filling, the crucial time when larval saugeye consumption per fish per day is significantly higher than at the time of stocking (Geiger et al. 1985, Culver 1988, Culver 1996). One of the reasons for this decline in zooplankton abundance during the culture period is the lack of sufficient edible phytoplankton species in the ponds (Michaletz et al. 1983). As such, fertilization of the ponds to stimulate phytoplankton growth is almost universally included in percid culture programs. However, determining optimal fertilization methods to maximize saugeye production is a daunting task. Pond fertilization typically enhances algal growth which in turn increases zooplankton forage base for growing saugeye. However, over- or under-fertilization can lead to pond conditions that are not conducive to saugeye growth. For example, over-fertilization can lead to over abundance as well as inappropriate species composition of phytoplankton which in turn can result in extreme pH values, lethal concentrations of unionized ammonia, low dissolved oxygen concentrations, lower
zooplankton biomass, etc., all of which can contribute to reduced fish growth and survival (Dobbins and Boyd 1976, Bergerhouse 1992, Emerson et al. 1975, Stickney 1994, Culver et al. 1993). Over-fertilization can also lead to greater chances of macrophyte infestation and floating algal mats that can interfere with fish survival and harvest from ponds (Darley 1982). Pond fertilization, therefore, is an active area of research among fisheries scientists, who especially seek optimal fertilization types and rates suitable for larval saugeye culture.

Different fertilization protocols such as: 1) organic fertilizer only (Fox et al. 1989); 2) inorganic fertilizer only (Culver 1991, Qin et al. 1994); 3) inorganic and organic fertilizers (Geiger et al. 1985, Fox et al. 1992, Qin and Culver 1992) are in vogue. Pond fertilization with organic matter such as alfalfa meal (Buttner and Kirby 1986), soybean meal (Fox and Flowers 1990), yeast (Beyerle 1979), and hay (Laarman and Reynolds 1974) is a traditional method for percid culture. The application of organic fertilizers stimulates mainly the heterotrophic food chain, providing forage base for zooplankton. It also promotes algal growth via release of nutrients during decomposition, creating an additional forage base for zooplankton. However, the low nitrogen to phosphorus ratio (N: P) of organic fertilizers may favor a filamentous Cyanobacteria-dominated algal community that impedes the growth of zooplankton. Indeed, several studies have reported the highly variable production of zooplankton following organic fertilization (Fox and Flowers 1990, Harding and Summerfelt 1993). Further, organic fertilizers increase biological oxygen demand in the ponds and at times can lead to fish kills following hypoxia in pond waters. Dissolved oxygen concentration as low as 1mg O₂/L has been reported from ponds fertilized with organic fertilizers (Qin 1994).
The application of inorganic fertilizers is a promising alternative to the conventional use of organic fertilizers. The inorganic fertilization method is cost-effective in terms of material cost and labor involved (Soderberg et al. 2000) and is more dependable than organic fertilizers with respect to fish yield and survival. Further, inorganic fertilizers can maintain higher dissolved oxygen concentration in the ponds, a crucial water quality parameter in the proper growth of saugeye, compared to organic fertilizers (Tice et al. 1996). Unlike organic fertilizers, the nutrient content and ratio in inorganic fertilizers are non-variable and thus nutrient loading into ponds can be precisely regulated.

The primary macronutrients regulating the growth of phytoplankton biomass are phosphorus, nitrogen and silicon (almost exclusively in diatoms). A number of laboratory and field studies have shown that the nitrogen to phosphorus ratio (N: P) plays a significant role in shaping phytoplankton community structure (Pearsall 1930, Tilman 1977, Schindler 1977, Rhee 1978, Smith 1983, Pick and Lean 1987, Levich and Bulgakov 1993). Further, it has been widely observed that low N: P ratios can favor the proliferation and dominance of Cyanobacteria and high N: P ratios can favor the growth of non-cyanobacterial algae. For example, Schindler (1977) reported the dominance of Cyanobacteria in a fertilized lake at an atomic N: P ratio of 11:1 as opposed to dominance by green algae at a higher N: P ratio (30:1). Likewise, Smith (1983) found that Cyanobacteria dominated in 12 lakes when the epilimnetic TN: TP ratio had values less than 29:1 by mass. At TN: TP ratios greater than 29:1 by mass, non-cyanobacterial species thrived.
In planktivorous larval fish culture, development of appropriate algal species (small chlorophytes and diatoms) most edible for zooplankton is a prerequisite for successful fish production. The development and dominance of inedible algae (large filamentous chlorophytes and Cyanobacteria) can pose many risks to larval fish culture. These algae are considered as ‘poor’ food for most zooplankton species, with their nutritional value relying on morphology, concentration and physiological state of the filaments (Lampert 1977, Gliwicz 1980, Watson and Kalff 1981, Gliwicz and Lampert 1990). Consequently, an inedible algae-dominated system results in decreased trophic transfer efficiency to higher trophic levels, which translates to reduced fish growth and survival in fish ponds. The presence of filamentous blue-green algae (Cyanobacteria) can interfere with the growth of zooplankton. Dawidowicz et al. (1988) found that Daphnia growth and reproduction cease when the filament concentration exceeds a certain threshold value which can range anywhere from 540 to 67,000 filaments/ml (Gliwicz 1990). Further, cyanobacterial blooms can lead to water quality problems such as depletion of oxygen concentrations, changes in pH, decreased light penetration, production of toxins by potentially toxic algae like Microcystis spp. and Cylindrospermopsis spp., etc., and hence are undesirable in fish ponds (Schrader and Dennis 2005).

To improve percid growth and survival via maintaining appropriate algal composition, Culver (1991) hypothesized that by manipulating nitrogen and phosphorus inputs, the proliferation of phytoplankton species most edible (greens and diatoms) to zooplankton can be favored in fish ponds. Culver (1991) examined percid culture practices and found that the ponds were over-fertilized with respect to phosphate
compared to the amount indicated in lake eutrophication studies. Preliminary experiments at Ohio’s Hebron State Fish Hatchery yielded promising results of developing appropriate algal composition by tailoring the inorganic N: P ratios in fish ponds (Helal 1990).

Since algae use N: P in an approximate mass ratio of 20:1, Culver (1991) suggested pond fertilization with sufficient liquid inorganic fertilizers to restore pond nitrogen (nitrate plus ammonia) concentration to 600 µg N/L and phosphate concentration to 30 µg P/L once per week. Culver et al. (1993) developed a fertilization and stocking protocol that included 1) pond filling immediately before stocking 2) complete exclusion of organic fertilizers 3) measuring the pond inorganic nitrogen and phosphorus concentrations prior to spraying to determine the amount of fertilizers to be added 4) weekly fertilization with liquid inorganic fertilizers by uniform spraying of phosphoric acid (H₃PO₄) and 28-0-0 inorganic fertilizer (NH₄NO₃ + Urea) and 5) stocking fry at the rate of 50-55 fry/m³. This method was fully incorporated into the percid production protocol at Hebron State Fish Hatchery, Hebron, Ohio, USA in 1990. Improved and consistent percid survival (average 65% survival) and yield (>60 kg/ha) were obtained following this fertilization regimen in the years 1991-1995 (Tew 2003)

However, analysis of fish survival and yield data from 1996-2000 revealed that survival (average 45% survival) had declined and become highly variable (Tew 2003). Tew (2003) hypothesized that at 30 µg P/L as the target phosphate concentration, over-fertilization of the ponds may have led to lower percid survival and yield at Hebron Hatchery. To test this hypothesis, Tew (2003) conducted fertilization experiments in which he compared the original target rate of 30 µg P/L with reduced fertilization of
20 µg P/L. Based on the 3-year fertilization studies, however, Tew (2003) reported that lowering phosphorus fertilization rate from 30 to 20 µg P/L did not affect larval percid survival and yield nor did zooplankton prey densities in 20 µg P/L-treated ponds differ from the 30 µg P/L-treated ponds.

In the current study, we attempted to explore the feasibility of further lowering the phosphorus fertilization rate to 10 µg P/L and compared this treatment to 20 µg P/L and 30 µg P/L treatments. This proposition was put forward for many reasons: 1) Analysis of phytoplankton data of 2001 from Hebron Hatchery showed that the phytoplankton biomass in 20 µg P/L treated ponds ranged from 2.6 to 14.7 mg/L (wet weight). Based on Heinonen’s (1980) criteria, these ponds exhibit eutrophic to hyper-eutrophic conditions. A eutrophied water body, characterized by algal blooms dominated by Cyanobacteria, low water clarity, occurrences of anoxia, fish kills etc., is unsuitable for fish culture. Schindler (1977) identified phosphorus as the primary cause for eutrophication. Hence, lowering current phosphorus application rate in percid ponds may decrease excessive phytoplankton biomass without significantly affecting fish culture. In other words, restoring the phosphate concentration to 10 µg P/L each week may provide enough phytoplankton biomass to support sufficient zooplankton forage base for larval percids. 2) Decreasing the excessive phytoplankton biomass via lowering the phosphorus fertilization rate may reduce the potential risk of developing deteriorated water quality parameters unfavorable for larval saugeye growth. 3) The ‘excess’ primary production, produced as a response to over-fertilization, eventually sinks and can lead to internal phosphorus release from the sediments posing a great risk of floating filamentous algal mats and macrophyte infestation in ponds. Dense mats of algae and thick strands of
vascular plants can interfere with fish growth and harvest and hence are undesirable in fish ponds. 4) The problem of filamentous algal and vascular plant infestation can be exacerbated in ponds that are double-cropped. For example, excess nutrients applied during saugeye culture in spring may accumulate in sediment and trigger the excessive growth of macrophytes during catfish culture in summer, which in turn will necessitate the use of algaecides and herbicides. Further, these chemicals can accumulate in the sediments and may negatively impact saugeye culture in spring (Tew 2003). To avoid such a negative feedback loop on fish culture in double-cropped ponds, reduction of phosphorus application rate may prove beneficial. 5) Lowering the fertilization application rates may be cost-effective in terms of the total amount of fertilizer used and labor involved. 6) Lowering the phosphorus application rate may reduce the potential discharge of nutrient-rich aquaculture effluent from hatcheries into surrounding natural waters during pond draining. To monitor and reduce discharge of nutrient-rich aquaculture effluent, United States Environmental Protection Agency (USEPA) has established effluent limitation guidelines for aquaculture facilities that produce at least 100,000 pounds per year in flow-through systems, recirculating systems and net pens (USEPA 2004). However, any measure adopted to reduce discharge of excessive nutrients into natural waters by fish culturists will contribute to conserving our natural waters. To this end, our phosphorus fertilization rate manipulation experiments in percid culture are appropriate.

Accordingly, we conducted fertilization experiments to test whether further decreasing the phosphorus application rate from 30 µg P/L to 10 µg P/L would decrease
phytoplankton biomass, maintain sufficient zooplankton forage base, improve water quality and consequently improve percid survival and yield.

Hypotheses

The following hypotheses were generated to study the effects of decreasing phosphorus fertilization rates in percid ponds:

1. Decreasing the pond phosphate application rate of 30 µg/L to 20 µg/L or 10 µg/L will increase percid survival and decrease variability in survival seen in recent years.

2. Lowering the phosphate application rate will not substantially decrease the instantaneous growth rate of saugeye.

3. Modification of phosphate application rate will not decrease the availability of saugeye’s preferred diet items.

4. The total zooplankton biomass and the turnover rate of zooplankton in the 10 µg/L and 20 µg/L ponds will exceed the biomass and turnover rate of zooplankton in the 30 µg/L phosphate treated ponds (due to sufficient edible algae and decreased biomass of feeding-interfering and potentially toxic large green algae and Cyanobacteria in the 30 µg P/L ponds).

5. Changing the phosphate application rate to 10 µg/L or 20 µg/L will decrease the biomass of relatively inedible phytoplankton taxa such as large gelatinous and colonial green algae as well as large filamentous, and potentially toxic, Cyanobacteria, while not decreasing the biomass of edible algae.

6. Decreasing the phosphate application rate to 10 or 20 µg/L will decrease the primary productivity of algae.
7. Lowering the phosphate application rate to 10 or 20 µg/L will improve the water quality in saugeye ponds relative to 30 µg/L treated ponds.

Objectives

We tested the stated hypotheses via the following study objectives:

1. Identify the optimal fertilization rate based on fish yield and percent survival results—As the efficacy of a pond fertilization treatment can be determined best by its effects on fish production (Mortimer 1954), we compared fish production data (percent survival and yield) among the three phosphorus fertilization treatments.

2. Determine the effect of phosphorus fertilization rate on the seasonal variation in the instantaneous growth rate of saugeye—We measured the length and weight twice per week of growing saugeye and estimated the instantaneous growth rate. Coupled with zooplankton abundance measurements, we could determine whether the timing of zooplankton bloom in low phosphorus treatments matched the growing appetite of saugeye especially during the critical third and fourth weeks of growth.

3. Determine the larval saugeye diet preferences relative to the zooplankton dynamics in the ponds with differing phosphorus fertilizer applications—We analyzed the fish stomach contents which facilitated comparison of fish and plankton interactions in three phosphate application rates.

4. Determine the seasonal variation of zooplankton composition, abundance and secondary productivity as function of fertilizer application rate—We determined the zooplankton community composition and abundance twice per week which showed whether enough forage base was present for growing percids or not and how zooplankton
community responded to variations in phytoplankton community composition as a direct result of different fertilization rates.

5. Determine the relative density and biomass of edible and inedible phytoplankton groups as a function of fertilizer application rate- We determined the phytoplankton community composition and biomass twice per week in each pond. These measurements enabled us to find the ratio of edible to inedible algae and determine whether decreased fertilization rate favored edible algae.

6. Compare the seasonal variation in the overall rates of primary production in ponds treated with different amounts of fertilizers- We measured gross and net primary productivity twice per week in each pond. Coupled with phytoplankton abundance measurements, the dynamics of phytoplankton responses to different phosphorus fertilization rates could be better understood.

7. Monitoring water quality parameters- We measured dissolved oxygen concentrations, pH, water transparency and temperature on a weekly basis. Comparison of water quality in ponds with different phosphate treatments helped us identify the optimal nutrient application rates.

MATERIALS AND METHODS

Study site and pond stocking

The experiments were conducted at Hebron State Fish Hatchery, Ohio, USA from 15 April to 18 May, 2004. Water for hatchery operations was obtained from a nearby eutrophic reservoir (Buckeye Lake) via a 1.5 mile section of the old Ohio Erie Canal. Two wells also provide water to the hatchery.
The rearing ponds at Hebron are approximately 3000 m² and contain 2500 m³ of water. Most ponds averaged 1 m in depth. Each pond has a separate filling and draining system and was filled about three days prior to stocking of fish with water filtered through 0.5 mm mesh screens to prevent introduction of eggs or larvae of undesirable fish.

We collected walleye eggs and sauger sperm from wild brood stock from Ohio reservoirs and fertilized them and incubated them in McDonald-type jars at the hatchery facility. We stocked 3- to 5- day old saugeye fry (counted by the volumetric displacement method) on 17 April 2004 at an average density of 53 fish /m³ and drained the ponds to harvest fingerlings after 4-5 weeks.

Experimental Design

In order to test the stated hypotheses, we used a complete randomized design consisting of 12 ponds, with four replicates of each of the three phosphate application rates: 10 µg P/L, 20 µg P/L and 30 µg P/L. In addition to our experimental ponds, 22 additional ponds were subjected to these same fertilization rates at the hatchery to yield a total of 13 ponds fertilized at 10 µg P/L, 11 ponds at 20 µg P/L and 10 ponds at 10 µg P/L. However, we focused on measuring final fish yield and survival in the additional ponds, and closely monitored fish growth, phytozooplankton dynamics and water quality only in our 12 experimental ponds.

We fertilized ponds weekly by uniform spraying of phosphoric acid (H₃PO₄) and liquid 28-0-0 inorganic fertilizer (NH₄NO₃ + Urea) to restore concentrations of 30, 20 or 10 µg P/L and 600 µg N/L in each pond. Prior to spraying the ponds, hatchery personnel
measured pond inorganic nitrogen (nitrate and ammonia) and phosphorus (reactive phosphate) concentrations to determine the amount of fertilizers to be added. Pond fertilization began on 28 April 2004 and continued for 4 weeks.

Sampling Methods

1. Saugeye survival and yield

We counted harvested fish by the volumetric displacement method and used stocking density and pond volume to estimate saugeye survival (%) and yield (g/m³).

2. Monitoring of saugeye length and weight and estimation of instantaneous growth rate

We collected approximately 10 fish per pond with a seine twice per week and preserved them in 10% formalin. In the laboratory, we measured total fish length to the nearest 0.1 mm, and the wet weight (after blot drying) to the nearest 0.01 g. We estimated instantaneous growth rate of saugeye (b) from weight and sample date using the equation:

\[ b = \frac{\ln W_2 - \ln W_1}{(t_2 - t_1)}. \]

\( W_1 \): fish wet weight (g) at time \( t_1 \) (day);
\( W_2 \): fish wet weight (g) at time \( t_2 \) (day)

3. Fish diet analyses

We determined fish stomach contents by dissecting 10 individuals from each experimental pond for each sampling date. Before dissection, we measured the total length (when possible) or standard length of each fish and weighed each fish before and
after the gut was removed. We placed the gut contents in a plankton counting wheel and scanned under a dissecting microscope at 50x. Prey items were counted, measured and identified to the lowest possible taxonomic level (usually genus or species).

4. Monitoring of zooplankton biomass and species composition

We sampled zooplankton using a metered (Model 2030R, General Oceanics, Inc., Miami, Florida), 0.5-m diameter, 64-µm mesh net fitted with a canning jar on the cod-end and mounted on a wooden pole. We towed the net from a row boat across the pond and raised and lowered it repeatedly to obtain a depth-integrated sample. The samples were concentrated with a sieve (64-µm mesh), transferred to labeled screw-cap plastic cups and preserved in the field in 4% formalin-sucrose solution (Haney and Hall 1973). The volume of the water column filtered by the net for each sample was calculated by multiplying the cross-sectional area of the net (0.196 m²) by the distance traveled (m) calculated from flow meter readings.

Zooplankton enumeration followed Kane (2004). Briefly, we diluted each concentrated sample to a known volume (~1500 to 9000 ml) and identified and counted all zooplankton taxa (rotifers, cladocerans and copepods (cyclopoids and calanoids)) in two or more sub-samples (with a total volume ranging from 5 to 50 ml) to genus and species (when possible) according to Balcer et al. (1984), Brooks (1959), and Wilson and Yeatman (1959) using a Wild dissecting microscope at 50x. We withdrew and analyzed sub-samples from the diluted sample until at least 100 individuals of the most common taxon were recorded. We used a calibrated ocular micrometer to measure the lengths of the first 20 individuals in each genus or species and calculated genus- or species-specific
average individual biomass from length-weight regressions (Culver et al. 1985) and the number of individuals per cubic meter. We summed genus or species-specific total biomasses over all taxa to provide a total crustacean zooplankton biomass (mg/L) for a given sampling pond, for a given date. We analyzed zooplankton data as total biomass and at the taxonomic level of class (e.g., Cladocera).

Secondary productivity (µg/L/Day) was calculated as a function of biomass and temperature for each zooplankton taxon (Frost 1997). The ratio of total zooplankton biomass to total productivity yielded the turn-over time (in days).

5. Monitoring of phytoplankton biomass and species composition

We collected phytoplankton samples weekly with an integrated tube sampler (10 cm diameter and 1.5 m long) by vertically lowering the open end of the sampler into the pond and then replacing the stopper at the other end to obtain the sample. We poured the collected water into a bucket and took a 250-ml sample, which was preserved in a canning jar with Lugol’s solution. In the laboratory, we mixed each sample thoroughly and poured it into a graduated cylinder and allowed it to settle for 3 days in a dark chamber. We then concentrated each sample to 30 ml by siphoning off 220 ml from the top and transferring the remaining sample into ~ 40-ml vials. We placed sub samples of 3-6 ml in tared Utermohl sedimentation chambers and weighed them to determine the exact counting volumes. We identified and counted phytoplankton genera using a Wild inverted microscope at 400x. We counted multiple transects until we recorded 100 algal units (cells, filaments or colonies) of the most common taxa, counting all algal units in at least two transects. We measured the cell dimensions for the first 20 algal units for each
genus enumerated and calculated the mean individual biomass for each taxon (mg wet weight/L). For filamentous algal taxa, however, we measured all filament lengths and summed and recorded them as total filament length for each taxon (Frost and Culver 2001). We calculated the mean cell volume for each taxon present using the algal dimensions for each species in a sample, using volumetric equations that best described the shape of each species. For colonies, we calculated the mean number of cells per colony and multiplied it by the average volume per cell to determine volume per colony. Subsequently, volumes were converted to biomass assuming the specific gravity of phytoplankton to be 1.0 g/cm³ (Munawar and Munawar 1976, Frost and Culver 2001). Consequently, all reported phytoplankton biomasses are wet weights (mg/L). We calculated total biomass of phytoplankton samples by summing the species-specific total biomass over all species present in a given sample. We analyzed phytoplankton data as total biomass, at the functional level of edible and inedible phytoplankton (Appendix A: Table A.1) and at the taxonomic level of phylum (e.g., Chlorophyta).

6. Primary productivity measurements

We measured gross primary productivity (GPP), net primary productivity (NPP) and respiration rate in situ with transparent (light) and opaque (dark) bottles. Water collected using an integrated tube sampler was gravity fed from a bucket affixed with three identical tubing drains into light and dark bottles. We incubated each pair of bottles in the corresponding pond from which water was collected at a depth of 0.5 meter for 6 hours in floating wire cage baskets. We used a modified Winkler method to determine oxygen concentration in the bottles (Wetzel and Likens 1979). We filled and fixed an
initial light bottle immediately before the start of incubation, providing the beginning dissolved oxygen concentration (DO) of each experiment. We calculated GPP from the DO differences between light and dark bottles, NPP by subtracting the DO in the initial bottle from the DO in the light bottle and respiration rate by subtracting the DO in dark bottle from the DO in the initial light bottle. We estimated primary productivity in 6 ponds initially and in all 12 ponds from the third sampling date.

7. Monitoring water quality

We measured dissolved oxygen using a YSI model-54A oxygen meter and pH with a pH probe (Orion 9256) attached to a Beckman 32 meter. We measured water transparency using a Secchi disk. We recorded precise temperature data from ponds using underwater, continuously monitoring temperature data loggers (Hobo loggers, Onset, Pocasset, MA) suspended at a depth of 0.5 meter.

STATISTICAL ANALYSES

We analyzed the results of the experiments as a completely randomized design with repeated measures using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Data were transformed when necessary to meet assumptions of normality. The treatment structure has a 3 x 6 factorial (3 types of treatments and 6 sampling dates) arrangement. The statistical model underlying the analyses is

$$Y_{ijk} = \mu + T_i + p_{ji} + D_k + TD_{ik} + e_{ijk}; \ i = 1, 2, 3; j = 1, 2, 3, 4, \ldots, 12; \ k = 1, 2, \ldots, 6$$
where $Y_{ijk}$ = measured response from the $i^{th}$ treatment, the $j^{th}$ pond and the $k^{th}$ date, $\mu$ and $T_i$ are fixed parameters such that the average response of the $i^{th}$ treatment is $\mu + T_i$, $D_k$ = fixed effect associated with $k^{th}$ date, $p_{j;i}$ = random effect associated with $j^{th}$ pond in the $i^{th}$ treatment, $TD_{ik}$ = fixed effect associated with the interaction of $i^{th}$ treatment and $k^{th}$ date and $e$ = random error associated with the $j^{th}$ pond in the $i^{th}$ treatment on $k^{th}$ date. We included fixed effects of treatments, dates, and treatment x date interaction in the model, and the random effects of ponds within treatments as the error term to test fixed effects. We included Kenward Roger specification in the model statement to estimate degrees of freedom. The REPEATED statement accounted for the effect of sampling day; the error term in the REPEATED statement was pond*treatments. We used the covariance structure that yielded the lowest Akaike’s information criterion for repeated measurements (Littell et al., 1998). We set the significance of the F-test of fixed effects at $P \leq 0.1$ and discussed trends when $0.1 < P \leq 0.15$. When protected by the F-test ($P \leq 0.10$), least squares means for fixed effects were separated using the PDIFF statement in SAS and deemed significant at $P \leq 0.1$.

**RESULTS**

Effects of phosphate fertilization rates on fish survival and yield

The survival and yield of juvenile saugeye from the three tested phosphate application rate treatments in experimental ponds averaged 82% and 56 kg/ha. Statistical analyses of saugeye growth and production data revealed no significant differences in mean final fish length, final wet weight, percent survival, number of fish harvested/m$^3$ or
yield (g/m³) among the three fertilization rate treatments in 12 experimental ponds (Table 1.1). We also found no significant difference in any of the mentioned variables except final weight (p=0.0940; Table 1.1) in the combined production data from our experimental ponds as well as 22 other ponds at Hebron hatchery that were subjected to the same fertilization rates. However, the final fish weight in 20 µg P/L treated ponds was significantly higher than that in 30 µg P/L ponds (p=0.0399).

Weekly monitoring of fish growth in 12 experimental ponds revealed no significant differences in length (p=0.2354) or wet weight (p=0.4433) among the three treatments although fish in 20 µg P/L treatments tended to grow heavier (Fig. 1.1). The instantaneous growth rate curves show a similar fish growth pattern (Fig. 1.2) with declining growth rate values after the first week, irrespective of treatments. No treatment effect was detected on instantaneous growth rate (p=0.4277); however, sampling date effects were observed (p=<0.0001).

Effects of phosphate fertilization rates on fish diet

The larval saugeye diet mainly included cyclopoid copepods (Acanthocyclops vernalis) and cladocerans (Bosmina sp.) but other prey items such as rotifers and calanoids were also found starting the first week and chironomids were observed in the diet beginning 10 days after stocking. Cyclopoids constituted the major portion (~80-95%) of the early diet and were replaced by Bosmina (60-80%) during the later weeks. However, in some cases, we observed the dominance of either Bosmina or Acanthocyclops vernalis in the stomachs of fish from the same pond.
The analysis of fish diet data obtained after fertilization of the ponds (17 days after stocking) showed no significant difference in the number of total prey items among treatments (p=0.8701). However, the number of total prey significantly declined over time (p=<0.0001) with a significant treatment x time interaction (p=0.0502). Likewise, we did not detect significant differences in the number of Cladocera (p=0.3171), Copepoda (p=0.9823), Rotifera (p=0.3108) and non-zooplankton group (p=0.2372) in the diet of saugeye among treatments. However, we observed the effect of sampling date on all the prey items (p= <0.10), with mainly cladocerans and to a lesser extent rotifers and non-zooplankton groups (e.g., chironomids, aquatic worms, etc.) becoming increasingly important over time. On the other hand, the number of cyclopoid copepods in the diet diminished over time and this temporal dietary pattern closely followed the overall zooplankton dynamics in the ponds (Fig. 1.3). No cannibalism was observed in 480 fish examined.

Effects of phosphate fertilization rates on zooplankton biomass and community composition

The major zooplankton taxa in the ponds were cladocerans, copepods and rotifers. We did not find any significant treatment effect on mean total zooplankton biomass (p= 0.1794); however, total zooplankton biomass significantly changed with time (p=0.0033). The 20 µg P/L treated ponds exhibited the largest fluctuations in mean total zooplankton biomass among the three treatments with a pronounced peak occurring late into the rearing season (Fig. 1.4). Copepods (mostly cyclopoids) and cladocerans dominated the zooplankton community irrespective of treatments, however their dominance differed
temporally. We observed a similar trend of a decrease in Copepoda biomass and an increase in Cladocera biomass over time in all the treatments (Fig. 1.5).

Significant treatment effects were detected on Copepoda biomass ($p = 0.0386$) with 20 µg P/L treated ponds having significantly higher Copepoda biomass (Table 1.2) dominated by cyclopoids in the initial weeks than 30 µg P/L or 10 µg P/L treatments. Among copepods, we found a significant treatment effect on cyclopoid copepods ($p= 0.0350$), but not on calanoid copepod biomass ($p = 0.2448$) which remained at a low level (< 0.05 mg/L) in all the treatments. The overall Cladocera biomass did not differ among treatments ($p= 0.2341$); however, we noticed a dramatic increase during week 4 in 20 µg P/L treated ponds compared to 30 µg P/L or 10 µg P/L treatments (Fig. 1.6).

Rotifer biomass (mainly *Asplanchna* spp. and *Keratella* spp.) remained at a low level (< 0.1 mg/L) in all the treatments throughout the duration of the experiment. We did not find any significant treatment effect for rotifer biomass ($p=0.3211$). Sampling date effects were, however, observed ($p=0.0001$) with an increase in rotifer biomass during the later weeks of the culture season (Fig. 1.7).

*Bosmina longirostris* dominated the cladoceran community throughout the duration of the experiment irrespective of treatments ($p= 0.2079$). *Acanthocyclops vernalis* predominated in the cyclopoid community, and a significant treatment effect ($p=0.0707$) was observed with 20 µg P/L treated ponds having higher biomass of *A. vernalis* than 10 or 30 µg P/L treated ponds (Table 1.2). Sampling date effects were found on both *Bosmina* and *A. vernalis* ($p< 0.1$). No treatment effect was observed on either calanoid ($p= 0.6824$) or cyclopoid ($p=0.8531$) nauplii.
The secondary productivity of total zooplankton was significantly affected by treatments \( (p=0.0967) \), with 20 \( \mu \)g P/L treated ponds having higher zooplankton productivity than 30 \( \mu \)g P/L \( (p=0.0367) \). However, we did not find significant differences in the productivity of calanoid copepods \( (p=0.1352) \), cyclopoid copepods \( (p=0.5477) \) and cladocerans \( (p=0.1093) \). The 20 \( \mu \)g P/L ponds had the highest cladoceran and calanoid productivity among treatments (Table 1.2). The turn-over time of the crustacean zooplankton population ranged from 3.1 to 8.6 days with no significant treatment effect \( (p=0.8294) \).

Effects of phosphate fertilization rates on phytoplankton biomass and species composition

The major phytoplankton taxa in the ponds were Cyanophyta \( (Anabaena, Aphanizomenon, Oscillatoria) \), Chlorophyta \( (Ankistrodesmus, solitary greens) \), Chrysophyta (mostly pennate diatoms), and Cryptophyta \( (Chroomonas, Cryptomonas, Rhodomonas \text{spp.}) \). The mean total phytoplankton biomass remained unaffected by phosphorus treatments \( (p=0.7933) \); however, the total biomass significantly decreased with time in all the treatments \( (p=0.0003) \). The 20 \( \mu \)g P/L treated ponds tended to have higher mean biomass and larger fluctuations in total phytoplankton biomass over time than 10 or 30 \( \mu \)g P/L treated ponds (Fig. 1.8).

Cyanophyta \( (Anabaena, Aphanizomenon and Oscillatoria \text{spp.}) \) dominated the phytoplankton community throughout the initial weeks irrespective of treatments (Fig. 1.9). The decrease in the biomass of inedible algae over time coincided with the increase in edible algal biomass in all treatments (Fig. 1.10); no treatment effects were detected on
either edible ($p=0.7435$) or inedible algal biomass ($p=0.5233$). Likewise, we could not detect significant differences in the biomass of individual algal groups- Chlorophyta ($p=0.6354$), Cyanophyta ($p=0.7514$) and Chrysophyta ($p=0.1300$). Cryptophyta biomass was marginally significant ($p=0.1186$) with respect to treatments; with 20 µg P/L-treated ponds having the highest biomass which significantly differed from 10 µg P/L-treated ponds ($p=0.0410$; Fig. 1.11). Significant sampling date effects ($p=0.0244$) and treatment x time interaction ($p=0.0337$) were detected on Cryptophyta biomass. While Chrysophyta biomass significantly increased with time ($p=0.0172$), Cyanophyta and Chlorophyta biomass significantly decreased with time ($p<0.0001$) (Fig. 1.11).

Effects of phosphate fertilization rates on primary productivity

Of the three different treatment groups, the highest gross and net primary production (GPP and NPP) rates were observed in 20 µg P/L-treated ponds (Fig. 1.12). In response to fertilizer application, GPP and NPP rates in 20 µg P/L ponds began to increase relative to the other treatments for a week, but declined sharply thereafter, at which point 30 µg P/L treated ponds maintained higher GPP and NPP rates up to several days before harvest. The 30 µg P/L treatment produced GPP and NPP rates that fluctuated the least; however, the maximum NPP rate achieved by the 30 µg P/L treated ponds was lower than the maximum NPP rate in the 10 µg P/L and the 20 µg P/L treated ponds.

There were no significant differences in net primary productivity (NPP, $p=0.3505$) or gross primary productivity (GPP, $p=0.2334$) rates across treatments; however these rates significantly decreased with time ($p<0.0001$).
With respect to respiration rates, the three treatment groups displayed a decreasing trend but with a peak occurring on May 7\textsuperscript{th} which coincided with the peak on GPP graph (Fig. 1.12). Overall, the 20 µg P/L treated ponds exhibited the highest sustained respiratory rate throughout the experiment. However, the respiration rates did not significantly differ among treatments (p=0.3899).

Effects of phosphorus fertilization rates on physico-chemical parameters

The mean pond water temperature varied between 14-27°C during the saugeye culture period (Fig. 1.13). Dissolved oxygen concentration at the surface never fell below 6.0 mg/L in the ponds but the bottom dissolved oxygen concentration dropped below 5.0 mg/L at least on one sampling date (Fig. 1.14). Neither surface nor bottom dissolved oxygen concentrations differed significantly among treatments (p=0.7357 and 0.9646), but sampling date effects were observed on both variables (p=0.001 and 0.0042 respectively). We also found a significant treatment x time interaction (p=0.0481) on bottom dissolved oxygen level.

The pH of pond water varied between 7.75-10.02. There was no significant difference in pH among treatments (p=0.3205) but sampling date effects were observed on pH (p=0.0001) (Fig. 1.15a). Secchi transparency was unaffected by phosphate fertilizer treatments (p=0.2081); however it significantly increased with time (p<0.0001). Irrespective of treatments, Secchi depth exceeded 1m by May 11 (2nd week) in all the ponds indicating the onset of a clear water phase (Fig. 1.15b).
DISCUSSION

The goal of fish culturists is to maximize fish production (survival and yield) by providing ideal physical, chemical and biological conditions using cost-effective and ecologically-sound methods. Provision of superior water quality and an adequate zooplankton forage base is essential for successful pond larval fish culture including juvenile saugeye culture (Culver 1991). The importance of pond fertilization of larval percid rearing ponds to stimulate phytoplankton growth which would in turn maintain an adequate zooplankton forage base has been long recognized. Much of the earlier research was directed to identify the suitable type of fertilizers that would optimize saugeye production in rearing ponds. Subsequently, liquid inorganic fertilizers emerged as a superior alternative in terms of cost-effectiveness, reliability of fish yield, precise control over nutrient loadings into the ponds, maintenance of factors conducive to fish growth such as high dissolved oxygen concentrations in the ponds etc., over other types of fertilizers (Culver 1991, Culver et al. 1993, Tice et al. 1996, Soderberg et al. 2000). Multi-year fertilization studies suggested that the original phosphorus fertilization rate of 30 µg P/L was unnecessary and that comparable results can be obtained from a reduced fertilization rate of 20 µg P/L without any negative impact on larval percid survival and yield (Tew 2003). Following this promising result, in the current study, we explored the feasibility of further lowering the phosphorus fertilization rate to 10 µg P/L.
Did lowering of the phosphorus fertilization rate affect fish production?

The efficacy of a pond fertilization treatment can be evaluated based on its effect on fish production (Mortimer 1954). Our experimental results clearly indicated that reducing the target phosphorus fertilization rate from 30 µg P/L to 10 µg P/L did not affect fish survival or yield in Hebron hatchery ponds. In other words, 10 µg P/L-treated ponds produced similar saugeye survival rates and yields as 30 µg P/L-treated ponds by providing sufficient zooplankton forage base without compromising other important parameters required for proper saugeye growth. Over-fertilization of ponds can lead to over-abundance as well as inappropriate species composition of phytoplankton which in turn can result in hypoxia, extreme pH values, lethal concentrations of unionized ammonia, lower zooplankton biomass, macrophyte infestation etc., all of which can contribute to reduced fish growth and survival (Emerson et al. 1975, Dobbins and Boyd 1976, Culver 1991, Bergerhouse 1992, Culver et al. 1993, Stickney 1994, Hessen et al. 2006, Tew et al. 2006). Moreover, pond experiments conducted with larval walleye in Pennsylvania showed the futility of over-fertilizing the ponds in an attempt to increase growth, survival and yield (Soderberg et al. 2000)

The survival and yield of juvenile saugeye from the three tested phosphate application rate treatments in experimental ponds averaged 82% and 56 kg/ha. In particular, the lowest fertilization rate of 10 µg P/L gave 89% survival and 57 kg/ha yield. Tew (2003) compared 30 µg P/L and 20 µg P/L fertilization targets at the same location using a similar percid production protocol from 2001-2003 and found similar survival (ranging from 40% to 60%) and yield (30-70 kg/ha). Similarly, average survival
and yield among three Ohio state fish hatcheries in 1991 using 30 µg P/L phosphorus treatments were 64% and 68 kg/ha respectively (Culver, 1996).

Comparison of our juvenile saugeye production results with other fertilization studies conducted elsewhere and documented in the literature, however, can be difficult as fish pond management practices such as fish stocking density, time of pond filling, culture duration etc., differ greatly among studies. Further, experiments with saugeye are fewer in comparison to those with walleye. However, studies have shown that walleye management practices can be equally applied to larval saugeye culture (Culver et al. 1993; Qin et al. 1994).

Tice et al. (1996) reported 56% survival and 37 kg/ha in yield when ponds were stocked with walleye fry at the rate of 16/m³ and subjected to unequal intervals of filling and stocking (4-20 days) with a culture duration of 40 days. Soderberg et al. (1997) reported walleye survival of 60.9% and 58.2 kg/ha when cultured at a stocking density of 20 fry/m³ for 45-47 days with phosphorus treatments of 66 and 30 µg/L but with unequal intervals of stocking and filling.

In our study, the ponds were filled with water 2-3 days before stocking at an average density of 53 fish/ m³ (350-400,000 fish/ ha) and drained to harvest within 31-34 days. In planktivorous fish culture, the timing of pond filling and stocking of ponds is an important factor as it affects plankton dynamics in the ponds (Culver et al. 1993, Culver 1996) upon which fish growth is dependent. Zooplankton populations decline within 3-4 weeks after filling in ponds with or without fish (Michaletz et al. 1983, Geiger et al. 1985, Qin et al. 1995). Therefore, the strategy for successful larval fish culture is to match the timing of zooplankton abundance with the period of highest prey consumption.
In other words, prey density should increase and be sustained when prey consumption per fish increases (usually after 2 weeks of stocking for saugeye) and peaks (Qin and Culver 1992). As such, maintaining high zooplankton biomass immediately after stocking is undesirable and may even facilitate overgrazing of phytoplankton which in turn may lead to decline in zooplankton population, depriving fish of prey items during crucial time of growth (Culver et al. 1993). Therefore, initiating pond filling just before fish fry are ready to be stocked, as conducted in the current study, offers the advantage of providing the maximum period of fish consumption of zooplankton before the inevitable decline in plankton abundance that occurs 4-5 weeks after stocking.

Stocking density is another important factor to be considered, as fish density and prey (zooplankton) density are tightly coupled. Fish density manipulation experiments (Qin et al. 1995) showed that at high fish density (500,000 fish/ha), the abundance of both large (*Daphnia*) and small-sized (*Bosmina*) zooplankton can be greatly suppressed depriving fish of prey items. At low fish density (250,000 fish/ha), though *Daphnia* abundance was greatly reduced, abundances of small-sized zooplankters and algae increased which provided adequate food for walleye. Interestingly, in fishless ponds, both zooplankton and phytoplankton abundances were significantly reduced via uncontrolled grazing by large and small-sized carnivorous and herbivorous zooplankton. These results suggest that choosing appropriate fish density will ensure maintenance of zooplankton populations without crashing during the culture period. Further, Tew (2003) in his examination of 12 years of fish production data at Hebron hatchery, found a positive correlation between yield and stocking density up to at least 400,000 fish/ha and
mean individual weight was unaffected by stocking densities in the range of 100-400,000/ha. The stocking density in the current study falls within this range.

Fish culture duration, which can impact production results greatly, varies within and among experimental studies. Too long a culture duration might result in exhaustion of prey items in the pond leading to starvation, whereas too short a duration might lead to harvesting of weak fish unsuitable for stocking. The variation in culture duration is usually more of a logistic issue mainly due to personnel and facilities limitations. In our study, we strived to schedule the draining of the ponds as close together as possible (within 3-4 days) to minimize culture duration effects on our saugeye production estimates.

Further, characteristics of water used for pond filling in fertilization studies might be drastically different between hatcheries, which makes comparison of fish production results difficult. Tice et al. (1996) obtained 26% survival and 19 kg/ha when ponds were stocked at 50 fish /m³. The pond waters had an average alkalinity of less than 20 mg/L and required liming of ponds. In our ponds, alkalinity varied between 80-110 mg/L and as such liming was not required. Not only does this variation in water chemistry confound comparison, but also emphasizes the fact that site-specific monitoring of water quality should be carried out and fertilization protocols need to be tailored to suit the site-specific water quality characteristics. Nevertheless, research at Ohio State Fish hatcheries spanning almost two decades has shown that inorganic fertilization at the rate of 30 µg P/L provides better saugeye survival and yield than organic fertilizers and, more recently, lowering of inorganic phosphorus fertilization rate from 30 to 20 µg/L from
2001-2003 and presently from 20 to 10 µg P/L has yielded comparable and favorable results.

*Did lowering of the phosphorus fertilization rate affect zooplankton dynamics and provide sufficient prey?*

One of the major challenges in pond saugeye culture is providing an adequate supply of food. Zooplankton (cladocerans, copepods and rotifers) is the major food for many larval fish (Parmley and Geiger 1985) including saugeye (Qin et al. 1995). Field experiments have shown that zooplankton abundance inevitably declines due to the combined effects of fish predation and lack of edible phytoplankton species in fish ponds (Geiger 1983a, Michaletz et al. 1983, Parmley and Gieger 1985). To fuel the growth of zooplankton, it is essential to have a constant and adequate supply of edible algae which in turn necessitates pond fertilization. In the current study, we examined the effect of three phosphorus fertilization rates on the zooplankton dynamics in the saugeye ponds.

Our results indicate that the average total zooplankton biomass did not significantly differ among the tested phosphorus fertilization rate treatments. This result contradicts our hypothesis that ponds with reduced phosphorus fertilization rate will have higher zooplankton biomass (via increased edible algae biomass), perhaps because we could not establish a bottom-up effect of phosphorus on phytoplankton biomass (see next section). Nevertheless, this result is important, as it suggests that decreasing phosphorus fertilization rate from 30 to 10 µg P/L in saugeye ponds will not have a negative impact on total zooplankton biomass.
The mean zooplankton biomass in our experimental ponds was 0.295 mg/L corresponding to a numerical density of ~over 100 prey/L. Based on laboratory studies, Li and Mathias (1982) recommended 100 daphnids/L for optimal walleye growth when larval fish density is less than 1 fish/L. However, the fish density was much lower (~0.05/L) in our ponds suggesting that overall prey availability was adequate in our ponds irrespective of treatments. Further, optimal prey density suggested by laboratory studies may not be applicable to field conditions because of the clumped distribution of prey usually observed in ponds (Johnston and Mathias 1994a). Prey clumping enables fish larvae to achieve maximal rates of consumption at much lower mean prey abundances than in a laboratory aquarium (Johnston 1999). Based on direct methods as well as a bioenergetics model, Johnston (1999) found that neither food consumption nor growth of walleye larvae was affected by zooplankton abundances varying from 0.1-0.5 mg dry wt/L in ponds.

The zooplankton in our ponds was dominated by copepods (mainly cyclopoids), small-sized cladocerans (Bosmina) and rotifers and the almost complete absence of large cladocerans (Daphnia), typical of eutrophic systems (Gliwicz 1969, 1977, Richman and Dodson 1983, Orcutt and Pace 1984). Fish predation is believed to be one of the major factors in structuring the zooplankton community in favor of small-sized zooplankters via selective predation on the large-sized ones (Brooks and Dodson 1965, Langeland 1982). However, in our ponds, Daphnia was observed at a biomass of < 0.05 mg/L only for 10 days after stocking and fish diet analyses gave no evidence of selective predation on Daphnia during this period. Hence, we could rule out the possibility of fish predation being responsible for the absence of Daphnia in the ponds.
Cyanobacterial dominance can also determine zooplankton community structure in aquatic systems. The reduction in the abundance of many *Daphnia* species associated with cyanobacterial blooms and the negative effect of Cyanobacteria on feeding, growth, and reproduction of *Daphnia* have been extensively reported in laboratory and field conditions (Schindler 1968, Jones et al. 1979, Lampert 1981a, 1981b, Edmondson and Litt 1982, Thompson et al. 1982, Jarvis 1986, Jarvis et al. 1987, DeMott et al. 1991). In our ponds, Cyanobacteria initially dominated the phytoplankton community (~ 45-67% of the total biomass) and possibly interfered with the proliferation of daphnids.

Though *Daphnia* was absent, other appropriate zooplankton taxa (copepods, rotifers and *Bosmina*) were present to serve as prey items for saugeye. In fact, studies have shown that copepods, rotifers and *Bosmina* are able to coexist with Cyanobacteria unlike daphnids (Lampert, 1987). Our fish diet analyses showed that cyclopoid copepods (mainly *Acanthocyclops vernalis*) were the major prey in all the experimental ponds for the first 3 weeks, followed by cladocerans (*Bosmina*). Though cyclopoid abundance was significantly higher in 20 µg P/L ponds, we did not find significant differences in the number of cyclopoids in the diet among treatments, suggesting the preferential predation on cyclopoids in all the treatments as observed in previous studies (Qin et al. 1994, Tew et al. unpublished data, cited in Tew et al. 2006). However, in a few cases, we observed the dominance of either *A. vernalis* or *Bosmina* in the diet among fish from the same pond on a given sampling date, which may be due to the patchy distribution of zooplankton in the ponds rather than fish preference (Lewis 1979).

Selective predation on cyclopoids enabled small-sized zooplankters such as *Bosmina* and rotifers to increase their population abundances in the ponds probably
because they were released from competition (Gilbert 1985a), and or direct consumption (Williamson and Gilbert 1980), to the benefit of saugeye. As cyclopoid abundance declined in the ponds, saugeye began to increasingly feed on *Bosmina*, rotifers, and non-zooplankton prey items (mainly chironomids). Thus, planktivory by fish enabled a constant supply of zooplankton forage irrespective of phosphorus treatments, as has been observed in prior studies (Culver et al. 1993, Qin et al. 1995).

*Did lowering of phosphorus fertilization rate affect phytoplankton biomass and species composition?*

We hypothesized that decreasing the phosphate fertilization rate from 30 µg P/L to 20 or 10 µg P/L would decrease the biomass of large filamentous, inedible algal species. The proposition of this hypothesis was supported by numerous experimental observations of N: P ratio being able to influence phytoplankton community structure and in particular, the observation that high N: P ratio can decrease the biomass of non-nitrogen fixing Cyanobacteria (Tilman 1977, Schindler 1977, Rhee 1978, Smith 1983, Pick and Lean 1987).

In the current study, however, we found that reducing the phosphorus fertilization rate (thereby increasing N: P ratio) did not result in a shift in the taxonomic composition from filamentous cyanobacteria to non-cyanobacterial algae (specifically edible greens). The large inoculum of Cyanobacteria from the source water, maintained its dominance over other phytoplankton groups, throughout the initial sampling dates in the ponds, irrespective of N: P ratio manipulations. It has been suggested that when Cyanobacteria dominate the phytoplankton community, further fertilization with phosphorus will only
enable their increased proliferation, perhaps because of their ability to store phosphate as polyphosphate granules which gives them a competitive advantage over other species of algae (Darley 1982, Qin and Culver 1994). Further, such observations contradicting widely accepted resource-ratio theory have been previously encountered by other researchers (Barica et al. 1980, Vaga 1986, Trimbee and Prepas 1987, Jensen et al. 1994, Qin and Culver 1996).

In fact, there is a school of thought opposing the paradigm of equilibrium-based resource-ratio theory in favor of considering “non-deterministic fluctuating resources base as merely one-axis of a habitat template model of community assembly” (Reynolds, 1999). In an excellent critical review of resource-ratio theory, Reynolds (1999) has put forward three points that negate the view of considering resource ratio as the sole driver and criterion for the selection of phytoplankton species: 1) The nutrient ratio is immaterial in the selection of species when the nutrient supply is greater than demand. This is illustrated in Sommer’s (1993) experiment in which he presented different resource ratios to 16 species of algae and found that sixteen species responded to varying resource ratios only at extreme ratios which suggest that only when resources get depleted is the ratio relevant in the selection of the species. However, nutrient ratio cannot be viewed as the driving force behind algal response to nutrient depletion. 2) The growth-rate limiting concentrations of nutrients (for phosphorus it is hardly more than 0.1 µmol/L and for nitrogen is 6-7 µmol/L) are often not encountered in real world situations and hence we cannot expect competition between algae for nutrients. Even when nutrients are depleted, outcome of species composition of the phytoplankton assemblage will be determined by inocula rather than by nutrient ratios 3) To date, no
molecular mechanism has been identified within a cell that can sense and respond to the resource ratio instead of an individual resource itself in the environment.

Though nutrient enrichment did not result in taxonomic shift of phytoplankton based on N: P ratios, our results suggest that, given the dominance of Cyanobacteria in the source water, there is little benefit in fertilizing the pond at higher rates, particularly at the beginning of the culture period, when Cyanobacteria are abundant. Further, as zooplankton abundance was unaffected by phosphorus treatments, lowering the phosphorus fertilization rate may be in fact beneficial in avoiding the potential risks (hypoxia, extreme pH, cyanobacterial toxins in water etc.) associated with excessive cyanobacterial abundance.

Another noteworthy observation was the ability of ponds to sustain zooplankton populations until the last week in spite of the decline in total phytoplankton biomass over time in all treatments and the occurrence of a clear-water phase (as indicated by Secchi depth) at 4-5 weeks after filling. Analysis of phytoplankton composition showed that the biomass of Cyanophyta and Chlorophyta decreased over time, but biomass of Chrysophyta (edible diatoms) increased in the later weeks irrespective of treatments. This suggests that even ponds with lowered fertilization rate of 10μg P/L were able to maintain a constant supply of edible phytoplankton species which is essential to maintaining an adequate zooplankton forage base for planktivorous saugeye.

Did lowering of the phosphorus fertilization rate improve water quality?

Besides biotic factors, abiotic factors such as pH, dissolved oxygen, temperature, etc., are important for determining the success of pond larval fish production, although
they are often overlooked (Smith and Piper 1975, Koenst and Smith 1976). In the current study, we monitored water quality parameters to test the hypothesis that lowering of phosphorus fertilization rate will improve water quality, assuming that higher nutrient loading will lead to eutrophic conditions with potential water quality deterioration. For example, excessive algal growth can lead to elevated pH levels (via photosynthesis), low dissolved oxygen concentrations, production of cyanobacterial toxins, etc., in nutrient-rich waters causing stress to the fish.

In the current study, mean pH measurements ranged from 7.98-9.32 and the highest pH value of 10.02 was recorded in a 30 µg P/L treated pond. Elevated pH levels can be lethal to both juvenile and adult fish (Jordan and Lloyd 1964, Daye and Garside 1975). Bergerhouse (1982) reported that the 6 h mortality threshold for pH is between 10.0-10.3 for 3-d-old walleyes and between 9.8-10.00 for 8-12-d-old walleye. pH changes are usually related to carbon dioxide flux created by photosynthesis and respiration and high photosynthetic rates typical of eutrophic systems can lead to elevated pH levels (Bergerhouse 1992, Stickney 1994). Though we did not detect significant differences among the three phosphorus treatments in the current study, lowering of fertilization rate may reduce the potential risk of elevated pH levels via possible reduction in excessive phytoplankton biomass. Tew (2006) found that ponds fertilized at 30 µg P/L had higher pH levels than those fertilized at 20 µg P/L.

Further, the fraction of total ammonium nitrogen that occurs as unionized ammonia (NH₃, which is highly toxic to fish) in the water is mainly determined by the pH and temperature. A change in one pH unit from 8.0 to 9.0 can result in ~ 10-fold increase in unionized ammonia concentration. In our experiment, the highest recorded total
ammonium nitrogen concentration of 0.108 mg/L as N (Source: Hebron hatchery data file) that occurred with a pH of 8.98 and an average temperature of 17°C would yield 0.025 mg/L of unionized ammonia which exceeds the maximum allowable concentration of unionized ammonia of 0.0125 mg/L recommended for fish ponds (Meade, 1985). However, further investigation needs to be conducted to determine the specific effect of elevated unionized ammonia concentration and pH on larval saugeye.

The dissolved oxygen concentration is another important parameter for larval fish culture (Middleton and Reeder, 2003). In the current study, both mean surface and bottom dissolved oxygen levels never fell below 5.0 mg/L. However, individual pond data showed that in more than one pond, DO fell below 5.0 mg/L during the 4th week and the lowest recorded DO of 1.8 mg/L was from a 30 µg P/L-treated pond. Though no threshold value of DO has been recommended for larval saugeye culture, DO concentrations at or above 5.0 mg/L are considered optimal for fish (Wheaton, 1977). Changes in DO are strongly tied to plankton dynamics and the low DO recorded during the final weeks may have been due to declining algal abundance and increasing zooplankton and fish biomass observed in our experimental ponds. Though no phosphorus treatment effect on DO or pH was detected, we believe that lowering of phosphorus fertilization rate will certainly not lead to deteriorated water quality conditions, but may only aid in improving water quality, given that the source water to Hebron hatchery is highly eutrophic with cyanobacterial dominance.

In summary, our fertilization study has shown that lowering of phosphorus fertilization rate from 30 to 10 µg P/L neither affected the larval saugeye production (in terms of survival, yield, and growth) nor the maintenance of adequate zooplankton forage.
base throughout the culture duration. Further, at a current stocking density of ~ 50 fish/m³, a fertilization protocol of 10 µg P/L and 600 µg N/L seems to be adequate to provide primary production necessary to maintain adequate zooplankton biomass and to avoid the potential risks of elevated pH, unionized ammonia, low dissolved oxygen concentration, etc., often associated with high nutrient input, especially given the dominance of Cyanobacteria in the source water at Hebron hatchery. As with most aquaculture field experiments, it is necessary to conduct more field trials over the years not only to determine and validate the effects of a particular fertilization rate (Wudtisin and Boyd, 2005) but also to evaluate whether the tested fertilization rate will decrease inter-annual variation in larval saugeye production observed at hatcheries. Further, field experiments at different locations would help in elucidating the universal applicability of the proposed fertilization protocol. Given the high demand for saugeye larvae for stocking in reservoir and lakes, more meaningful and practical research needs to be carried out to maximize saugeye production using sound ecological and cost-effective methods.
References


Qin, J., and Culver, D. A. 1996. Effect of young-of-the-year walleye (Percidae: 
Stizostedion vitreum) on plankton dynamics and water quality in ponds.

Qin, J., Culver, D. A. and Yu, N. 1994. Comparisons of larval walleye and saugeye
(walleye x saugeye hybrid) growth and impacts on zooplankton in experimental ponds.


Rhee, G. Y. 1978. Effects of N: P atomic ratios and nitrate limitation on algal growth,


Williamson, C.E., and Gilbert, J. J. 1980. Variation among zooplankton predators: The potential of Asplanchna, Mesocyclops, and Cyclops to attack, capture and eat various


### Table 1.1. Juvenile saugeye growth and production data in ponds treated with three phosphorus fertilization rates (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery, Ohio, USA in 2004. Values are presented as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>10 µg P/L</th>
<th>20 µg P/L</th>
<th>30 µg P/L</th>
<th>p-value</th>
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<tr>
<td><strong>Experimental ponds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># of ponds</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>28.6±1.0</td>
<td>30.7±1.0</td>
<td>28.3±1.0</td>
<td>0.2965</td>
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<tr>
<td>Final fish weight (g)</td>
<td>0.17±0.02</td>
<td>0.21±0.02</td>
<td>0.17±0.02</td>
<td>0.4564</td>
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<tr>
<td>Percent survival</td>
<td>88.5±1.2</td>
<td>81.6±15.9</td>
<td>74.3±6.5</td>
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<tr>
<td># harvested/m³</td>
<td>45.9±4.6</td>
<td>38.1±4.5</td>
<td>44.2±4.4</td>
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<tr>
<td>Yield (g/m³)</td>
<td>8.24±0.77</td>
<td>7.99±1.36</td>
<td>7.88±1.27</td>
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<td><strong>All hatchery ponds</strong></td>
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<td># of ponds</td>
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<td>11</td>
<td>10</td>
<td></td>
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<tr>
<td>Total length (mm)</td>
<td>31.4±0.6</td>
<td>32.0±0.6</td>
<td>30.3±0.7</td>
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<td>Final fish weight (g)</td>
<td>0.20±0.01</td>
<td>0.23±0.01</td>
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<tr>
<td>Percent survival</td>
<td>66.9±5.7</td>
<td>64.3±5.9</td>
<td>74.1±6.5</td>
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<td># harvested/m³</td>
<td>35.8±3.2</td>
<td>33.9±3.3</td>
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<tr>
<td>Yield (g/m³)</td>
<td>6.98±0.74</td>
<td>7.60±0.78</td>
<td>7.84±0.85</td>
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Table 1.2. Treatment means of all response variables, and significance level associated with treatment and date for each variable, as determined by repeated measures of ANOVA.
<table>
<thead>
<tr>
<th>Response variables</th>
<th>Treatment Means</th>
<th>Probability values</th>
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<tr>
<td></td>
<td>10 µg P/L</td>
<td>20 µg P/L</td>
</tr>
<tr>
<td>Biotic Variables</td>
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<tr>
<td>1) Zooplankton (mg/L)</td>
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<tr>
<td>Rotifera</td>
<td>0.004</td>
<td>0.001</td>
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<tr>
<td><em>Bosmina</em></td>
<td>0.219</td>
<td>0.299</td>
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<td>Cladocera</td>
<td>0.093</td>
<td>0.111</td>
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<tr>
<td><em>Acanthocyclops vernalis</em></td>
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</tr>
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<td>Cyclopooid nauplii</td>
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<td>Copepoda</td>
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<td>Total zooplankton</td>
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<td>0.401</td>
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<tr>
<td>Secondary Production (mg/L/day)</td>
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<td>Cladocera</td>
<td>0.060</td>
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<tr>
<td>Cyclopoidea</td>
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<td>Calanoida</td>
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<td>Total crustacean zooplankton</td>
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<td>Turn-over time (days)</td>
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<td>2) Phytoplankton (mg/L)</td>
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<tr>
<td>Cyanophyta</td>
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<td>0.776</td>
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<td>Chlorophyta</td>
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<td>0.855</td>
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<td>Chrysophyta</td>
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<td>0.175</td>
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<td>Cryptophyta</td>
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<td>2.97</td>
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<tr>
<td>Edible algae</td>
<td>3.94</td>
<td>4.74</td>
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<tr>
<td>Inedible algae</td>
<td>0.897</td>
<td>0.788</td>
</tr>
<tr>
<td>Total phytoplankton</td>
<td>6.32</td>
<td>7.16</td>
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<tr>
<td>Primary Production (mgO₂/L/Day)</td>
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</tr>
<tr>
<td>Gross production</td>
<td>1.71</td>
<td>2.12</td>
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<tr>
<td>Net production</td>
<td>0.90</td>
<td>1.38</td>
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<tr>
<td>Respiration</td>
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<td>Abiotic variables</td>
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<tr>
<td>Dissolved oxygen (mg/L)</td>
<td></td>
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<tr>
<td>Surface</td>
<td>8.77</td>
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<tr>
<td>pH</td>
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<td>8.51</td>
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<tr>
<td>Secchi (cm)</td>
<td>83.35</td>
<td>79.98</td>
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Fig. 1.1. Comparison of saugeye total length (mm) and wet weight (g) among three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L). The values of each date are the mean from 10 fish from each of the 4 replicate ponds of each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 1.2. Comparison of instantaneous saugeye growth rate curves among three phosphate fertilization rate treatments (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery in 2004. The values of each date are the mean ± standard error of 10 fish from 4 replicate ponds per treatment.
Fig. 1.3. Effects of the three phosphorus fertilization rates (10, 20 and 30 µg P/L) on saugeye dietary composition in the experimental saugeye production ponds (pond nos. B2, B3, B5 and C10 (10 µg P/L), B8, B9, C8 and C9 (20 µg P/L), and B4, B6, B7 and C3 (30 µg P/L)) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from 4 replicate ponds for each treatment.
Fig. 1.4. Seasonal variation in mean total zooplankton biomass (dry weight) in saugeye culture ponds across three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 1.5. Effects of the three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) on zooplankton composition in the experimental saugeye production ponds (pond nos. B2, B3, B5 and C10 (10 µg P/L), B8, B9, C8 and C9 (20 µg P/L), and B4, B6, B7 and C3 (30 µg P/L)) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment.
Zooplankton Biomass (mg/L)

Date

Cladocera
Cyclopoida
Calanoida
Rotifera

10 µg P/L

20 µg P/L

30 µg P/L
Fig. 1.6. Seasonal variation in cladoceran biomass (dry weight) in saugeye culture ponds across three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 1.7. Seasonal variation in rotifer biomass (dry weight) in saugeye culture ponds across three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 1.8. Seasonal variation in mean total phytoplankton biomass (wet weight) in saugeye culture ponds across three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean
Fig. 1.9. Effects of the three phosphorus fertilization rates (10, 20 and 30 µg P/L) on phytoplankton composition in the experimental saugeye production ponds (pond nos. B2, B3, B5 and C10 (10 µg P/L), B8, B9, C8 and C9 (20 µg P/L), and B4, B6, B7 and C3 (30 µg P/L)) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment.
Fig. 1.10. Effects of the three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) on edible/inedible algal biomass (wet weight) in the experimental saugeye production ponds (pond nos. B2, B3, B5 and C10 (10 µg P/L), B8, B9, C8 and C9 (20 µg P/L), and B4, B6, B7 and C3 (30 µg P/L)) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates of each treatment.
### Phytoplankton Biomass (mg/L)

- **10 µg P/L**
  - Apr-27: 12 mg/L, Inedible algae: 7 mg/L, Edible algae: 5 mg/L
  - 30 May: 5 mg/L, Inedible algae: 2 mg/L, Edible algae: 3 mg/L
  - 4 May: 4 mg/L, Inedible algae: 2.5 mg/L, Edible algae: 1.5 mg/L
  - 7 May: 3 mg/L, Inedible algae: 1.5 mg/L, Edible algae: 1.5 mg/L
  - 11 May: 2 mg/L, Inedible algae: 1 mg/L, Edible algae: 1 mg/L
  - 14 May: 1 mg/L, Inedible algae: 0.5 mg/L, Edible algae: 0.5 mg/L
  - 18 May: 0.5 mg/L, Inedible algae: 0.25 mg/L, Edible algae: 0.25 mg/L

- **20 µg P/L**
  - Apr-27: 20 mg/L, Inedible algae: 10 mg/L, Edible algae: 10 mg/L
  - 30 May: 15 mg/L, Inedible algae: 7.5 mg/L, Edible algae: 7.5 mg/L
  - 4 May: 12 mg/L, Inedible algae: 6 mg/L, Edible algae: 6 mg/L
  - 7 May: 10 mg/L, Inedible algae: 5 mg/L, Edible algae: 5 mg/L
  - 11 May: 8 mg/L, Inedible algae: 4 mg/L, Edible algae: 4 mg/L
  - 14 May: 6 mg/L, Inedible algae: 3 mg/L, Edible algae: 3 mg/L
  - 18 May: 4 mg/L, Inedible algae: 2 mg/L, Edible algae: 2 mg/L

- **30 µg P/L**
  - Apr-27: 25 mg/L, Inedible algae: 15 mg/L, Edible algae: 10 mg/L
  - 30 May: 18 mg/L, Inedible algae: 12 mg/L, Edible algae: 6 mg/L
  - 4 May: 15 mg/L, Inedible algae: 9 mg/L, Edible algae: 6 mg/L
  - 7 May: 12 mg/L, Inedible algae: 8 mg/L, Edible algae: 4 mg/L
  - 11 May: 9 mg/L, Inedible algae: 6 mg/L, Edible algae: 3 mg/L
  - 14 May: 6 mg/L, Inedible algae: 4 mg/L, Edible algae: 2 mg/L
  - 18 May: 3 mg/L, Inedible algae: 2 mg/L, Edible algae: 1 mg/L
Fig. 1.11. Effects of the three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) on different algal groups in the experimental saugeye production ponds (pond nos. B2, B3, B5 and C10 (10 µg P/L), B8, B9, C8 and C9 (20 µg P/L), and B4, B6, B7 and C3 (30 µg P/L)) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates. The vertical bars refer to standard error associated with the mean.
Fig. 1.12. Effects of the three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) on gross primary production, net primary production and respiratory rate in the experimental saugeye production ponds (pond nos. B2, B3, B5 and C10 (10 µg P/L), B8, B9, C8 and C9 (20 µg P/L), and B4, B6, B7 and C3 (30 µg P/L)) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates. The vertical bars refer to standard error associated with the mean.
Fig. 1.13. Variation in mean pond water temperature in the experimental ponds during the saugeye culture season at Hebron State Fish Hatchery in 2004.
Fig. 1.14. Seasonal variation in dissolved oxygen concentration at the surface and bottom of the saugeye culture ponds across three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 1.15. Seasonal variation in pH (1.15a) and Secchi depth (1.15b) in saugeye culture ponds across three phosphorus fertilization rate treatments (10, 20 and 30 µg P/L) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
CHAPTER 2

EXPERIMENTAL EVALUATION OF THE TROPHIC-LEVEL IMPACTS OF THE USE OF FLURIDONE AND COPPER SULFATE IN CATFISH PONDS

INTRODUCTION

Algaecides and herbicides are routinely applied to catfish ponds to curtail the growth of nuisance aquatic algae and macrophytes. Overabundance of algal (often dominated by Cyanobacteria) and macrophyte communities is a common occurrence in catfish ponds, mostly resulting from various pond management practices including addition of artificial fish feed (Boyd 1974, Zimba et al. 2001, Zimba et al. 2002, Schrader and Dennis 2005). Routine inputs of fish feed rich in nitrogen and phosphorus result in eutrophied pond waters leading to nuisance algal and macrophyte infestation. Of the total amount of phosphorus contained in fish feed, it has been estimated that 11% dissolves in water and as much as 66% accumulates in the sediments (Ackefors and Sodergren 1985), which in turn can fuel the growth of aquatic plants.

The resulting dense growth of algae and macrophytes can be detrimental to fish culture in many ways. Most importantly, plants can interfere with feeding and harvest of fishes. Feed pellets may get caught up in dense mats of algae or thick strands of vascular
plants, making them inaccessible to fish, resulting in wasted feed. Likewise, harvesting equipment may get entwined in algal mats or dense strands of macrophytes resulting in equipment damage and/or futile efforts to capture fish (Boyd and Tucker 1998). Further, during harvest, fish could get smothered and killed under thick mats of floating algae especially those of macrophytic filamentous algae such as *Hydrodictyon* spp., *Spirogyra* spp., *Zygnema* spp., *Chara* spp., etc. Harvesting of fish can thus become laborious, time consuming, and even result in economic losses via harvesting equipment damage and fish mortality.

The excessive growth of algae and vascular plants can also negatively affect water quality parameters such as dissolved oxygen concentration, pH, temperature, light penetration, etc., resulting in an unfavorable environment for aquatic biota (Bowes et al. 1979, Honnell et al. 1993). For example, overabundance of phytoplankton and vascular plants can result in a deficit in dissolved oxygen at night, which can elevate carbon dioxide levels and decrease the pH, all of which can cause stress to the fish.

Furthermore, algal blooms dominated by Cyanobacteria are highly undesirable in catfish ponds (Schrader and Dennis 2005). Besides potentially leading to water quality problems such as hypoxia, low water clarity, extreme pH values, etc., cyanobacterial blooms may include potentially toxic algae such as *Microcystis* spp. and *Cylindrospermopsis* spp., which can produce toxins (microcystins, cylindropermopsins) and/or odorous compounds (methyl isoborneol and geosmin) that can cause off-flavor in cultured fish (Brown and Boyd 1982, Armstrong et al. 1986, Paerl and Tucker 1995, Zimba et al. 2002). Huge economic losses to catfish producers (sometimes as much as $60 million annually) can result from off-flavor (Sinedlar 1987, Engle et al. 1995, Tucker
Furthermore, cyanobacterial dominance can directly affect fish feeding and growth (Bayne et al. 1991, Burke and Bayne 1986) impacting fish survival and yield from aquaculture ponds.

For the above reasons, fish culturists resort to chemical control of macrophytes and algae for successful catfish pond culture. However, the use of chemicals is a risky proposition in aquaculture, as it can create many unfavorable environmental conditions for profitable fish culture. Deterioration of water quality is a major concern associated with the use of the herbicides and algaecides, from death and decay of plants, which can deplete dissolved oxygen in the pond waters resulting in reduced fish growth (Tucker and Boyd 1978, Perschbacher et al. 1997) or even mortality. Decomposition of the dead plants can also result in elevated total ammonia concentrations in the ponds (Tucker et al. 1983, Boyd and Tucker 1998). Further, elimination of phytoplankton via algaecidal use will also decrease the rate at which ammonia is removed from the pond water, and result in the production of nitrite via nitrification of ammonia (Tucker et al. 1984a). Elevated levels of both ammonia (specifically unionized ammonia) and nitrite can be toxic to fishes (Colt and Armstrong 1979, Huey et al. 1980, Boyd and Tucker 1998).

Another concern is the potential negative impact of herbicides and algaecides on non-target species in the fish ponds. Pesticides targeted to eliminate the overabundant phytoplankton or macrophyte community can negatively affect the zooplankton population, either by direct toxicity or by a decrease in the algal forage base (McKnight et al. 1983, Perschbacher et al. 1997). Changes in the zooplankton community dynamics may affect the efficacy of pesticide treatment; for example, a decrease in zooplankton foraging may counteract the direct toxic effect of pesticides on algae.
In addition to possible changes in water chemistry and plankton community structure and composition, herbicidal and algaecidal use could also result in potential bioaccumulation of chemicals in fish and plankton, accumulation in sediments, variability in fish production and the possibility of discharge of potentially harmful aquaculture effluents to natural water bodies when the ponds are drained (Boyd and Massaut 1999).

Finally, herbicides and algaecides are very expensive to purchase and apply, so they are an additional economic burden to fish producers. It is therefore imperative to conduct site-specific monitoring on the overall effectiveness and possible effects of the application of herbicides and algaecides in fish ponds.

Though a number herbicides and algaecides are available to control nuisance plants in aquatic ecosystems, only a few of them are permitted in the USA for use in aquaculture ponds (Federal Joint Subcommittee on Aquaculture 1994). Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4-(1H)-pyridinone), the active ingredient in the aquatic herbicide, AVAST (SePRO Corporation, Carmel, IN, USA) has been registered for use in fish ponds (Schnick et al. 1986). Fluridone is a slow-acting, systemic herbicide that controls the growth of several submerged and emergent aquatic vascular plants, with application rates ranging from 5 µg/L to the USEPA registered maximum labeled rate of 150 µg fluridone/L (Mille 1990, Sprecher et al. 1998, Netherland et al. 2002). Fluridone inhibits carotenoid biosynthesis in plants via blocking phyotene desaturation, which eventually results in the photo-oxidation of the unprotected chlorophyll (Bartels and Watson 1978, Mille et al. 1990, Sandmann 1994, Sprecher et al. 1998). Susceptible plants thus become chlorotic and slowly die.
At the Hebron State Fish Hatchery, Ohio, fluridone is applied during summer to catfish rearing ponds that are infested with vegetation. Application of fluridone effectively helps in reducing fish mortality and successful harvesting of fishes from drained ponds (J. Stafford, Ohio Division of Wildlife, pers. comm.). But any herbicide application requires identification of plants, and evaluation of the effectiveness of the chosen herbicide. Neither has a survey of plants present prior to application nor an evaluation of the effectiveness of fluridone in controlling them been conducted at Hebron State Fish Hatchery. Because of the high expense and selectivity of fluridone, it is necessary to evaluate its effectiveness in controlling plants and examine whether its use is appropriate for aquaculture of juvenile catfish.

As for algaecides, copper sulfate pentahydrate (CuSO₄·5H₂O) is the only one approved by the US Environmental Protection agency for application in catfish ponds (USEPA 2003). Copper is an essential plant micronutrient at trace levels but is also considered a priority pollutant by the U.S. Environmental Protection Agency (USEPA 1980). Exposure to high levels of copper inhibits growth or kills plants by disrupting a variety of cellular functions including photosynthesis, respiration, chlorophyll synthesis and cell division (Fisher et al. 1981, Baron et al. 1995, Cid et al. 1997, Eide and Guerinot 1997).

Although the use of copper sulfate as an algaecide in aquatic systems is widespread, its short residence time in the water column often renders it inefficient in controlling phytoplankton blooms (Button et al. 1977, Zimba et al. 2002). In natural waters, copper exists mainly as: 1) the free ionic form (Cu²⁺), 2) various complexes, and 3) sorbed onto particulates including biological components. The relative proportions of
these copper species determine the concentration, mobility and bioavailability of copper in the environment. In the aqueous phase, this speciation of copper is regulated by processes such as inorganic and organic complexation, precipitation and biological uptake which are in turn dictated by various chemical properties like pH, dissolved organic carbon, hardness, alkalinity, etc. of the water. Several studies have shown that the free ionic form (Cu$^{2+}$) is the most bioavailable and hence the most toxic form to biota (Pagenkopf et al. 1974, Sunda and Guillard 1976, Xue and Sigg 1990). Nevertheless, depending on the water chemistry, the free copper ion activity in the water column can be substantially reduced within a short time after application, thereby decreasing the effectiveness of copper sulfate in controlling nuisance algal blooms.

Moreover, as copper does not degrade, repeated applications of copper sulfate to ponds within and over years during catfish culture can lead to the accumulation of high levels of copper in the sediment (Han et al. 2001, Huggett et al. 2001, Zimba et al. 2002, Liu et al. 2006). The persistence of copper in the sediments and the possible re-suspension into the overlying water column during pond filling can be detrimental to the culture of more copper-sensitive fish species in the same pond, at a different time of the year.

Further, of late, discharge into natural waters of pollutants arising from anthropogenic activities, including aquaculture, has caused great concern among scientists and managers alike. To monitor and reduce discharge of potentially harmful aquaculture effluent, United States Environmental Protection Agency (USEPA) has established effluent limitation guidelines (ELGs) currently for those facilities that produce at least 100,000 pounds of fish per year in flow-through systems, recirculating
systems and net pens (USEPA 2004). Toward this end, information regarding the effects of algaecidal copper and herbicidal fluridone use in aquaculture facilities is important for compliance with ELGs and application for National Pollution Discharge Elimination System permits. Taken together, it is necessary to evaluate the overall effectiveness of copper sulfate and fluridone in controlling nuisance algal and macrophyte species and its dynamics in catfish ponds.

The current study investigated the efficacy of fluridone and copper sulfate application at controlling plants and algae and their possible effects on water quality, seasonal dynamics and species composition of phytoplankton and zooplankton, and overall catfish production. We also assessed the spatio-temporal fate of copper in the ponds to better understand its dynamics in catfish ponds. Specifically, we tested the following hypotheses:

1. Application of fluridone will effectively control macrophytes in catfish ponds.
2. The residence time of copper sulfate in the water column will be short.
3. Application of copper sulfate and fluridone will result in the deterioration of the water quality of catfish ponds.
4. Fluridone and copper sulfate applied individually and in combination will decrease the biomass of phytoplankton and also change its community composition.
5. Fluridone and copper sulfate applied individually and in combination to catfish ponds will alter the community composition of zooplankton by direct or indirect toxicity.
6. Application of fluridone and copper sulfate to catfish ponds will not directly affect the overall fish production.
7. Combined application of fluridone and copper sulfate will result in synergistic effects on measured ecological variables.
The stated hypotheses were tested via the following objectives:

1. Determine the efficacy of fluridone in controlling aquatic plant infestations and nuisance filamentous algae during catfish culture in summer.

The stated objective was met by the following three measurements:

   a) We sampled macrophytes, identified them using OSU herbarium specimens, and, based on the herbicide manufacturer’s list of susceptible plants, determined whether fluridone is suitable for application at Hebron hatchery for their control.

   b) We measured the half-life of fluridone in Hebron ponds under natural conditions using high performance liquid chromatography. The longer its half-life in the water column, the greater will be the efficacy of fluridone in controlling nuisance plants.

   c) We quantified macrophyte (vascular plants and macroalgae) biomass before and after fluridone application in the experimental ponds which revealed whether or not the use of fluridone is effective at Hebron hatchery.

2. Measure the efficacy and effectiveness of the use of copper sulfate in catfish ponds-

   Accurate depiction of fate of copper in catfish ponds was studied using the following measurements:

   We measured the change in the free copper ion activity in the water column during the week of application and the following week of non-application of copper sulfate using an ion-specific copper electrode. Similarly, we measured the relative short-term changes in two operationally defined copper species: dissolved copper and total copper. The ‘dissolved copper’ refers to copper remaining in water after filtering the
samples through a 0.45-µm PVDF membrane (SUNSRI Co., NC, USA), whereas the ‘total copper’ refers to the amount of copper determined by hot acid digestion of unfiltered water samples.

3. Determine the effect of fluridone and copper sulfate applied (together and separately) on the water quality in catfish ponds

   The water quality parameters considered for this study were dissolved oxygen, pH and transparency and we measured the changes in these variables in response to fluridone and copper sulfate application weekly in 16 catfish ponds. As temperature influences both dissolved oxygen concentrations and pH, we collected precise temperature data from ponds using underwater, continuously monitoring temperature data loggers (Onset Co., Pocasset, MA, USA).

4. Determine the relative effectiveness of fluridone and copper sulfate in reducing phytoplankton abundance and changing its community structure

   Because both chemicals are routinely added to ponds, we measured phytoplankton biomass and species composition weekly to determine the variations in overall biomass and species composition in response to fluridone and copper sulfate applications, applied individually and together.
5. Determine the direct and indirect effects of fluridone and copper sulfate on the biomass and species composition of zooplankton

We determined zooplankton biomass, species composition and secondary productivity weekly to determine the changes in overall biomass and species composition in response to fluridone and copper sulfate applications.

6. Evaluate the effects of fluridone and copper sulfate on overall fish production

We obtained data on fish survival and yield from hatchery personnel that enabled evaluation of the direct and indirect effects of application of fluridone and copper sulfate on fish production.

7. Evaluate possible synergistic effect of fluridone and copper sulfate on measured variables

Our randomized block experimental design (See Methods section) effectively allowed us to analyze possible synergistic effects of combined application of fluridone and copper sulfate on all measured ecological variables.

To the best of our knowledge, no study has been conducted to examine the effects of simultaneous application of an herbicide and an algaecide in catfish ponds on phytoplankton, zooplankton, water quality, and fish production. The overall objective of this study was thus to evaluate whether the use of these chemicals is justified in catfish ponds and to develop best management practices that are ecologically sound and cost-effective for algaecide and herbicide use.
MATERIALS AND METHODS

Study site and pond stocking

All experiments were conducted at Hebron State Fish Hatchery, Ohio, USA from June-September of 2004 during channel catfish production season. Water for hatchery operation was obtained from Buckeye Lake through two feeder pipes (315 L/sec capacity) in a 1.5 mile section of the old Ohio Erie Canal. Two wells (23 L/sec capacity) also provide water to the hatchery.

Each pond has a separate filling and draining system and is filled with water about three days prior to stocking of fish. The water is filtered through 0.5-mm mesh screens to prevent introduction of eggs or larvae of undesirable fish. The grow-out ponds are approximately 3000 m² in area and contain 2500 m³ of water. Most ponds averaged 1 m in depth.

We stocked yearling channel catfish in 16 experimental ponds on 23 and 24 June 2004 at an average density of 2 fish /m³ and ponds were drained to harvest after 12-13 weeks.

Experimental Design

In order to test the stated hypotheses, we used a randomized complete block design with a total of 16 ponds and 4 blocks; with each block having ponds with 3 different treatments- fluridone only, copper sulfate only, fluridone and copper sulfate and 1 control pond without herbicide or algaecide, but stocked with catfish at the same density as the others.
Pond filling began on 22 June 2004 and continued for a week until ponds were full (by 30 June 2004). We applied copper sulfate pentahydrate (CuSO$_4$.5H$_2$O) to achieve a target concentration of 1 mg CuSO$_4$.5H$_2$O/L (= 0.25 mg Cu/L) to eight of the total sixteen ponds by uniform surface spraying from a truck. The first copper sulfate application occurred on 25 June, 2004 when the ponds were half-full and thereafter it was applied every other week. We applied fluridone once during the catfish rearing season on 1 July, 2004 to eight ponds at the rate of 71.90 g/1000 m$^3$ uniformly in the ponds using a spray applicator from a row boat. In addition to copper sulfate and fluridone, ponds received commercial dry catfish feed (Silver Cup, S.C Trout Pellet, Nelson and Sons, Utah) daily. Further, all of the experimental ponds have received copper sulfate applications during catfish culture in previous years.

Sampling and Analytical procedures

Macrophyte sampling and biomass estimation

We sampled macrophytes using 0.25 m$^2$ plastic quadrats from 5 random locations from each pond once before and 79 days after fluridone application, and immediately rinsed plants to remove debris and silt. We identified all macrophyte samples by comparison to Ohio State herbarium specimens. For biomass estimations of the total macrophyte community, we dried samples from each quadrat in an oven at 105$^\circ$C and weighed to the nearest ±0.1g.
Determination of half-life of fluridone

Water sampling: We sampled water samples for fluridone in amber glass bottles on days after treatment (D. A. T. = 0, 1, 2, 6, 8, 12, 36, 50 and 79 days) after rinsing each bottle with pond water from that location prior to taking the sample. We took water samples from three different locations (shallow end, middle and deep end) in the pond with an integrated tube sampler, and composited them for each pond and date. We kept the samples in a cooler with ice and transported them to the laboratory, and stored them at 4ºC until analysis.

Standard Solutions: We purchased a stock solution of fluridone (100 µg/ml) from Accustandard (New Haven, CT, USA) and prepared working standard solutions from the stock solution by sequential dilution with methanol (100%) to yield final concentrations of 0.25, 0.5, 1, and 10 µg/ml. We protected the stock standard solution and working standard solutions from light by wrapping bottles in foil, and stored them in a refrigerator at 4ºC.

Extraction Procedure: We extracted fluridone from pond water samples using a Bakerbond Sep Octadecyl (C18) extraction column with a pore size of 60 Å (Mallinckrodt Baker Inc, NJ, USA). We pumped a 50-ml aliquot of each water sample through the C18 column and discarded the eluate. We eluted fluridone from the adsorbent with 4 ml methanol and collected it in pre-weighed amber glass injection vials.

High Performance Liquid Chromatography Analysis: We performed the analyses on a Shimadzu (Kyoto, Japan) chromatographic system equipped with a LC-10AT VP pump, an SIL-10AD VP auto-sampler, an SPD-10A UV detector and SCL 10A VP controller.
We acquired and processed the fluridone analysis data using CLASS-VP (v.7.0) software. We pumped the mobile phase, methanol: water (65:35, v/v) at a flow rate of 0.200 ml/min through the column at 40°C with a sample injection volume of 15µl. The retention time for fluridone was approximately 7.5 minutes. We monitored the fluridone peak by UV absorbance at 254 nm, with a sensitivity of 0.125AUFS. We quantified fluridone in each sample by plotting fluridone to external standard peak height ratios as a function of concentration.

Fluridone Dissipation Analysis: We described the dissipation (decay) of fluridone in each treated pond using the first-order rate equation

\[ \ln \left( \frac{A_t}{A_0} \right) = -kt \] [1], where \( A_t \) is the concentration of the herbicide contained in the pond water at time \( t \), \( k \) is the rate constant (days), and \( A_0 \) is the initial water herbicide concentration. We fit this equation to the fluridone concentration data using the Marquardt's compromise method (using PROC NLIN in SAS), which involved iterative calculation of parameters \( A_0 \) and \( k \). We computed half-life for the fluridone from the formula \( DT_{50} = \frac{0.693}{k} \), [2] where \( k \) is the rate constant computed in Equation 1.

Determination of fate of copper in catfish ponds

Water Sampling: Using the same pond sampling protocol described previously for fluridone, we collected water samples for copper measurements in polyethylene bottles. In the laboratory, we partitioned each water sample into 3 sub-samples: 1) an unfiltered, unacidified sample for free copper ion measurements 2) a filtered, acidified (using 20%
HNO₃) sample for total dissolved copper measurements, and 3) an unfiltered, acidified sample for total copper measurements. We stored all samples at 4°C until analysis.

Measurement of free copper ion activity: We measured free copper ion activity in water using a copper ion selective electrode (Cu-ISE, ORION 9429) coupled with a reference electrode (ORION 900200) at 25°C. We polished the Cu-ISE for 30 s with aluminum oxide strips each day before use. We changed the reference electrode outer filling solution (Orion 90003) and inner filling solution (Orion 90006) daily. We soaked both electrodes successively for 10 min in 0.025M HNO₃ and 0.1 M Na₄EDTA before use. We prepared the calibration buffers with 1mM IDA (iminodiacetic acid), 6mM NaOH, 2.5mM KHC₈H₄O₄ (potassium acid phthalate) and 0.01M CaCl₂, and varied the pH by incremental additions of nitric acid. We recorded the millivolt readings of the calibration buffers and the samples after they became stable (±0.3 mv for 3 minutes). We plotted the electrode potential determined by Cu-ISE against free copper ion activity (which is frequently reported as the negative logarithm of the molar concentration \([\text{pCu} = -\log (\text{Cu}^{2+})]\)) calculated by using an equilibrium speciation model (MINEQL+ ; Schecher and McAvoy 1998) to determine the free copper ion activity in the samples.

Total dissolved and total copper measurements: We analyzed total dissolved copper and total copper (after hot acid digestion) in the pond waters samples using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP).

Water quality parameters

We measured dissolved oxygen concentrations (in the mornings) using YSI model-54A oxygen meter, pH (in the afternoons) with a pH probe (Orion 9256) attached
to a Beckman 32 meter and water transparency with a Secchi disk once every week. We recorded precise temperature data from ponds using underwater, continuously monitoring temperature data loggers (Hobo loggers, Onset, Pocasset, MA) suspended at a depth of 0.5 m.

Zooplankton Biomass and Community Composition Analysis

We sampled zooplankton using a metered (Model 2030R, General Oceanics, Inc., Miami, Florida), 0.5-m diameter, 64-µm mesh net fitted with a canning jar on the cod-end and mounted on a wooden pole. We towed the net from a row boat across the pond and raised and lowered it repeatedly to obtain a depth-integrated sample. The samples were concentrated with a sieve (64-µm mesh), transferred to labeled screw-cap plastic cups and preserved in the field in 4% formalin-sucrose solution (Haney and Hall 1973). The volume of the water column filtered by the net for each sample was calculated by multiplying the cross-sectional area of the net (0.196 m$^2$) by the distance traveled (m) calculated from flow meter readings.

Zooplankton enumeration followed Kane (2004). Briefly, we diluted each concentrated sample to a known volume (~1500 to 9000 ml) and identified and counted all zooplankton taxa (rotifers, cladocerans and copepods (cyclopoids and calanoids)) in two or more sub-samples (with a total volume ranging from 5 to 50 ml) to genus and species (when possible) according to Balcer et al. (1984), Brooks (1959), and Wilson and Yeatman (1959) using a Wild dissecting microscope at 50x. We withdrew and analyzed sub-samples from the diluted sample until at least 100 individuals of the most common taxon were recorded. We used a calibrated ocular micrometer to measure the lengths of
the first 20 individuals in each genus or species and calculated genus- or species-specific average individual biomass from length-weight regressions (Culver et al. 1985) and the number of individuals per cubic meter. We summed genus or species-specific total biomasses over all taxa to provide a total crustacean zooplankton biomass (mg/L) for a given sampling pond, for a given date. We analyzed zooplankton data as total biomass and at the taxonomic level of class (e.g., Cladocera). For each zooplankton taxon, we calculated secondary productivity (µg/L/Day) as a function of biomass and temperature (Frost 1997).

Phytoplankton Biomass and Community Composition Analysis

We collected phytoplankton samples weekly with an integrated tube sampler (10 cm diameter and 1.5 m long) by vertically lowering the open end of the sampler into the pond and then replacing the stopper at the other end to obtain the sample. We poured the collected water into a bucket and took a 250-ml sample, which was preserved in a canning jar with Lugol’s solution. In the laboratory, we mixed each sample thoroughly and poured it into a 250-ml graduated cylinder and allowed it to settle for 3 days in a dark chamber. We then concentrated each sample to 30 ml by siphoning off 220 ml from the top and transferring the remaining sample into ~ 40-ml vials. We placed sub samples of 3-6 ml in tared Utermohl sedimentation chambers and weighed them to determine the exact counting volumes. We identified and counted phytoplankton genera using a Wild inverted microscope at 400x. We counted multiple transects until we recorded 100 algal units (cells, filaments or colonies) of the most common taxa, counting all algal units in at least two transects. We measured the cell dimensions for the first 20 algal units for each
genus enumerated and calculated the mean individual biomass for each taxon (mg wet weight/L). For filamentous algal taxa, however, we measured all filament lengths and summed and recorded them as total filament length for each taxon (Frost and Culver 2001). We calculated the mean cell volume for each taxon present using the algal dimensions for each species in a sample, using volumetric equations that best described the shape of each species. For colonies, we calculated the mean number of cells per colony and multiplied it by the average volume per cell to determine volume per colony. Subsequently, volumes were converted to biomass assuming the specific gravity of phytoplankton to be 1.0 g/cm³ (Munawar and Munawar 1976, Frost and Culver 2001). Consequently, all reported phytoplankton biomasses are wet weights (mg/L). We calculated total biomass of phytoplankton samples by summing the species-specific total biomass over all species present in a given sample. We analyzed phytoplankton data as total biomass and at the taxonomic level of phylum (e.g., Chlorophyta).

Catfish survival and yield

We counted harvested fish by the displacement method and used stocking density and pond volume to estimate catfish survival (%) and yield (g/m³).

STATISTICAL ANALYSES

We analyzed the results of the experiments as a completely randomized block design with repeated measures using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Data were transformed when necessary to meet assumptions of normality.
The treatment structure has a 4 x 11 factorial (4 types of treatments and 11 sampling dates) arrangement. The statistical model underlying the analyses is

$$Y_{ijk} = \mu + T_i + b_j + Tbij + D_k + TD_{ik} + e_{ijk}; \quad i = 1, 2, 3, 4; \quad j = 1, 2, 3, 4; \quad k = 1, 2, ..., 11$$

where $Y_{ijk}$ is the measured response from the $i^{th}$ treatment, the $j^{th}$ block and the $k^{th}$ date, $\mu$ and $T_i$ are fixed parameters such that the average response of the $i^{th}$ treatment is $\mu + T_i$, $D_k$ is the fixed effect associated with the $k^{th}$ date, $TD_{ik}$ is the fixed effect associated with the interaction of the $i^{th}$ treatment and the $k^{th}$ date, $b_j$ is the random effect associated with the $j^{th}$ block, $Tbij$ is the random interaction effect associated with the $i^{th}$ treatment and the $j^{th}$ block, and $e$ is the random error associated with the $j^{th}$ pond in the $i^{th}$ treatment on the $k^{th}$ date. A pond within a block is the experimental unit and sampling date is the repeated measure. We included fixed effects of treatments, sampling dates, and treatment x date interaction and the random effects of blocks in the model. We used Kenward Roger specification in the model statement to estimate degrees of freedom. The RANDOM statement accounted for the random effect of the block and the REPEATED statement accounted for the effect of sampling date; the error term in the REPEATED statement was block*treatments. We used the covariance structure that yielded the lowest Akaike’s information criterion for repeated measurements (Littell et al. 1998). We set the significance of the F-test of fixed effects at $P \leq 0.1$ and discussed trends when $0.1 < P \leq 0.15$. When protected by the F-test ($P \leq 0.10$), least squares means for fixed effects were separated using the PDIFF statement in SAS and deemed significant at $P \leq 0.1$. 

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RESULTS

Macrophytes

Of the twenty-five species of plants identified from the experimental ponds (Table 2.1), only 3 are reported on the manufacturer’s list of susceptible plants to be fully controlled by AVAST (SePRO Corporation, Carmel, IN, USA). As initial macrophyte coverage was not uniform with respect to species composition and density in the experimental ponds (Table 2.2), interpretation and statistical analysis of treatment results are difficult. Nevertheless, based on the overall percent change in biomass (Table 2.2), we conclude that fluridone + copper treatment was more effective than the fluridone-only treatment in controlling macrophytes in Hebron ponds, probably because the macrophytic alga *Chara* is known to be controlled by copper but not by fluridone.

Fluridone dissipation in catfish ponds

Initial fluridone concentrations in the 8 treated ponds ranged from 0.33 mg/L to 0.07 mg/L (on DAT 1, Fig. 2.1). The herbicide concentrations rapidly declined to concentrations ranging from 0.008 mg/L to 0.002 mg/L on DAT 79. Fluridone half-life was computed for each treated pond, and varied from a maximum of 10.8 days to a minimum of 1.6 days in the ponds.

Copper speciation in catfish ponds

The mean total copper concentration (T-Cu) ranged from 0.24–0.53 mg/L during the days of application and was significantly higher than the concentrations in the control
and fluridone-only ponds (0.007-0.010 mg Cu/L; p=0.0002) (Fig. 2.2a). Although the T-Cu concentration in the treated ponds decreased the following week after application (0.05-0.02 mg Cu/L), it still remained significantly higher than the control ponds (p<0.0001).

The dissolved copper (D-Cu) fraction constituted < 60% of the T-Cu measured in the treated ponds, with mean concentration (0.28-0.14 mg Cu/L) during the days of application being significantly higher than in the control and fluridone-only ponds (0.01-0.002 mg Cu/L; p=0.0002) (Fig. 2.2b). The week following copper application, D-Cu concentrations dropped, but remained significantly remained higher than D-Cu in control ponds (p<0.0001).

The background levels of free copper ion activity expressed as pCu (= -log Cu^2+) as measured in the control and fluridone-only ponds ranged from 13.72 (=1.91x10^{-14}M) to 12.34 (= 4.57 x10^{-13}M). In the copper-treated ponds, we found significantly higher p-Cu values (7.73 (=1.86x10^{-8}M) to 8.99 (=1.02x10^{-9}M)) during the days of application, but constituted 0.4-0.06 % of the total D-Cu (p<0.0001; Fig. 2.2c). pCu values in the copper-treated ponds dropped the week following application, but remained significantly higher than background levels (p=0.0001).

Effects of fluridone and copper sulfate on water quality parameters

Mean pH varied from 7-9 in the catfish ponds with control ponds tending to have higher pH than copper and fluridone-treated ponds (Fig. 2.3, Table 2.3). Although we did not find a significant treatment effect (p=0.1510), a significant sampling date effect on pH was detected (p<0.0001).
We observed high fluctuations in surface dissolved oxygen concentrations in the ponds during the first five weeks, but they subsided thereafter (Fig. 2.4a). The mean bottom dissolved oxygen concentrations also showed a similar trend but the oscillations were less pronounced than surface dissolved oxygen concentrations (Fig. 2.4b). Copper-treated ponds tended to have higher surface and bottom dissolved oxygen concentrations among the four treatments (Table 2.3). We detected a significant treatment effect (p=0.0777), a sampling date effect (p<0.0001) and a treatment x time interaction (p=0.0129) for surface dissolved oxygen concentrations. However, we only detected a significant sampling date effect (p<0.0001) on the mean bottom dissolved oxygen concentration.

Mean Secchi depth across treatments ranged from 30 to 80 cm during the experimental period. We found a significant treatment effect (p=0.0160), a sampling date effect (p<0.0001) and a treatment x time interaction effect (p=0.0601) on Secchi depth. Copper-treated ponds had significantly lower Secchi depth readings (Table 2.3; Fig. 2.5) than control ponds (p=0.0166), fluridone only ponds (p=0.0032) and fluridone + copper ponds (p=0.0148).

The average temperature in the catfish ponds fluctuated between 22 and 31°C during the culture season (Fig. 2.6). The lowest temperature of 20.9°C was recorded towards the end of the culture period.

Effects of fluridone and copper sulfate on phytoplankton dynamics

Dominant algal genera included *Ankistrodesmus, Actinastrum, Chlamydomonas, Crucigenia, Kirchneriella, Scenedesmus, Cyclotella, Nitzschia, Synedra, Chroomonas,*
Rhodomonas, Cryptomonas, Aphanizomenon, Anabaena, Chroococcus, Microcystis and Oscillatoria. The total phytoplankton wet biomass (Fig. 2.7) over the entire twelve weeks remained unaffected by treatments \( (p=0.2633) \); however, we found that the total phytoplankton biomass in fluridone-treated ponds during the first seven weeks following chemical application was significantly lower than control and copper-treated ponds \( (p=0.0974; \text{Table 2.3}) \).

We observed drastic changes in the phytoplankton community composition in response to fluridone and copper sulfate treatments (Fig. 2.8). We detected significant differences in Chlorophyta biomass \( (p<0.0001) \), Cyanophyta biomass \( (p=0.0083) \) and Chrysophyta biomass \( (p<0.0001) \) among treatments, but not on Cryptophyta biomass \( (p=0.6424) \).

The application of fluridone and copper sulfate affected the Cyanophyta community. Pair-wise comparisons show that copper only, fluridone only and fluridone + copper-treated ponds had significantly lower Cyanophyta biomass than control ponds (Table 2.3; Fig. 2.9). In sharp contrast to the effect on Cyanophyta biomass, copper sulfate had a positive impact on Chlorophyta and Chrysophyta biomass. Copper-only treated ponds had significantly higher Chlorophyta biomass than fluridone only ponds \( (p<0.0001) \) or control ponds \( (p<0.0001; \text{Fig. 2.10a}) \). Combined application of fluridone and copper sulfate also significantly increased Chlorophyta biomass compared to control ponds \( (p=0.0004) \) and fluridone only ponds \( (p=0.0003) \), but not as much as in copper-only treated ponds \( (p=0.0121) \). Similarly, copper-only treated ponds had relatively higher Chrysophyta biomass than other treatments and interestingly, fluridone-only ponds had the lowest Chrysophyta biomass among the treatments (Table 2.3; Fig. 2.10b).
Effects of fluridone and copper sulfate treatments on zooplankton dynamics

The dominant zooplankton groups in the ponds consisted of cladocerans (mainly *Bosmina* sp., and to a lesser extent, *Daphnia galeata mendotae, D. parvula, Ceriodaphnia* sp., and *Diaphanosoma* sp.), copepods (*Acanthocyclops vernalis, Leptodiaptomus siciloides, Mesocyclops edax* and *Skistodiaptomus oregonensis*) and rotifers (*Asplancha* sp., and *Brachionus* sp.). The total zooplankton biomass was similar among treatments (p=0.8551; Fig. 2.11); however, it varied significantly with sampling dates (p<0.0001).

We observed changes in the zooplankton community composition in response to fluridone and copper sulfate treatments (Fig. 2.12). We detected a significant chemical treatment effect on Cladocera biomass (p=0.0002) and Copepoda biomass (p<0.0001). Pair-wise comparisons showed that all copper-treated ponds (copper-only and fluridone + copper) had significantly lower cladoceran biomass than did non copper-treated ponds, suggesting the direct toxicity of copper on Cladocera. (Table 2.3; Fig. 2.13a). Further, fluridone only and control ponds had similar cladoceran biomass (Fig. 2.13a), which confirms the toxic effect of copper on Cladocera.

In sharp contrast, Copepoda attained significantly higher biomasses in copper-treated ponds (copper-only and fluridone + copper) compared to control ponds and fluridone only ponds (p< 0.0001; Table 2.3, Fig. 2.13b). Within Copepoda, we observed differential responses to applications of fluridone and copper sulfate. Calanoid copepod biomass was significantly higher in copper-treated ponds than fluridone-only and control
ponds (p< 0.001)(Table 2.3, Fig. 2.14), but cyclopoid copepod biomass was not (p = 0.3187).

We also detected differential species-specific responses within Copepoda. While the biomass of cyclopoid copepod *Acanthocyclops vernalis* was significantly higher in copper-treated ponds (p = 0.0177) during the initial weeks, the biomass of *Mesocyclops edax* was significantly lower in copper-treated ponds (p=0.0399, Fig. 2.15a-b) However, the biomass of both the dominant calanoid copepods- *Leptodiaptomus siciloides* and *Skistodiaptomus oregonensis* was significantly higher (p<0.1, Fig. 2.16a-b) in copper-treated ponds.

Although the secondary productivity of total zooplankton was unaffected by treatments (p=0.8138), we found significant differences in the secondary productivity of Copepoda (p<0.0001) and Cladocera (p=0.0004) among treatments. Copper-treated ponds had the lowest cladoceran productivity, but had the highest copepod productivity (Table 2.3)

Effects of fluridone and copper sulfate treatments on catfish survival and yield

Catfish survival and yield in the experimental ponds averaged 87% and 99.8 g/m³, respectively. Of the four treatments, fluridone + copper-treated ponds tended to have higher percent survival, yield and number harvested/m³; however we did not find statistically significant differences in any of these variables among treatments (Table 2.3).
The goal of application of chemicals to fish ponds is to maximize fish production using cost-effective and ecologically sound methods. As the use of chemicals is a risky proposition in aquaculture due to possible unwarranted ecological effects, it is imperative to conduct site-specific monitoring to evaluate its effectiveness on target species and possible effects mainly on pond water quality, non-target species and overall fish production.

The purpose of the current study was to elucidate the efficacy and effects of routinely applied herbicide fluridone and the algaecide copper sulfate in catfish ponds at Hebron State Fish Hatchery, Ohio, USA. However, the use of fluridone and copper sulfate is not limited to fish ponds; both are widely used to eliminate nuisance aquatic plants and algae in natural lakes, streams, reservoirs, ponds, canals, etc. (MacKenthun and Cooley 1952, Horne and Goldman 1974, Elder and Horne 1978, West et al. 1979, McKnight 1981, Fox et al. 1994, Smith and Pullman 1997, Sprecher et al. 1998).

Although a wealth of information from laboratory assays exists in literature, a comprehensive examination of the ecological impacts of fluridone and copper sulfate on natural ecosystems is lacking. In order to corroborate the predictions of potential environmental effects of contaminants from laboratory tests, manipulative studies in natural ecosystems are necessary; however lack of replication and suitable controls limits such studies (Effler et al. 1980). In the current study, replicated aquaculture ponds offered the unique opportunity to assess the long-term success (experimental duration of 85 days) and the direct and indirect perturbative effects of environmentally realistic
concentrations of fluridone and copper sulfate in a natural ecosystem as well as to examine the community-level responses to the stress caused by these chemicals. Further, the effect of pesticide mixtures that can lead to synergistic or antagonistic effects, is one of the least studied areas in ecotoxicology (Relyea and Hoverman 2006); there is hardly any published work on the effects of the simultaneous application of fluridone and copper sulfate in aquatic systems. Taken together, the results of this study have yielded meaningful results relevant not only to aquaculture but to other freshwater systems in general, thus significantly contributing to the emerging field of ecotoxicology.

Our experimental results revealed that fluridone applied at the rate of 0.07 mg/L was not completely effective in decreasing the macrophyte biomass in the catfish ponds at Hebron State Fish Hatchery. The effectiveness of fluridone in aquatic systems depends on numerous factors such as initial treatment dosage, time of application, length of exposure, water chemistry, etc. (West et al. 1983, Muir and Grift 1982, Netherland et al. 1997). Further, various environmental fate processes such as photolysis, microbial degradation, adsorption onto particulate matter, etc., can rapidly reduce the target concentrations below nominal values in aquatic systems (West et al. 1979, Muir and Grift 1982, Mossler et al. 1989, Netherland and Getsinger 1995). The reported aqueous half-life of fluridone ranges from 5 to 60 days, with an average of 20 days (West et al. 1983, Osborne et al. 1989). The rapid dissipation of the fluridone (estimated half-life of 10.8-1.6 days) could be one of the reasons for ineffectiveness in our experimental ponds. Further, in the current study, fluridone was applied only once during the 80-day study period. In studies elsewhere, fluridone was applied multiple times which would ensure maintaining target concentrations for longer period of time and thereby increase the
effectiveness of plant control. Also, the huge variation among ponds with respect to initial coverage of macrophytes could have altered the herbicide’s effectiveness.

In addition to its rapid dissipation, fluridone is a selective herbicide that is ineffective against the macroalga Chara (Netherland et al. 1997). Though the biomasses of individual plant species were not estimated quantitatively in this study, we observed high abundances of Chara in many experimental ponds. The application of fluridone and copper proved to be the best treatment combination to decrease macrophyte biomass in the experimental ponds, largely because of the effectiveness of copper sulfate in reducing the biomass of Chara (Murray-Gulde et al. 2002). Further, in a field trial with the aquatic weed, hydrilla, Shearer and Nelson (1999) found that the combination of copper (a contact herbicide) and fluridone (a systemic herbicide) was more effective than fluridone alone in controlling plant biomass. In fact, an increase in the effectiveness of other herbicides such as diquat and endothall, when used in combination with copper has also been reported (Sutton et al. 1972, Pennington et al. 2001). Perhaps the presence of copper increases the uptake of fluridone by plants via increased cell permeability as has been suggested for diquat by Sutton et al. (1972).

Although copper sulfate, the only algaecide approved by USEPA for use in catfish ponds, has been used extensively for decades (Han et al. 2001, Huggett et al. 2001, Liu et al. 2006), very few studies have thoroughly investigated the fate and effects of copper sulfate in aquaculture facilities. Prior studies have shown that copper quickly precipitates from water and concentrations returned to pretreatment levels within a week (Tucker and Boyd 1978, Masuda and Boyd 1993). Using rigorous sampling procedures, McNevin and Boyd (2004) reported the decrease of copper concentrations in treated fish
ponds to pretreatment levels within 48 h. However, in all these studies, only total copper concentrations in the water column were determined, whereas total concentrations in any matrix do not reflect bioavailability (Vulkan et al. 2001) and copper toxicity is a function of free copper (Cu$^{2+}$), not of total copper (Sunda and Guillard 1976).

In the current study, therefore, changes in free copper ion activity were determined in the water column following copper sulfate application at the rate of 1mg/L of pond water. The free copper ion activity ranged from 1.02 x 10^{-10} M to 6.10 x 10^{-8} M in the copper-treated ponds while in the control ponds, free copper ion activity was several orders of magnitude lower (< 10^{-12} M). Our weekly measurements further revealed that free copper ion activity was significantly higher than background levels during the week of non-application, i.e., one week after application.

In accordance with earlier studies (Monteiro et al. 1995, Jak et al. 1996, Le Jeune et al. 2006), the elevated free copper ion activity in the copper-treated ponds, which falls in the range of toxic concentrations for algae (>10^{-6}M to 10^{-11}M) (McKnight et al., 1983), elicited a differential response within the phytoplankton community. Copper treatments reduced Cyanophyta biomass, whereas biomass of Chlorophyta and Chrysophyta increased. On the other hand, biomass of Cryptophyta was unaltered by copper additions, suggesting there is a broad range of sensitivity to copper among algal taxa (Bossuyt and Janssen 2004, Le Jeune et al. 2006), which has been attributed to various copper regulatory mechanisms adopted by different species and differences in water chemistry parameters (Takamura et al. 1989, Janssen and Heijrick 2003).

The observed reduction in the biomass of Cyanophyta, the primary target of algaecidal copper treatment, is consistent with previous reports (Horne and Goldman
1974, Rai et al. 1981, Kerrison et al. 1988, Mann et al. 2002). However, reduction in overall biomass of a major group of plankton does not necessarily mean reduction or elimination of all nuisance species within that group. The extent of toxic effects of copper to aquatic organisms depends on the sensitivity of the organism and on the concentration of copper and its bioavailability. Within Cyanophyta, species-specific responses were observed to copper treatments in the present study. The biomass of *Oscillatoria* sp. declined following copper treatment whereas *Microcystis* sp. was unaffected by elevated copper ion activity. *Microcystis* has the potential to form toxic blooms and can affect dissolved oxygen concentration, pH, extent of light penetration, etc. Thus, the finding of reduction in overall Cyanophyta biomass can be misleading as to the effectiveness of copper treatment in fish ponds and other freshwater aquatic systems.

In response to algaecidal copper sulfate treatment, the phytoplankton community composition shifted towards species tolerant to elevated copper ion activity, which is consistent with the reports from different freshwater environments (Say and Whitton 1980, Moore et al. 1979, Austin et al. 1985, Monteiro et al. 1995). Specifically, in the current study, *Scenedesmus, Ankistrodesmus, Kirchneriella*, solitary chlorophytes and pennate diatoms dominated the phytoplankton community in the copper treated ponds; most of these taxa are known for their tolerance to copper (Van den Berg et al. 1979, Nalewajko et al. 1997).

In spite of differential response of phytoplankton taxa, the total phytoplankton biomass remained unaffected by copper treatments as has previously been shown in metal-polluted lakes (Yan 1979). Total phytoplankton biomass and dynamics in
contaminated environments is best analyzed in the light of community composition data (Roussel et al. 2007). Examination of the effects of contaminants at the community level in natural ecosystems sheds light on both direct and indirect effects of contaminants at different trophic levels, which are overlooked in laboratory or mesocosm experiments. In our pond study, the decrease in copper-sensitive taxa, combined with the indirect effect of decreased grazing pressure by Cladocera might have been instrumental in preserving the total phytoplankton biomass.

In the current study, we observed significant decreases in Cyanophyta biomass in ponds treated only with fluridone 2 weeks after application. Similar results were obtained by Parka et al. (1978) and Arnold (1979) in their pond experiments. Kamarianos et al. (1989) reported elimination of blue-green algae in a carp pond following one time application of fluridone at the rate of 0.042 mg/L. Mille et al. (1990) found a significant decrease in algal biomass, chlorophyll-a, and total carotenoid contents in axenic cultures of Oscillatoria agardhii Gomont when exposed to increasing fluridone concentrations (0 to 0.1 mg/L). Other laboratory experiments have also shown reduction of chlorophyll in some greens and euglenoid algae (Vaisberg and Schiff 1976, Sandmann 1984).

In addition to reduction in Cyanophyta biomass, we observed decreases in total phytoplankton for seven weeks following fluridone application, which suggest the algaecidal potential of fluridone. While fluridone may have had direct toxicity on phytoplankton in the first seven weeks after treatment, the algal population showed recovery later in the culture season, perhaps from the nutrients released from decaying macrophytes. Slijkerman et al. (2005) found that the application of linuron reduced
macrophyte abundance but increased green algae abundance in a macrophyte-green algae dominated system.

Contradictory findings exist in literature regarding the effects of fluridone on total phytoplankton biomass in natural ponds. Arnold (1979) reported reductions in total phytoplankton by more than 50% in one pond treated with 1mg/L fluridone. Similarly, Kamarianos (1989) observed drastic reductions in total phytoplankton density in a carp pond following one time application of fluridone at 0.042 mg/L. However, many authors found no evidence for any effect of fluridone on total phytoplankton in pond experiments (Leva and Lembi 1978, Parka et al. 1978). More recently, Struve et al. (1991) reported that two applications of fluridone at the rate of 0.125 mg/L made 25 days apart in 20 m³ isolated columns of water did not affect phytoplankton densities or chlorophyll-a.

Perhaps, in addition to fluridone application rates, the differences in macrophyte coverage and half-life of fluridone between ponds could account for the differences in the effects of fluridone on phytoplankton between studies. Moreover, none of the previous studies had as many replicates as we have in the current study, nor did they quantify the macrophyte abundance in the ponds.

Though examination of natural communities for pesticide effects would yield meaningful results, interpretation of results from complex communities with many confounding trophic links is difficult. Even more confounding is the interpretation of effects of a mixture of toxicants on natural communities, as found in our study.

Here, the response of Chlorophyta and Chrysophyta in fluridone + copper- treated ponds was different from copper-only ponds. The dramatic increase in chlorophytes and chrysophytes in copper-only ponds following copper treatment was not observed in
ponds treated with both fluridone and copper. Possibly, the antagonistic action of fluridone and copper prevented significant increases in copper-resistant species within the two groups. We speculate that the algaecidal potential of fluridone may have been strong enough to suppress the proliferation of copper-resistant species. Also the nutrients released during macrophyte decay following fluridone treatment may have influenced species interactions and the relative success of different phytoplankton species.

The response of zooplankton community to copper additions was similar to that of the phytoplankton community. Though the total zooplankton biomass was unaffected by copper additions, differences in sensitivity of zooplankton groups were observed. In the current study, Cladocera was the most sensitive group of zooplankton to copper. This response is in accordance with findings of other authors (Jak et al. 1996, Yan et al. 2004). In sharp contrast, Copepoda biomass increased dramatically in copper-treated ponds, which reveal their tolerance to copper. Within Copepoda, the biomass of calanoid copepods increased significantly in the copper-treated ponds, but cyclopoid biomass did not. This not only shows the higher copper tolerance of calanoids but also their superior ability to compete with other zooplankton groups at least in the absence of cladocerans. The biomass of rotifers was unaffected by the chemical treatments.

Species-specific responses to copper treatment were observed within each major group of zooplankton. Within Cladocera, direct toxicity of copper was observed on all the species (Bosmina sp., Daphnia parvula, Daphnia galeata, Diaphanosoma sp.) in the copper-treated ponds relative to controls. The high sensitivity of daphnids to copper has been confirmed by different studies (de Oliveira-Filho et al. 2004, Nor 1987, WHO 1998). Laboratory toxicity tests have shown that 48 h-E (L) C₅₀s (effective or lethal
concentration that can kill 50% of the population) can be as low as 0.007 mg/L for some daphnids (Oikari et al. 1992). Among cyclopoid copepods, the biomass of Mesocyclops edax was significantly reduced in copper-treated ponds compared to controls whereas Acanthocyclops vernalis showed some initial tolerance to copper but declined in abundance in all the ponds including controls. The two calanoid copepod species, Leptodiaptomus siciloides and Skistodiaptomus oregonensis became dominant in the copper-treated ponds by the 6th week of treatment. The resistance of copepods and the sensitivity of Cladocera to copper observed in the current study are consistent with results obtained by Leland and Kent (1981) in natural plankton communities of a stream and in copper-rich mine effluents (Oliveira 1985). Thus, copper acted as a “community-structuring agent” (Blanck et al. 1998), facilitating the shift in the plankton community composition to copper-resistant species, which in turn is a reliable indicator of toxic stress (Gray, 1989).

In the current study, fluridone treatment had no obvious effects on the zooplankton community. Hamelink et al. (1986) reported an acute median lethal concentration (LC50) of 4.3 mg/L of fluridone for aquatic invertebrates. However, in chronic studies, they observed no effects when daphnids (Daphnia magna) were exposed to fluridone concentrations of 0.2 mg/L. Kamarianos et al. (1989) reported no changes in zooplankton population in a fish pond following application of 0.042 mg/L of fluridone. Hamelink et al. (1986) based on laboratory toxicity assays, posited that a target concentration of 0.1 mg/L, based on the recommended application rate, will not affect Daphnia magna or similar non-target aquatic organisms. Our finding reveals that
fluridone applications at higher concentrations also do not affect the zooplankton community; the highest concentration recorded in our study was 0.35 mg/L.

We did not find significant changes in dissolved oxygen concentrations or pH following fluridone treatments, in agreement with earlier studies (Struve et al. 1991, Arnold 1979). Fluridone is a slow-acting herbicide, so does not cause sudden die-offs of plants and hence will not detrimentally affect dissolved oxygen concentrations in ponds (Arnold 1979, McCowen et al. 1979). Moreover, in the present study, even though the plankton community composition was changed following copper and fluridone treatment, the total phytoplankton biomass remained unaffected which might have prevented catastrophic decline in dissolved oxygen levels in the ponds.

We also found no significant differences in fish yield or survival across treatments. Various studies have shown that fluridone when applied at the recommended maximum application rate of 0.15 mg/L has an adequate margin of safety for fish (Hamelink et al. 1986, Kamaranos et al. 1989, Paul et al. 1994). Likewise, copper sulfate, when typically applied at about 1% (w/w) of the total alkalinity of pond water (i.e., at the rate of 1 mg CuSO₄.5H₂O/L in our ponds given that the total alkalinity ranged from 80-110 mg/L (Culver et al. 1993)) is not likely to be toxic to fish (Boyd, 1990). However, toxicity of copper to fish is species and life-stage-specific and depends on various physico-chemical properties of water including pH, alkalinity, hardness, dissolved organic matter, temperature, etc. Straus and Tucker (1993) reported 96-h LC₅₀ of 0.05 mg Cu/L at 16 mg/L of total alkalinity (as CaCO₃) and 0.95 mg Cu/L at 127 mg/L of total alkalinity for fingerling channel catfish. Similar inverse relationships among copper toxicity, water temperature and water hardness have been suggested for
channel catfish (Perchbacher and Wurts 1999, Perschbacher, 2005). Therefore, monitoring of water quality parameters before and after copper sulfate application is recommended to avoid fish kills.

Though our study reveals that copper and fluridone treatment did not have negative effect on catfish survival or yield, we recommend site-specific monitoring of macrophytes before choosing and applying herbicides in catfish ponds to avoid unnecessary addition of chemicals, many of which are expensive. Further, application of copper to fish ponds should be done cautiously because of its prolonged persistence in the sediments. Multiple treatments of copper sulfate over years can lead to the accumulation of high levels of copper in the sediment (D.A. Culver, unpublished data). This can be detrimental to the culture of other species of fishes, which may be sensitive to copper, in the same ponds at a different time of the year.

Our experimental results clearly indicate that environmentally realistic concentrations of copper and fluridone can directly and indirectly shape aquatic communities and can affect non-target groups like zooplankton. Since plankton constitute the foundation of aquatic food webs, any changes in their community structure can affect functioning and overall health of the entire aquatic ecosystem. However, one should be cautious when drawing conclusions regarding the effects of chemicals in natural communities. Species interactions can, and do, modify the direct and or indirect effect of toxicants in an ecosystem. For instance, the observed population decrease of a species in response to a chemical may be either due to the direct toxicity of the chemical or due to the negative influence of other tolerant species in the community. Any change in the population dynamics of one species in response to a chemical stressor can affect
food web interactions and trophic structure of the ecosystem which would make the interpretation of results from ecotoxicological studies difficult. Nevertheless, we feel that this study has shed light on the basic effects and responses within a natural aquatic system to the applications of fluridone and copper sulfate. Further, this study emphasizes the importance of examining the effects of toxicants in natural ecosystems as opposed to laboratory assays, which had been the mainstay of the field of toxicology until recently. However, laboratory experiments oversimplify field conditions, where ecological interactions modify the effects of toxicants in numerous ways; ecologically relevant effects including indirect effects on non-target organisms can be elucidated only from ecotoxicological studies such as the one undertaken in this paper. Moreover, knowledge of environmental fate and effects of pesticides in natural ecosystems will aid in formulating management policies and procedures associated with ecological risk assessments. With approximately 63,000 pesticides registered in the United States (Ramade 1988) and many more introduced into the market annually, there is a pressing need for more ecotoxicological studies on natural communities to allow us to better understand the perturbative effects of chemicals added to the aquatic environment.
References


<table>
<thead>
<tr>
<th>No.</th>
<th>Scientific name</th>
<th>Common name</th>
<th>Effectiveness of fluridone</th>
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<tbody>
<tr>
<td>1.</td>
<td><em>Acer rubrum</em></td>
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<td>Milkweed</td>
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</tr>
<tr>
<td>3.</td>
<td><em>Conyza canadensis</em></td>
<td>Canadian horseweed</td>
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</tr>
<tr>
<td>4.</td>
<td><em>Cirsium arvense</em></td>
<td>Canada Thistle</td>
<td>?</td>
</tr>
<tr>
<td>5.</td>
<td><em>Cyperus esculentus</em></td>
<td>Chufa flatsedge</td>
<td>?</td>
</tr>
<tr>
<td>7.</td>
<td><em>Eleocharis obtusa</em></td>
<td>Spike rush</td>
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</tr>
<tr>
<td>8.</td>
<td><em>Eleocharis acicularis</em></td>
<td>Spike rush</td>
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<tr>
<td>9.</td>
<td><em>Fraxinus pennsylvanica</em></td>
<td>Green Ash</td>
<td>?</td>
</tr>
<tr>
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<td><em>Lemna minor</em></td>
<td>Duckweed</td>
<td>C</td>
</tr>
<tr>
<td>11.</td>
<td><em>Najas minor</em></td>
<td>Allioni Bushy Pond weed</td>
<td>C</td>
</tr>
<tr>
<td>12.</td>
<td><em>Phalaris arundinacea</em></td>
<td>Reed Canary grass</td>
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</tr>
<tr>
<td>13.</td>
<td><em>Polygonum amphibium</em></td>
<td>Smart weed</td>
<td>P</td>
</tr>
<tr>
<td>14.</td>
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<td><em>Polygonum pennsylvanicum</em></td>
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</tr>
<tr>
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<td><em>Polygonum punctatum</em></td>
<td>Smart weed</td>
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<tr>
<td>19.</td>
<td><em>Rumex crispus</em></td>
<td>Curly Dock</td>
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</tr>
<tr>
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<td><em>Salix interior</em></td>
<td>Willow</td>
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</tr>
<tr>
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<tr>
<td>22.</td>
<td><em>Setania sp.</em></td>
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</tr>
<tr>
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<td><em>Typha latifolia</em></td>
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<td>25.</td>
<td><em>Chara</em></td>
<td>Algae</td>
<td>NC</td>
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</table>

Table 2.1. List of macrophytes identified in 16 experimental ponds at Hebron State Fish Hatchery in 2004. Based on the manufacturer’s list (AVAST!, SePRO Corporation, Carmel, IN, USA), the effectiveness of fluridone on each species is indicated by ?-Don’t know, C-controlled, P-Partially controlled and NC- Not controlled.
<table>
<thead>
<tr>
<th>Ponds</th>
<th>Treatments</th>
<th>Macrophyte biomass (g/m²) at DAT 0</th>
<th>Macrophyte biomass (g/m²) at DAT 79</th>
<th>Change in biomass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>Control</td>
<td>91.8</td>
<td>102.7</td>
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</tr>
<tr>
<td>C4</td>
<td>Fluridone</td>
<td>318.0</td>
<td>142.7</td>
<td>-55.1</td>
</tr>
<tr>
<td>C5</td>
<td>Copper</td>
<td>13.6</td>
<td>131.7</td>
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<tr>
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<td>Fluridone+Copper</td>
<td>80.6</td>
<td>55.7</td>
<td>-31.0</td>
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<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E3</td>
<td>Control</td>
<td>323.0</td>
<td>213.0</td>
<td>-34.1</td>
</tr>
<tr>
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<td>Fluridone</td>
<td>191.3</td>
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<td>Copper</td>
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<tr>
<td>G8</td>
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<td>6.3</td>
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<td>-100</td>
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<td>45.6</td>
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Table 2.2. Percent change in macrophyte biomass in response to fluridone at 79 days after treatment (DAT) in experimental catfish ponds at Hebron State Fish Hatchery in 2004. Each value represents the mean of 5 quadrats. + indicates increase whereas – indicates a decrease in biomass.
Table 2.3. Treatment means of all response variables, and significance level associated with treatment and date for each variable, as determined by repeated measures of ANOVA. + F indicates fluridone only treatment, + C indicates copper only treatment, + F+C indicate fluridone and copper treatment and -F-C indicates control.
### Response variables

#### Treatment means

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<td>Dissolved oxygen (mg/L)</td>
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<td></td>
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#### Probability values

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#### Biotic variables

**Zooplankton (mg/L)**

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<th>Acanthocyclops vernalis</th>
<th>Mesocyclops edax</th>
<th>Leptodiaptomus siciloides</th>
<th>Skistodiaptomus oregonensis</th>
<th>Calanoida</th>
<th>Copepoda</th>
<th>Total zooplankton</th>
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<td>0.027</td>
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**Secondary Productivity (mg/L/day)**

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**Phytoplankton (mg/L)**

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<th>Chrysophyta</th>
<th>Cryptophyta</th>
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**Fish Production**

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<td>0.59</td>
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Fig. 2.1. Fluridone dissipation in 8 fluridone-treated ponds (pond nos. B4, C4, D2, E4, F5, F6, F7 and F8) over time at Hebron State Fish Hatchery in 2004. The best-fit exponential equation is plotted as a trend line. \( A_t = A_0 \cdot \exp(-k \cdot t) \) is the concentration of the herbicide contained in the pond water at time \( t \), \( k \) is the rate constant (days), and \( A_0 \) is the initial water herbicide concentration.
Fig. 2.2. Seasonal variation in free copper ion activity (pCu = -log Cu$^{2+}$) (2.2a), dissolved copper (2.2b) and total copper concentration (2.3c) in the copper-treated catfish ponds (pond nos. B4, C5, D2, D3, F5, F6, F9, and G7) at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment. The vertical bars refer to standard error associated with the mean. The arrows indicate the dates of copper sulfate application at the rate of 1 mg CuSO$_4$.5H$_2$O/L.
a) Date
6/25 7/1 7/8 7/15 7/22 7/29 8/19 8/26

pCu
0 2 4 6 8 10 12 14

Control
Copper
Fluridone
Fluridone+Copper

Total Dissolved Copper (mg/L)

b) Date
6/25 7/1 7/8 7/15 7/22 7/29 8/19 8/26

Total Copper (mg/L)
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8

Control
Copper
Fluridone
Fluridone+Copper

c) Date
6/25 7/1 7/8 7/15 7/22 7/29 8/19 8/26

Total Copper (mg/L)
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8
Fig. 2.3. Seasonal variation in pH in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.4. Seasonal variation in dissolved oxygen concentration at the surface (2.4a) and bottom (2.4b) of the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Dissolved oxygen (mg/L)

Control
Copper
Fluridone
Fluridone+Copper

Date
1-Jul 6 8 15 22 29 5-Aug 12 19 26 2-Sep 10 17

Dissolved oxygen (mg/L)

Control
Copper
Fluridone
Fluridone+Copper

Date
1-Jul 6 8 15 22 29 5-Aug 12 19 26 2-Sep 10 17
Fig. 2.5. Seasonal variation in Secchi depth (cm) in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.6. Seasonal trends in mean temperature (at 6 hour intervals) in experimental catfish ponds at Hebron State Fish Hatchery in 2004.
Fig. 2.7. Seasonal variation in mean total phytoplankton biomass (wet weight) in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.8. Effects of chemical treatments on phytoplankton community composition in the experimental ponds at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates.
Fig. 2.9. Seasonal variation in mean Cyanophyta biomass (wet weight) in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.10. Seasonal variation in mean Chlorophyta (2.10a) and Chrysophyta (2.10b) biomass (wet weight) in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicate ponds for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.11. Seasonal variation in mean total zooplankton biomass (dry weight; mg/L) in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.12. Effects of fluridone and copper sulfate treatments on zooplankton community composition in the experimental catfish ponds at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment.
Fig. 2.13: Seasonal variation in relative dry weight biomass of Cladocera (2.13a) and Copepoda (2.13b) in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.14. Seasonal variation in relative dry weight biomass of Calanoida in the ponds subjected to three chemical treatments (fluridone-only (pond nos. C4, E4, F7, and F8), copper-only (pond nos. C5, D3, F9, and G7), and fluridone + copper (pond nos. B4, D2, F5, and F6)) and control ponds (pond nos. B3, E3, G6, and G8) during catfish culture at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.15. Response of two cyclopoid copepods- *Acanthocyclops vernalis* (2.15a) and *Mesocyclops edax* (2.15b) (dry weight; mg/L) to fluridone and copper sulfate treatments in catfish ponds at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment. The vertical bars refer to standard error associated with the mean.
Fig. 2.16. Response of two calanoid copepods—*Leptodiaptomus siciloides* (2.16a) and *Skistodiaptomus oregonensis* (2.16b) (dry weight; mg/L) to fluridone and copper sulfate treatments in catfish ponds at Hebron State Fish Hatchery in 2004. The values for each date are the mean from four replicates for each treatment. The vertical bars refer to standard error associated with the mean.
CHAPTER 3

THE EXTENT AND EFFECTS OF COPPER CARRY-OVER IN DOUBLE-CROPPED
AQUACULTURE PONDS

INTRODUCTION

Copper is an essential micronutrient and a component of numerous enzymes involved in many important physiological processes in both eukaryotes and prokaryotes (Flemming and Trevors 1989, Scheinberg 1991, de-Oliveira-Filho et al. 2004). Although a vital element, copper has been long recognized as a toxicant to biota at elevated concentrations, hence the widespread use of copper as an algaecide, fungicide, bactericide, herbicide, molluscicide and therapeutant in aquatic systems (WHO 1993, Xue and Sunda 1997, Mastin and Rogers 2000, de-Oliveira-Filho et al. 2004, Roussel et al. 2007).

A copper-based product, copper sulfate has been widely used as an algaecide for over a century in channel catfish culture to limit the occurrence of nuisance algal blooms especially those of Cyanobacteria (Reimer and Toth 1970, Tucker and Boyd, 1978, Han et al. 2001, Huggett et al. 2001, McNevin and Boyd 2004, Liu et al. 2006, Liu and Zhao, 2007). Supplemental feeding of catfish with feeds rich in nitrogen and phosphorus and timing of fish culture through the warm summer months together almost guarantee the occurrence of cyanobacterial blooms in catfish ponds (Boyd 1974, Schrader and Dennis
2005). As cyanobacterial blooms can lead to deteriorated water quality conditions such as hypoxia, low water clarity, reduced fish growth, off-flavor in fish, etc., the use of an algaecide is inevitable in catfish ponds (Tucker and Boyd 1978, Zimba et al. 2002, Schrader and Dennis 2005). To date, copper sulfate is the only USEPA-permitted algaecide for use in catfish ponds (USEPA, 2003). Considering the enormous growth of catfish industry in USA over the years from using 1000 hectares in the 1960s to more than 64,750 hectares in 2007 and the recent implementation of effluent limitation guidelines for concentrated aquatic animal production facilities that include aquaculture facilities, the fate and effects of routinely applied chemicals including copper sulfate have received enormous attention (Boyd et al. 2000, Liu et al. 2007, USDA 2007, USEPA 2004). However, the long-term effects of copper sulfate application to catfish ponds are unknown, especially in “double-cropped” ponds.

Double-cropping of fishes refers to the use of the same pond to raise two species of fish at different times of the year and is a common fish management practice in many fish hatcheries. The application of chemicals in double-cropped ponds may result in chemical residues remaining in the sediment from one species’ culture that could detrimentally affect the culture of another species of fish. However, the carry-over effect of these chemicals from one cropping to the other has received insufficient attention in aquaculture research.

The carry-over effect of copper sulfate in double-cropped ponds demands special attention for three main reasons: 1) Persistence- Copper from algaecide treatments is short-lived in the water column and eventually sinks into the sediments (Button et al. 1977, Huggett et al. 2001, Zimba et al. 2002). Because copper, like other trace metals,
does not degrade, repeated applications of copper sulfate within and over years to ponds during catfish culture can lead to the accumulation of high levels of copper in the sediment (Han et al. 2001, Huggett et al. 2001, Zimba et al. 2002, Liu et al. 2006). The persistence of copper in the sediments can be detrimental to the culture of more copper-sensitive fish species in the same pond, at a different time of the year. 2) Environmental mobility- Trace metals can move from one environmental compartment to another; for example, copper can move from the sediment into the overlying water by physical processes such as advection, turbulent mixing, diffusion, etc., by biological process such as bioturbation, and by geochemical processes such as adsorption/desorption and precipitation/dissolution. Indeed, oceanographic and limnological studies indicate that trace metals like copper are remobilized from the sediments into the overlying water column, depending on the physico-chemical characteristics (pH, redox potential, organic matter, etc.) at the sediment-water interface (Klinkhammer 1980, Fischer et al. 1986, Prepas and Murphy 1988, Paulson et al. 1991, Balistrieri et al. 1992). Any remobilization of copper into the water column from the sediments of double-cropped ponds poses a risk to the culture of fish at a different time of the year. 3) Toxicity- Copper can be potentially toxic to the biota in an aquatic system even at low concentrations, and its bioavailability is governed by its speciation in the environment. In natural waters, copper exists mainly as: 1) the free ionic form (Cu^{2+}), 2) various complexes, and 3) sorbed onto particulates, including biological components. In the aqueous phase, copper speciation is regulated by processes such as inorganic and organic complexation, precipitation and biological uptake, but the free ionic form (Cu^{2+}) is the most bioavailable and hence the most toxic form to biota (Pagenkopf et al. 1974, Sunda
Free copper ion activity as low as $10^{-10}$ M has been found to be toxic to algae (McKnight et al. 1983). Gagneten et al. (2001) showed that population dynamics of a zooplankton species was significantly affected at concentrations as low as 5 µg/L ($7.87 \times 10^{-8}$ M) of copper. However, very few field studies have been conducted to investigate the direct and indirect effects of low concentrations of copper on the aquatic biota.

Ohio’s State Fish Hatcheries have been double-cropping channel catfish ($Ictalurus punctatus$) and larval percids (walleye [$Sander vitreum$], saugeye [walleye $♀ \times$ sauger $♂ Sander canadensis$] for many years. Generally, ponds are used sequentially to raise channel catfish in summer (late June-Sept) and larval percids in spring (April-May). Copper sulfate is routinely applied during catfish culture to curtail the growth of nuisance algae. It is typically applied at the rate of 1% (w/w) of the total alkalinity several times during the culture period (Boyd, 1990). For example, if the alkalinity is 100 mg/L as CaCO$_3$, then copper sulfate (CuSO$_4 \cdot 5$H$_2$O) is added at the rate of 0.99g/m$^3$ (2.7 lbs/acre-foot) to achieve the target concentration of 1 mg CuSO$_4 \cdot 5$H$_2$O/L (equivalent to 0.25 mg Cu/L). It is estimated that nearly all of the applied copper sinks into the sediment (Liu et al. 2006), which may pose a risk for the culture of larval percids in April.

Superior water quality, high algal productivity, and an abundant zooplankton forage base are prerequisites for successful culture of the larval, planktivorous percids that are used to stock inland waters to enhance sport fishing. Inconsistent fish survival and yield observed in the recent years has made it difficult for fish managers to implement their annual stocking plans in Ohio’s waterways (Tew, 2003). Conventionally, factors such as over-fertilization, low dissolved oxygen concentration,
high pH, unionized ammonia, excessively high fish stocking density, cannibalism, etc., are identified to explain the inconsistent percid production at the hatcheries (Loadman et al. 1986, Loadman et al. 1989, Tew 2003)

Recently, however, at Hebron State Fish Hatchery, Ohio, double-cropped ponds were found to have almost five times higher sediment copper concentration than single-cropped ponds (ponds that were used solely for percid culture) (Tew, 2003). The high copper concentration in the sediments of double-cropped ponds correlated with lower percid percent survival, leading to the speculation that sediment-bound copper resulting from copper sulfate applications during catfish culture may be negatively impacting percid culture in double-cropped ponds (Tew, 2003)

Fish production data from 2000-2004 reveal a decline in percid survival, number harvested/m$^3$ and yield (g/m$^3$) at Ohio’s Senecaville State Fish Hatchery (Fig. 3.1). Low algal productivity and low zooplankton abundance have been observed during percid culture in Senecaville ponds (Todd Beisser, Ohio Division of Wildlife, pers. communication). Copper sulfate has been extensively applied to Senecaville ponds over the years as an algaecide and as a therapeutant for successful catfish culture in summer. Double-cropped aquaculture ponds at Senecaville offer the opportunity to examine the persistence, environmental mobility and toxicity of low-levels of copper in freshwater aquatic systems. The specific questions we sought to answer in this study were: 1) Is there a high copper concentration in the sediment? 2) Will the copper-loaded sediment act as a source for copper for the overlying water at a later time? 3) How does resuspended copper speciate in the water column? 4) Will low levels of copper have any
effect on biota? In order to answer these questions, we used a combination of field and laboratory experiments.

Field investigation of disturbed ecosystems can shed useful information on the fate and extent of direct and indirect effects of toxicants on natural communities. However, such investigations, especially of sub-lethal effects of toxicants such as copper in natural ecosystems where abiotic and biotic interactions significantly affect their bioavailability and toxicity can be challenging. Manipulative ecotoxicological studies in natural ecosystems could possibly yield meaningful results; however, high cost and lack of controls and replication greatly limit such studies. Further, field monitoring data may not always yield decisive results establishing the direct and indirect effect of low levels of toxicants like copper on biota. In such circumstances, scaling down the study to simple laboratory experiments can be a first step in focusing on a relevant aspect that can complement field studies and possibly lay the foundation for testable hypotheses in the future. In the current study, along with field investigation, we sought to determine the chronic effect of copper on a native cladoceran species through a laboratory experiment as an initial step toward the goal of understanding the sub-lethal effects of copper on plankton biomass and fish production. In the context of larval planktivorous percid culture, cladocerans are an important dietary item for percids and as such assessment of chronic effects of copper on a cladoceran species in double-cropped pond is appropriate. Further, to the best of our knowledge, chronic toxicity tests with a resident cladoceran species, *Daphnia parvula*, in the surface water of origin are rarely conducted and moreover, chronic toxicity data on *D. parvula* are lacking.
In the current study, using an integrated approach of field investigation and laboratory experiments, we tested the following hypotheses:

1. Copper concentrations are high in the sediments of double-cropped ponds at Senecaville State Fish Hatchery compared to ponds at a reference site.

2. Copper in the sediment gets resuspended into the water column during percid culture and speciates depending on the chemical characteristics of the water column.

3. Depending on the nature of trace metal speciation, copper in the water column may have a direct effect on phytoplankton biomass and a direct or indirect effect on zooplankton biomass and fish production.

The stated hypotheses were tested via the following objectives:

1) Determine the extent of copper accumulation in the sediment

   We measured total copper concentration in the sediment to determine the extent of copper contamination in the sediment from previous applications of copper sulfate during summer catfish culture.

2) Determine whether copper is resuspended into the water column and characterize the speciation of resuspended copper in the pond waters

   Copper speciation in the water column was studied by examining three pools of copper: 1) Total copper, which refers to the sum of the dissolved copper and the copper liberated from the particulate phase during extraction of unfiltered water samples in hot, dilute mineral acid  2) Total dissolved copper, which refers to copper remaining in water
after filtering the samples through a filter with a pore size of 0.45µm, and 3) Free copper, which is the soluble cupric ion (Cu²⁺) measured using an ion-selective electrode.

We measured copper concentrations in the pond waters and in the hatchery’s source water (acts as the control) during the percid culture season, to determine the ‘source’ of copper in the pond waters. We measured the change in the free copper ion (Cu²⁺) activity in the water column thrice in a series of 10 ponds during the percid culture season. Similarly, we measured the concentrations of two operationally defined copper species: dissolved copper and total copper, in a series of 10 ponds during percid culture.

3) Determine the potential effect of resuspended copper on phyto/zooplankton biomass and fish survival and yield

We determined phyto/zooplankton biomass weekly during percid culture season and acquired fish harvest data to examine the potential effect of low concentrations of copper on plankton dynamics and percid survival and yield. We conducted chronic toxicity effects of copper on a resident cladoceran species to determine the direct effect of copper on its reproductive output.

MATERIALS AND METHODS

Experimental Design

In order to test the hypotheses, we conducted field monitoring for copper concentrations in pond waters and sediments at two hatcheries, one acting as the
‘contaminated site’ (Senecaville) with all the ponds suspected to have received heavy copper loadings and with a history of poor fish survival and yield, and the other acting as a ‘reference’ (Hebron), where the ponds are suspected to be less contaminated with copper and with relatively higher fish survival and yield. We also monitored the source water for Senecaville hatchery ponds for copper concentrations, which would shed light on the background levels of copper at the investigation site. A total of 10 ponds were chosen at Senecaville for copper monitoring. As part of another project, these ponds were treated with three phosphorus fertilization rates (weekly restoration of dissolved phosphate to 10, 20 and 30 μg P/L), with constant nitrogen fertilization rate by weekly restoration of inorganic N to 600 μg N/L. However, we believe that these fertilization rates would have a minimal effect on copper dynamics in the ponds.

We determined phytoplankton biomass and species composition weekly in the 10 Senecaville ponds and compared them to data obtained from the source water (Seneca Lake) to examine whether phytoplankton responded to fertilization with phosphorus and nitrogen. We also determined zooplankton biomass and species composition weekly to investigate whether an adequate forage base was present for larval, planktivorous percids and whether zooplankton abundance at Senecaville was comparable to that at our reference site.

Study site

Field monitoring was conducted at Senecaville State Fish Hatchery and Hebron State Fish Hatchery, Ohio, USA from April-May of 2005 during the larval percid production season. Water for Senecaville hatchery operation was obtained from Seneca
Lake, a mesotrophic reservoir and for Hebron hatchery operation from Buckeye Lake, a eutrophic reservoir.

Each pond at both hatcheries has a separate filling and draining system and is filled with water about three days prior to stocking of fish. The water is filtered through 0.5-mm mesh screens to prevent introduction of eggs or larvae of undesirable fish. The ponds at Senecaville are approximately 4500 m² in area and contain 4500 m³ of water, while the ponds at Hebron are approximately 3000 m² in area and contain 2500 m³ of water.

We stocked 3- to 5- day-old saugeye fry at an average density of 33 fish/m³ at Senecaville and at 53 fish/m³ at Hebron and drained the ponds to harvest fingerlings after 4-5 weeks.

Sampling and Analytical procedures

Determination of the extent of copper accumulation in the sediment

Total copper concentrations in sediments: We collected and air-dried sediments (0.5 cm of top layer) for total copper measurements from 3 random locations in each pond at Senecaville after percid culture. We then homogenized the air-dried sediments and digested ~ 0.5 g with 10 ml of concentrated nitric acid until the production of brown fumes stopped. After cooling, we diluted each digestion tube to 75 ml with double-deionized water and mixed thoroughly. After suspended particles settled out (~16 h), we transferred the top 70 ml of solution into 120 ml Nalgene bottles and filtered each sample for Inductively Coupled Plasma Atomic Emission Spectroscopy analysis.
Because we had data on sediment copper concentration from ponds at our reference site (Hebron) from 2004, we did not sample sediment at Hebron in 2005.

Determination of the speciation of resuspended copper in the water column

Water sampling: We sampled water samples for copper measurements in polyethylene bottles after rinsing each bottle with water from that pond prior to taking the sample. We took water samples from three different locations (shallow end, middle, and deep end) in the pond with an integrated tube sampler, and composited them for each pond and date. We kept the samples in a cooler with ice and transported them to the laboratory within 8 h, where we partitioned each water sample into 3 sub-samples: 1) Unfiltered, unacidified sample for free copper ion measurements, 2) Filtered, acidified (using 20% HNO₃) sample for total dissolved copper measurements, and 3) Unfiltered, acidified sample for total copper measurements. We stored all samples at 4°C until analysis.

Measurement of free copper ion activity: We measured free copper ion activity in water using a copper ion selective electrode (Cu-ISE, ORION 9429) coupled with a reference electrode (ORION 900200) at 25°C. We polished the Cu-ISE for 30 s with aluminum oxide strips before use each day. We changed the reference electrode outer filling solution (Orion 90003) and inner filling solution (Orion 90006) daily. We soaked both electrodes successively for 10 min in 0.025M HNO₃ and 0.1 M Na₄EDTA before use. We prepared the calibration buffers with 1mM IDA (iminodiacetic acid), 6mM NaOH, 2.5mM KHC₈H₄O₄ (potassium acid phthalate) and 0.01M CaCl₂. We varied the pH of the calibration buffer by incremental additions of nitric acid and recorded the
millivolt readings. After calibration, we recorded the millivolt readings in the samples after they became stable (±0.3 mv for 3 minutes). We plotted the electrode potential determined by Cu-ISE against free copper ion activity (which is frequently reported as the negative logarithm of the molar concentration \([pCu = -\log (Cu^{2+})]\)) estimated from published values of pH and corresponding pCu for the same calibration buffer that we used (Sauvé, 1999).

Total dissolved and total copper measurements: We analyzed total dissolved copper and total copper (after hot acid digestion) in the pond waters samples using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

Monitoring of Plankton Biomass and Composition

Zooplankton Biomass and Community Composition Analysis: We sampled zooplankton using a metered (Model 2030R, General Oceanics, Inc., Miami, Florida), 0.5-m diameter, 64-µm mesh net fitted with a canning jar on the cod-end and mounted on a wooden pole. We towed the net from a row boat across the pond and raised and lowered it repeatedly to obtain a depth-integrated sample. The samples were concentrated with a sieve (64-µm mesh), transferred to labeled screw-cap plastic cups and preserved in the field in 4% formalin-sucrose solution (Haney and Hall 1973). The volume of the water column filtered by the net for each sample was calculated by multiplying the cross-sectional area of the net (0.196 m²) by the distance traveled (m) calculated from flow meter readings.

Zooplankton enumeration followed Kane (2004). Briefly, we diluted each concentrated sample to a known volume (~1500 to 9000 ml) and identified and counted
all zooplankton taxa (rotifers, cladocerans and copepods (cyclopoids and calanoids)) in two or more sub-samples (with a total volume ranging from 5 to 50 ml) to genus and species (when possible) according to Balcer et al. (1984), Brooks (1959), and Wilson and Yeatman (1959) using a Wild dissecting microscope at 50x. We withdrew and analyzed sub-samples from the diluted sample until at least 100 individuals of the most common taxon were recorded. We used a calibrated ocular micrometer to measure the lengths of the first 20 individuals in each genus or species and calculated genus- or species-specific average individual biomass from length-weight regressions (Culver et al. 1985) and the number of individuals per cubic meter. We summed genus or species-specific total biomasses over all taxa to provide a total crustacean zooplankton biomass (mg/L) for a given sampling pond, for a given date. We analyzed zooplankton data as total biomass and at the taxonomic level of class (e.g., Cladocera).

Phytoplankton Biomass and Community Composition Analysis

We collected phytoplankton samples weekly with an integrated tube sampler (10 cm diameter and 1.5 m long) by vertically lowering the open end of the sampler into the pond and then replacing the stopper at the other end to obtain the sample. We poured the collected water into a bucket and took a 250-ml sample, which was preserved in a glass jar with Lugol’s solution. In the laboratory, we mixed each sample thoroughly and poured it into a 250-ml graduated cylinder and allowed it to settle for 3 days in a dark chamber. We then concentrated each sample to 30 ml by siphoning off 220 ml from the top and transferring the remaining sample into ~ 40-ml vials. We placed sub samples of 3-6 ml in tared Utermohl sedimentation chambers and weighed them to determine the
exact counting volumes. We identified and counted phytoplankton genera using a Wild inverted microscope at 400x. We counted multiple transects until we recorded 100 algal units (cells, filaments or colonies) of the most common taxa, counting all algal units in at least two transects. We measured the cell dimensions for the first 20 algal units for each genus enumerated and calculated the mean individual biomass for each taxon (mg wet weight/L). For filamentous algal taxa, however, we measured all filament lengths and summed and recorded them as total filament length for each taxon (Frost and Culver, 2001). We calculated the mean cell volume for each taxon present using the algal dimensions for each species in a sample, using volumetric equations that best described the shape of each species. For colonies, we calculated the mean number of cells per colony and multiplied it by the average volume per cell to determine volume per colony. Subsequently, volumes were converted to biomass assuming the specific gravity of phytoplankton to be 1.0 g/cm³ (Munawar and Munawar 1976, Frost and Culver 2001). Consequently, all reported phytoplankton biomasses are wet weights (mg/L). We calculated total biomass of phytoplankton samples by summing the species-specific total biomass over all species present in a given sample. We analyzed phytoplankton data as total biomass and at the taxonomic level of phylum (e.g., Chlorophyta).

Laboratory Assay of Copper Toxicity to *Daphnia*:

Sampling and preparation of test medium: We sampled water from Seneca Lake (source water for double-cropped fish ponds at Senecaville State Fish Hatchery) in pre-washed polyethylene 20-L cubitainers (Fisher Scientific) after rinsing each cubitainer with lake water prior to taking the sample. We took water samples from three different
locations in the lake with an integrated tube sampler, and composited them. We kept the samples in a cooler with ice and transported them to the laboratory, where we filtered the water through 0.45-μm filters (GE Magna, Nylon membrane filter, Fisher Scientific). We added 3-N-morpholino-propanesulfonic acid at 750 mg/L as a pH buffer to the filtered water. 3-N-morpholino-propanesulfonic acid is reported to be non-complexing for metals and is recommended by the U.S. Environmental Protection Agency (Kandegedara and Rorabacher 1999, USEPA 1991). We then adjusted the pH of the water to ~8.0 with sodium hydroxide and one third of the total volume was then kept aside to serve as control. The remaining solution was spiked with copper to obtain test solutions of two different copper concentrations with approximate free copper ion activity of 10^{-11} and 10^{-10} M. We stored the spiked media and control at 4°C in plastic amber bottles. We equilibrated appropriate volumes of these media at room temperature, 48 hours before use.

Sampling of test organisms: We sampled zooplankton using a 0.5-m diameter, 64-μm mesh net fitted with a canning jar on the cod-end and mounted on a wooden pole. We towed the net across the lake and raised and lowered it repeatedly to obtain a depth-integrated sample. We transferred the sample to labeled screw-cap plastic cups. In the laboratory, we identified *D. parvula* according to Balcer et al. (1984) and Brooks (1959) and isolated several individuals and cultured them in the filtered natural water of origin in a temperature-controlled room (20°C) with light and dark cycle12h:12h. We used the individuals of the third brood progeny for the experiment.

Chronic toxicity test: We performed the experiment following protocol 202 of the Organization for Economic Co-operation and Development with few modifications for
the native cladoceran species. We transferred individually 10 juvenile animals (< 48hr old) per concentration to polyethylene cups containing 50ml of the test medium (i.e., 10 replicates per concentration). We exposed animals to two test concentrations and a control. We carried out the 14-day experiment in a temperature-controlled room (20±1°C) with a light: dark cycle of 12h:12h. We fed the test organisms daily with the alga *Chlorella* sp. (2 x 10^6 cells /test vessel), and renewed the test medium every other day. During medium renewal, we placed the parent individual into a fresh test vessel with the new medium, while the old medium was poured into a counting wheel and scanned under a dissecting microscope to record live juveniles and parent mortality. During the 14-day test, we measured the free copper ion activity and dissolved copper concentration in both the fresh (i.e., stock solution) and used media (Table 3.2.).

Saugeye survival and yield

We counted harvested fish by the displacement method and used stocking density and pond volume to estimate saugeye survival (%) and yield (g/m³).

Statistical Analyses

We compared data from Senecaville ponds, reference site and source water using analysis of variance and analyzed toxicity test data using repeated measures of ANOVA. We used PROC MIXED procedure in SAS (SAS Institute Inc., Cary, NC) to conduct statistical analyses. We set the significance of the F-test of fixed effects at P ≤0.1 and when protected by the F-test (P ≤ 0.10), least squares means for fixed effects were separated using the PDIFF statement in SAS and deemed significant at P ≤ 0.1.
RESULTS

Relative sediment copper content in the Senecaville and reference hatchery ponds

The copper concentration in the sediments from 10 ponds at Senecaville ranged from 301 mg/kg dry weight to 466 mg/kg with a mean concentration of 361 mg/kg (Fig. 3.2). We found significantly lower copper concentrations in the sediment in 11 ponds at the reference site (p <0.1, ANOVA; Fig. 3.2), ranging from 52 to 219 mg/kg (mean = 127 mg/kg).

Copper speciation in pond waters

The seasonal mean total copper concentration (T-Cu) in the pond waters at Senecaville Hatchery during percid culture ranged from 5.4 to 8.6 μg/L, whereas in the source water, the T-Cu was 2.1 μg/L, which suggests the remobilization of copper applied previously during catfish culture from the sediment (Fig. 3.3). At our reference site, the T-Cu in the ponds ranged from 5.6 to 12.5 μg/L (Fig. 3.4).

The seasonal mean dissolved copper concentration (D-Cu) in the pond waters during percid culture at Senecaville ranged from 4.9 to 6.9 μg/L, whereas in the source water, the D-Cu was <1 μg/L, which again suggests the remobilization of copper from the pond sediment (Fig. 3.3). At our reference site, the D-Cu in the ponds ranged from 1.4 to 8.5 μg/L (Fig. 3.4). In Senecaville pond waters, the D-Cu constituted, on average, 83% of the total copper, while at the reference site, D-Cu constituted only half (average of 52%) of the T-Cu.
Further, both the mean total copper and dissolved copper concentration in Senecaville ponds varied temporally (Fig. 3.5).

The free copper ion activity expressed as pCu ranged from 10.7 to 11.1 (1.8 x 10^{-11} to 7.1 x 10^{-11} M) in Senecaville pond waters, whereas at the reference site (Hebron Hatchery), pCu ranged from 11.2 to 12 (6.7 x 10^{-12} to 9.4 x 10^{-13} M) during the first week of saugeye culture (Fig. 3.6). The mean free copper ion (Cu^{2+}) activity in Senecaville pond waters decreased with time; however it remained significantly higher than at the reference site (p < 0.1, ANOVA). At both sites, free copper ion activity constituted < 0.1% of the total dissolved copper.

Phytoplankton community dynamics in Senecaville pond waters vs. Seneca Lake

The major phytoplankton taxa in the ponds during percid culture were Chlorophyta (Actinastrum, Ankistrodesmus, Chlamydomonas, Dictyosphaerium, Scenedesmus, solitary greens), Chrysophyta (Dinobryon, centric and pennate diatoms), Cryptophyta (Chroomonas, Rhodomonas), and Cyanophyta (Aphanizomenon, Microcystis). As we did not find any significant difference among phosphorus treatments on total phytoplankton biomass or individual taxa (p > 0.1, ANOVA), we pooled the phytoplankton biomass of all the ponds and compared it to the lake phytoplankton biomass. Though ponds were fertilized with phosphorus and nitrogen weekly, total phytoplankton biomass of the ponds was significantly lower than the biomass in the source water (Seneca Lake) (Fig. 3.7). In other words, pond phytoplankton failed to respond to fertilization treatments in the ponds. Further, we found that the biomass of individual groups except Cyanophyta was similar between the lake and the ponds (Fig.
3.8). Cyanophyta biomass was significantly lower in the ponds than in the lake (p <0.1). We detected significantly lower phytoplankton biomass in the ponds with higher free copper ion activity (pCu <11) than ponds with lower free copper ion activity on the first sampling date (Fig. 3.9).

Zooplankton community dynamics in Senecaville ponds vs. reference ponds

The total zooplankton biomass in Senecaville ponds remained below 0.05 mg/L (< 100 individuals/liter) throughout the first four weeks of the culture season irrespective of phosphorus treatments (Fig. 3.10). The total zooplankton biomass in our reference site remained above 2.0 mg/L (> 100 individuals/liter) throughout the culture period and was significantly higher than in Senecaville ponds (Fig. 3.10; p<0.1, ANOVA).

The major zooplankton taxa at both locations were copepods and cladocerans; however, the percent contribution of these taxa to total biomass differed between locations. At Senecaville, the biomass was dominated by cyclopoid copepods, accounting for more than 55% of the total biomass during most of the culture period. *Bosmina* dominated the cladoceran community but accounted for less than 20% of the total zooplankton biomass until the final week of culture period, at which time their biomass dramatically increased (Fig. 3.11a). At our reference site, although zooplankton biomass was dominated by cyclopoid copepods initially, cladocerans accounted for more than 50% throughout the culture period (Fig. 3.11b).
Copper effects on the reproductive output of *Daphnia parvula*:

The reproductive output of *D. parvula* in the control was significantly higher than in the test copper additions (p <0.05, ANOVA), suggesting that dissolved copper concentrations as low as 6.3 μg/L and 9.9 μg/L can negatively affect the reproductive output of a cladoceran species (Table 3.2).

Saugeye survival and yield

The saugeye survival and yield in Senecaville ponds averaged 45% and 3 g/m³. Except for percent survival, saugeye yield, number/m³, final fish length and weight at Senecaville were lower than at the reference site (Table 3.1.)

DISCUSSION

Sediments are a repository for contaminants and potentially serve as a source of pollution for the overlying aquatic ecosystem (Calmano et al. 1996, Burton 2002). Copper, routinely added as an algaecide, can accumulate and remain intact indefinitely in the aquaculture pond sediment, posing a risk of copper pollution. About 99% of the copper applied as algaecide gets trapped in the sediment (McNevin and Boyd 2004). The concentration of total copper measured in all the double-cropped ponds at Senecaville (>300 mg/kg) is relatively high when compared to the values obtained in studies elsewhere. Liu et al (2007) found a total copper concentration as high as 200 mg/kg only in one 5-year old catfish pond in Alabama, whereas two other ponds (1-year and 25-years old) had concentrations of 25 and 11 mg/kg, respectively. Nine 0.4 ha earthen ponds in
Thad Cochran Warmwater Aquaculture Centre in Mississippi that received 59 applications of 5.68 kg CuSO$_4$.5H$_2$O/ha over 3 years had a total Cu concentration of 172.5 mg/kg (Han et al. 2001). McNevin and Boyd (2004) found an average total copper concentration of 120 mg/kg in the top 2 cm of sediment in twelve 0.4 ha catfish ponds. Boyd et al. (1994) found an average sedimentary copper concentration of 8.6 mg/kg in 358 freshwater fish ponds. The wide range of total copper concentration in the aquaculture pond sediments in different studies could be attributed to variables such as the rate and the frequency of copper applications, aquaculture management practices such as sediment removal, dredging, etc. Though accurate records of the frequency and rates of application of copper sulfate in Senecaville ponds are not available, our study shows that copper applied over the years has accumulated in the sediment and exceeds the average values found in aquaculture sediments in studies elsewhere.

Further, based on the guideline values provided by the U.S. Environmental Protection Agency (EPA) in its National Sediment Quality Survey (1997) for many toxicants including copper, the total copper concentrations at Senecaville exceeds the specified ‘probable effects level’ (108 mg/kg) and the median value for ‘effects level’ (270 mg/kg). However, the total metal concentration in any matrix does not reflect bioavailability and, therefore, toxicity to flora and fauna. Moreover, when making comparisons with guideline values, variables such as important solid phases that govern copper speciation, physiochemical characteristics and other site-specific conditions need to be considered. The various fractions of the sediment generally analyzed for trace metals like copper are the easily exchangeable fraction, and the oxide-bound, carbonate-bound and organic matter-bound fractions. The easily exchangeable fraction more
closely reflects the bioavailable fraction, and several studies have shown that this fraction is typically less than < 10% of the total copper values in the sediment, suggesting that only very small amounts of copper will be leached into water column (Han et al. 2001, Liu et al. 2007). However, Liu et al. (2007) found that when only a small fraction of the total copper was leachable from fish pond sediments, the percentage of bioaccessible copper (based on physiological based extraction test (PBET)) was as high as 40-85%, suggesting a potential toxicity hazard when such contaminated sediments are ingested by animals. Hence, proper disposal of dredged copper contaminated fish pond sediments is warranted.

Our study further indicated that copper accumulated in the sediment from repeated applications during catfish culture in summer acted as a source of copper for the water column during saugeye culture in spring. Remobilization of trace metals like copper from sediments into the overlying water column resulting from changes in the physiochemical characteristics at the sediment water interface has been widely observed in field (freshwater and marine systems) and laboratory experiments (Elder and Horne 1978, Davison 1985, Balsitrieri et al 1992, Kerner and Geisler 1995, Prepas and Murphy 1988, Petersen et al. 1997, Simpson et al. 1998, Ahn et al. 2003, Van Hullebusch et al. 2003, Van Greithyusen et al. 2005). For example, oxidation of reduced sediment phases such as sulphides may release trace metals such as copper into the water phase. In the current study, the observed temporal changes in the dissolved metal concentration in the water column may have been caused by the temporal changes in the solid phases of the pond sediments, which have been reported in other systems such as floodplain lake sediments subject to seasonal flooding (Van Griethuysen et al. 2005). However, further
investigation of the important solid phases that govern copper speciation and the temporal processes that regulate copper mobilization at sediment-water interface in aquaculture pond sediments need to be conducted to fully understand the risk of copper pollution to the overlying water column.

In the current study, though the concentration of dissolved copper in Senecaville ponds was lower than at the reference site, the free copper ion activity was higher in Senecaville ponds than in the reference ponds. Given that the source water for Senecaville ponds comes from a mesotrophic reservoir and that for the reference ponds comes from a eutrophic reservoir, the higher free copper ion activity at Senecaville may be in fact due to the lower complexation capacity of Senecaville waters. Xue et al. (1996) compared free copper ion activity in lakes with different algal productivity, and found copper complexation to be weaker in an oligotrophic lake than in two eutrophic lakes, resulting in higher free copper ion activity in the former even when total dissolved copper concentrations were similar between lakes. Strong ligands from algae either as extracellular secretions from living algae or intracellular exudates from dying algae can influence the copper speciation in aquatic systems (McKnight and Morel, 1979, 1980, Xue et al.1996). Our results further suggest the importance of free copper ion activity determinations, to which toxicity to organisms is directly related; however, a majority of published studies only report some operationally defined fraction such as total copper or total dissolved copper. Moreover, regulatory agencies such as the United States Environmental Protection Agency (US EPA) use total dissolved metal concentrations to reflect the bioavailable fractions to develop metals criteria. The range of dissolved copper concentrations in Senecaville ponds does not exceed the ‘criteria maximum
concentration’ (13 μg/l)-a water quality criterion specified by US EPA for copper, a priority toxic pollutant.

However, acute and chronic toxic effects of copper on plankton at such low concentrations in laboratory experiments have been reported. Gagneten and Vila (2001) found significant effects on the population dynamics of *Ceriodaphnia dubia* at copper concentrations of 5 μg/l. Laboratory toxicity tests have shown that 48-h lethal concentrations at which 50% of the organisms die (LC50) can be as low as 7 μg/L for some daphnids (Oikari et al. 1992). Species mean acute value and genus mean acute values calculated by USEPA (2007) from numerous laboratory assays were as low as 2.37 μg/L (*Daphnia pulicaria*) and 4.05 μg/L (*Daphnia*), respectively. Untersteiner et al. (2003) found chronic effects such as decreased swimming velocity of *Daphnia magna* at concentrations of 10 μg/L. Very few studies have reported the chronic effects on zooplankton in relation to free copper ion activity. In laboratory studies, decreased grazing activity in the estuarine copepods *Acartia tonsa*, *A. hudsonica* and *Temora longicornis* has been observed at free cupric ion activities of ~ 10^{-10} M (Sharp and Stearns 1997). Sunda et al. (1987) found negative effects on the survival of *A. tonsa* at a cupric ion activity of 10^{-10} M. However, the copper speciation in estuarine waters and the behavior of zooplankton in the estuarine environment could be drastically different from those found in the freshwater aquaculture system under study here.

Similarly, a free copper ion activity as low as 10^{-10} M can be toxic to some freshwater phytoplankton species (McKnight et al. 1983). Laboratory assays have shown sub-lethal effects such as deflagellation, inhibition of photosynthesis and nitrogen fixation, etc., and even complete growth inhibition to several algal species at
concentrations below 10 µg/L (Elder and Horne 1978, Steeman-Nielsen and Wium-Andersen 1970). Likewise, changes in natural algal communities at copper concentrations below 10 µg/L have been reported (Leland and Carter 1985, Guasch et al. 2002).

It is difficult to use the results from laboratory assays to conclusively establish the potential toxic effects of copper on plankton biomass and species composition in complex, natural ecosystems, especially at the low copper concentrations observed in the ponds in the current study. Nevertheless, our field investigation of plankton biomass suggests the possibility of toxic effects of copper. Liquid phosphorus and nitrogen fertilizers are routinely added to saugeye rearing ponds to enhance phytoplankton growth, which in turn would maintain an adequate zooplankton forage base for growing saugeye. Contrary to the results obtained in earlier aquaculture research (Tew, 2003), phytoplankton biomass in Senecaville did not respond to repeated applications of phosphorus and nitrogen fertilizers. Further, cyanobacterial biomass was lower in Senecaville ponds than in Seneca Lake (source water for the ponds). The high sensitivity of Cyanobacteria to copper has been extensively reported (Horne and Goldman 1974, Rai et al. 1981, Kerrison et al. 1988, Mann et al. 2002). We also found significantly lower phytoplankton biomass in those ponds that had higher free copper ion activity. Based on these field observations, we speculate that copper may have had a negative effect on phytoplankton biomass and species composition. However, further manipulative experiments need to be conducted to establish a cause and effect relationship between copper and changes in phytoplankton biomass and species composition. Also, further investigation needs to be carried out to determine whether lower zooplankton biomass in
Senecaville rearing ponds, atypical of saugeye rearing ponds, can be attributed to direct or indirect effects of copper.

Accordingly, we conducted laboratory experiments to assess the chronic effects of copper on a resident cladoceran species. Cladocerans are ecologically important freshwater invertebrates that are widely used as test organisms in aquatic toxicity tests (Bossuyt et al. 2004). Among cladocerans, *Daphnia magna* Straus has been the choice for toxicology tests for over sixty years, mainly due to their ecological relevance, short life cycle, parthenogenetic reproduction, high fecundity, ease of culturing in the laboratory, etc. (Anderson 1944, Adema 1978, Munzinger and Monicelli 1991, Koivisto 1995, Untersteiner et al. 2003). However, the use of this species for toxicity tests for basic toxicology research and regulatory testing has received much criticism. *Daphnia magna* is not ubiquitously present in all aquatic systems, limiting its use as an “ecologically representative zooplankton species” in toxicity tests (Kovisito et al. 1992). Further, the life-history strategy of producing large clutches of small neonates of large-sized zooplankters such as *D. magna* is quite opposite to the strategy of smaller cladocerans that produce small clutches of larger neonates. Such differences in life-history traits may also affect the organism’s response to a toxicant (Kovisto 1995).

Also, using an indigenous species rather than a surrogate such as *D. magna* in toxicity assays to estimate the potential toxicity of a chemical has been widely cited as better approximating field responses (Rand and Petrocelli 1985, Chapman 1983). Likewise, the use of water from the specific body of water in question as a toxicity test medium rather than the customary use of standardized laboratory water can eliminate some bias while predicting field responses from laboratory toxicity tests (Chapman 1983,
Bossuyt et al. 2004). Finally, laboratory toxicity tests are often criticized for using toxicant concentrations that are rarely encountered in natural environments; thus the use of concentrations that closely match field concentrations can reveal more ecologically relevant information.

In our laboratory experiment, we incorporated the above-mentioned modern approaches in laboratory toxicity tests to determine the effect of copper on the reproductive output of a cladoceran species. We chose *Daphnia parvula*, an indigenous species found in Seneca Lake, as the test organism because the lake provides the source water for the double-cropped ponds at Senecaville and we also used its water as the toxicity test medium. We also strived to match the concentration of copper found in the contaminated ponds as determined by our field measurements. Our experiments revealed that the reproductive output of *D. parvula* is negatively affected at dissolved copper concentrations as low as 6.3 and 9.9 $\mu$g/L. This result indicates the importance of conducting toxicity tests, especially regulatory testing using species other than *Daphnia magna* or *Ceriodaphnia dubia*, to assess the safety of chemicals in aquatic systems and to develop appropriate water quality criteria.

In summary, we found a significant build-up of copper in the sediment from repeated applications of copper as copper sulfate during catfish culture in summer. During juvenile saugeye culture in the next spring season, copper in the sediment was remobilized into the overlying water column, resulting in higher total dissolved copper concentration in the pond waters than the source water. Our chronic toxicity experiment revealed that low copper concentrations observed in the current study can affect population dynamics of a native cladoceran species. More research needs to be carried
out to conclusively establish the negative direct and indirect effect of remobilized copper
to plankton biomass and juvenile saugeye production. Although the ecotoxicology of
copper is too complex to be fully understood using simple laboratory or mesocosm
studies, this study underscores the potential of replicated aquaculture ponds to examine
the *in situ* processes that govern copper accumulation in, and remobilization from, the
sediment, and its speciation, bioavailability, and toxicity to organisms at different trophic
levels.


Table 3.1. Comparison of saugeye production data (mean ± standard error) from Senecaville State Fish Hatchery and Hebron State Fish Hatchery in 2005. Data Source: Todd Beisser, Senecaville State Fish Hatchery and Jim Stafford, Hebron State Fish Hatchery, Ohio Department of Natural Resources.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Concentration 1</th>
<th>Concentration 2</th>
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</thead>
<tbody>
<tr>
<td>Fresh media</td>
<td>1.5x10^{-11}M</td>
<td>5.7x10^{-11}M</td>
<td>3.0x10^{-10}M</td>
</tr>
<tr>
<td>Free copper ion activity</td>
<td>(pCu=10.8)</td>
<td>(pCu=10.2)</td>
<td>(pCu=9.5)</td>
</tr>
<tr>
<td>Dissolved copper concentration</td>
<td>2.0 μg/L (3.15x10^{-8} M)</td>
<td>6.3 μg/L (9.92x10^{-8} M)</td>
<td>9.9 μg/L (1.56x10^{-7} M)</td>
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<tr>
<td>Used Media</td>
<td>8.6x10^{-12}M</td>
<td>2.7x10^{-11}M</td>
<td>1.1x10^{-10}M</td>
</tr>
<tr>
<td>Free copper ion activity</td>
<td>(pCu=11.1)</td>
<td>(pCu=10.6)</td>
<td>(pCu=10.0)</td>
</tr>
<tr>
<td>Dissolved copper concentration</td>
<td>2.1 μg/L (3.31x10^{-8} M)</td>
<td>5.0 μg/L (7.87x10^{-8} M)</td>
<td>8.0 μg/L (1.26x10^{-7} M)</td>
</tr>
<tr>
<td>Reproductive output - No. of live offspring produced/ parent animal alive at the end of the test ± standard error</td>
<td>5 ± 1.0</td>
<td>1 ± 0.7</td>
<td>2 ± 0.8</td>
</tr>
</tbody>
</table>

Table 3.2. Reproductive output of *Daphnia parvula* in control and test concentrations of Cu.
Fig. 3.1. Percid production as percent survival (%; open bar), yield (g/m$^3$; cross-hatched bar) and number harvested/m$^3$ (dotted bar) in all ponds used for percid culture at Senecaville State Fish Hatchery, Ohio from 2000-2004. Data Source: Todd Beisser, Senecaville State Fish Hatchery, Ohio Department of Natural Resources.
Fig. 3.2. Total copper concentration in the sediments (mg/kg) in 10 ponds (1= Senecaville State Fish Hatchery pond no. 21, 2= pond no. 22, 3= pond no. 23, 4= pond no. 24, 5= pond no. 25, 6= pond no. 26, 7= pond no. 27, 8= pond no. 28, 9= pond no. 29, and 10= pond no. 30) at Senecaville State Fish Hatchery after percid culture in 2005. The dotted line indicates the mean sediment copper concentration (127 mg/kg) in 11 ponds (Pond nos. B3, B4, C4, C5, D2, D3, E3, E4, F5, F6, and G6) at Hebron State Fish Hatchery in 2004.
Fig. 3.3. Seasonal mean total water copper (open bar) and dissolved copper (hatched bar) concentration (μg/L) in 10 ponds (1= Senecaville State Fish Hatchery pond no. 21, 2= pond no. 22, 3= pond no. 23, 4= pond no. 24, 5= pond no. 25, 6= pond no. 26, 7= pond no. 27, 8= pond no. 28, 9= pond no. 29, and 10= pond no. 30) at Senecaville State Fish Hatchery during percid culture season from April-May in 2005. The gray bar indicates the total water copper concentration in the source water (Seneca Lake) for Senecaville Hatchery ponds.
Fig. 3.4. Seasonal mean total water copper (open bar) and dissolved copper (hatched bar) concentration (μg/L) in 10 ponds (1= Hebron State Fish Hatchery pond no. B3, 2= pond no. B4, 3= pond no. C4, 4= pond no. C5, 5= pond no. D2, 6= pond no. D3, 7= pond no. E3, 8= pond no. E4, 9= pond no. F5, and 10= pond no. F6) at Hebron State Fish Hatchery during percid culture season from April-May in 2005.
Fig. 3.5. Seasonal variation in mean total copper (solid line) and dissolved copper (dashed line) concentration (μg/L) from 10 ponds (Senecaville State Fish Hatchery pond nos. 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) at Senecaville State Fish Hatchery during percid culture in 2005. The vertical bars refer to standard error associated with the mean.
Fig. 3.6. Free copper ion activity (Moles/Liter) in 10 ponds at Senecaville (open bar) (1= Senecaville State Fish Hatchery pond no. 21, 2= pond no. 22, 3= pond no. 23, 4= pond no. 24, 5= pond no. 25, 6= pond no. 26, 7= pond no. 27, 8= pond no. 28, 9= pond no. 29, and 10= pond no. 30) and at reference site (hatched bar) (1= Hebron State Fish Hatchery pond no. B3, 2= pond no. B4, 3= pond no. C4, 4= pond no. C5, 5= pond no. D2, 6= pond no. D3, 7= pond no. E3, 8= pond no. E4, 9= pond no. F5, and 10= pond no. F6) during the first week of saugeye culture in 2005.
Fig. 3.7. Seasonal variation in total phytoplankton biomass (wet weight) in Senecaville ponds (pond nos. 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) (solid line) and the source water (Seneca Lake; dotted line) during percid culture in 2005. The vertical bars refer to one standard error associated with the mean of 10 ponds.
Fig. 3.8. Seasonal variation in phytoplankton composition in 10 ponds at Senecaville State Fish Hatchery (pond nos. 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) (a) and the source water (Seneca Lake; b) during percid culture from April-May in 2005.
Fig. 3.9. Variation in phytoplankton biomass in 10 ponds (pond nos. 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) as a function of free copper ion activity during the first week of saugeye culture at Senecaville State Fish Hatchery in 2005.

Fig. 3.10. Seasonal variation in the mean total zooplankton biomass in 10 ponds at Senecaville State Fish Hatchery (pond nos. 21, 22, 23, 24, 25, 26, 27, 28, 29 and, 30) (solid line), and in 11 ponds at Hebron State Fish Hatchery (pond nos. B3, B4, C4, C5, D2, D3, E3, E4, F5, F6, and G6) (dotted line) during saugeye culture in 2005.
Fig. 3.11. Seasonal variation in zooplankton taxonomic composition (as biomass) in 10 ponds at Senecaville State Fish Hatchery (pond nos. 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) (a) and in 11 ponds at Hebron State Fish Hatchery (pond nos. B3, B4, C4, C5, D2, D3, E3, E4, F5, F6, and G6) (b) during saugeye culture in 2005.
APPENDIX A
<table>
<thead>
<tr>
<th>Phylum</th>
<th>Genus/Category</th>
<th>Algal Type</th>
<th>Edible/Inedible</th>
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</thead>
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<td>Edible</td>
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<td>Characium</td>
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<td>Chlamydomonas</td>
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<tr>
<td></td>
<td>Chlorophyte filament</td>
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</tr>
<tr>
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<td>Closteriopsis</td>
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<tr>
<td></td>
<td>Coelastrum</td>
<td>Colonial</td>
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<tr>
<td></td>
<td>Colonial chlorophyte with sheath</td>
<td>Colonial</td>
<td>Edible</td>
</tr>
<tr>
<td></td>
<td>Colonial chlorophyte without sheath</td>
<td>Colonial</td>
<td>Edible</td>
</tr>
<tr>
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<tr>
<td></td>
<td>Crucigenia</td>
<td>Colonial</td>
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<td>Dictyosphaerium</td>
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<tr>
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<td>Gontium</td>
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<td></td>
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<tr>
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<td>Oocystis</td>
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<tr>
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<td>Schroederia</td>
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<td>Solitary</td>
<td>Edible</td>
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<tr>
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<td></td>
<td>Spiny Green</td>
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<td>Spirogyra</td>
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<td>Staurastrum</td>
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<td></td>
<td>Synura</td>
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</tr>
<tr>
<td></td>
<td>Tetraedron</td>
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<td>Edible</td>
</tr>
<tr>
<td></td>
<td>Treubaria</td>
<td>Solitary</td>
<td>Edible</td>
</tr>
</tbody>
</table>

**Chrysophyta (Chrysophyceae)**

|              | Dinobryon      | Colonial | Edible |
|              | Mallomonas     | Solitary | Edible |

**Chrysophyta (diatoms)**

<p>|              | Asterionella   | Colonial | Edible |
|              | Centric Diatom | Solitary | Edible |
|              | Cocconeis     | Solitary | Edible |
|              | Coscinodiscus | Solitary | Edible |
|              | Cyclotella    | Solitary | Edible |
|              | Čymbella      | Solitary | Edible |
|              | Fragilaria    | Colonial | Edible |</p>
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<thead>
<tr>
<th>Phylum</th>
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<th>Growth Form</th>
<th>Edibility</th>
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<td>Chroomonas</td>
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<td>Anabaena</td>
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<td>Aphanizomenon</td>
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</tr>
<tr>
<td></td>
<td>Aphanocapsa</td>
<td>Colonial</td>
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<tr>
<td></td>
<td>Aphanothece</td>
<td>Colonial</td>
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<td>Chroococcus</td>
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<td>Merismopedia</td>
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Table A.1. The classification of edible and inedible status of all algal genera and general categories, separated by Phylum that were counted when analyzing phytoplankton samples.
BIBLIOGRAPHY


Boyd, C. E. 1990. Water quality in ponds for Aquaculture. Alabama Agricultural experimental Station, Auburn University, Alabama. 482pp


survival of walleyes reared in ponds fertilized with organic or inorganic materials. Progressive Fish-Culturist 58: 135-139.


