CHAPTER 2

Control of *Prymnesium parvum* using Ammonium Sulfate or Copper Sulfate in Plastic-Lined Ponds for Koi Carp Production

DENNIS G. SMITH

Abstract

Prymnesium parvum is a toxic alga that has been responsible for numerous fish kills in reservoirs, rivers, and hatcheries in Texas and many other locations around the world. We compared the effectiveness of ammonium sulfate and copper sulfate treatments in controlling *P. parvum* and its associated toxicity in plastic-lined ponds for rearing koi carp, a strain of common carp *Cyprinus carpio,* fingerlings. Treatments were 9.5-mg/L ammonium sulfate, 2-mg/L copper sulfate, and control (no chemical treatment). After the initial treatments, treatments were reapplied to all ponds if weekly bioassay results revealed ichthyotoxin in any pond. All ponds were harvested after 75 or 76 days of koi carp rearing. Bioassay and cell density results revealed that ammonium sulfate and copper sulfate were effective in reducing *P. parvum* density and toxicity. Fish production was highest in ammonium sulfate ponds, followed by the copper sulfate ponds and was zero in the control ponds. The copper sulfate treatment ponds had a mean net loss of 0.9 kg in fish biomass while the ammonium sulfate treatment ponds had a net gain in mean fish biomass of 266.8 kg. Both chemical treatments were effective in controlling *P. parvum*; however, ammonium sulfate is recommended because fish production was significantly ($P \leq 0.05$) better in ponds treated with that chemical.

Introduction

The presence of the halophilic phytoflagellate *Prymnesium parvum* and its associated toxins in Dundee State Fish Hatchery (DSFH) ponds during spring 2001 resulted in the combined loss of over 5 million fry and fingerling striped bass *Morone saxatilis* and palmetto bass (female *M. saxatilis* × male *M. chrysops*). In addition, significant numbers of smallmouth bass *Micropterus dolomieu* and largemouth bass *M. salmoides* brood stock, adult rainbow trout *Oncorhynchus mykiss*, and fingerling koi carp and channel catfish *Ictalurus punctatus* also perished.

Various control methods, including applications of ammonium sulfate, copper sulfate and mud, nutrient manipulation, and reduction in salinity, have been employed to control *P. parvum* throughout the world with mixed results (Guo et al. 1996). Of these, ammonium sulfate and copper sulfate generally are most frequently recommended (Sarig 1971). Ammonium sulfate, when added to a pond, raises the total ammonia level. It has been suggested that the un-ionized portion of the total ammonia is responsible for causing the *P. parvum* cells to swell and lyse (Shilo and Shilo 1953, 1962). This is supported by the fact

that ammonium sulfate treatments are more effective in controlling *P. parvum* at higher temperatures and pH levels (Shilo and Shilo 1953). At higher temperature and pH levels, a greater proportion of the total ammonia exists in the un-ionized form (Emerson et al. 1975), which helps explain why ammonium sulfate treatments are not always effective (Guo et al. 1996). Copper sulfate is usually effective at eliminating *P. parvum* from ponds; however, it is an indiscriminate algaecide and its use may result in anoxic conditions due to decomposition of dead algae or reduction of oxygen production from photosynthetic activity. Copper sulfate also is toxic to a variety of fish species (Irwin 1997) and its toxicity to phytoplankton and fish is influenced by alkalinity (Boyd 1990).

Because the DSFH water supply has high levels of alkalinity (88-90 mg/L as $CaCO_3$) and none of the published literature on control of *P. parvum* was conducted in plastic-lined ponds, we evaluated the effectiveness of ammonium sulfate and copper sulfate at the recommended doses for controlling *P. parvum* in plastic-lined ponds. The specific objectives were to determine the effects of ammonium sulfate (10 mg/L) and copper sulfate (2 mg/L) on *P. parvum* density and toxicity and koi carp production in plastic-lined ponds.

Materials and Methods

This study was conducted at the DSFH near Wichita Falls, Texas from August through mid-October 2001. The experimental design was three replicates of each of ammonium sulfate (9.5 mg/L), copper sulfate (2 mg/L), and untreated control. Treatment concentrations were selected from the recommendations in published literature (Sarig 1971). The recommended minimum dose of 10 mg/L ammonium sulfate was not used due to an investigator error that led to an initial treatment rate of 9.5 mg/L which also was used in follow-up treatments for consistency. All treatments were based upon full pond volumes.

Nine 0.4-ha plastic-lined ponds were utilized for this study. The catch basin of each pond was cleaned of sediment, and containment screens were installed prior to filling ponds. Fill water was filtered through 500-µm socks to prevent contamination by wild fish. Pond filling was started on the same day and all ponds reached full volume at least two days prior to stocking of fish. Once full, no additional water was added to the ponds in an attempt to prevent reintroduction of *P. parvum* from Lake Diversion, the water source for the hatchery. The water characteristics were alkalinity 88 mg/L as $CaCO_3$, hardness 900 mg/L as $CaCO_3$, pH 8, and 4 ppt salinity. Ponds were randomly assigned to the treatment and control groups. Initial treatments were applied to the ponds two days prior to stocking of fish. Water samples were collected from each pond prior to treatment and again the day after treatment for bioassay and *P. parvum* enumeration.

Temperature, dissolved oxygen, and pH were measured and recorded twice daily with a Yellow Springs Instrument® (YSI) model 600XL sonde attached to an YSI model 610DM data logger. Once every seven days, water was collected from each pond to perform cell enumeration, conduct a bioassay, and measure nitrogen and phosphorous concentrations using established protocols (Appendix A; Appendix B; APHA 1995). Total ammonia was measured with an ion specific Accumet® brand electrode connected to a Denver Instruments® model 250 meter, and phosphorous concentrations were determined using the

stannous chloride method (APHA 1995). Test fish for the bioassay were wild-caught red shiner *Cyprinella lutrensis* that were maintained in a laboratory culture. A positive bioassay result (half or more of the test fish dying in any one bioassay container using water from a treated pond) triggered follow-up treatments of all ponds. If follow-up treatments were required, bioassays and cell enumerations were performed again the day after the treatments to monitor treatment effects. Copper concentrations in ponds were not measured because materials required for the testing were not available to investigators during the study.

Koi carp fingerlings (≈ 27 mm TL) were obtained from the A. E. Wood Fish Hatchery in San Marcos, Texas on 2 August 2001 and transported to DSFH. These fish were stocked into ponds at 250,000 fish/ha. Feeding of fish commenced the day after stocking and fish were fed a daily ration of 6% body weight based on the initial stocking weight. The fish were fed equal rations twice per day using a mechanical feed blower. Eleven days after stocking and every seven days thereafter, fish in each pond were sampled by seining. At least one hundred fish were collected from each pond, counted, and weighed in aggregate to determine number fish per kg. The number fish per kg and the initial number of fish stocked were used to estimate the biomass of fish in each pond. Feed amounts were adjusted according to biomass estimates each week. Thirty randomly selected fish from each sample also were measured for total length.

The fish were harvested by completely draining the ponds, allowing the fish to concentrate in the pond catch basins, and removing them with a plastic basket attached to a crane. At harvest, the total biomass, mean total length, and number of fish per kg were determined for each pond. The total biomass and number per kg were used to estimate the total number fish harvested. Six ponds (two each from the treatments and control) were harvested on 16 October 2001 and the remaining three were harvested the following day. These production data were compared using a t-test.

Results and Discussion

Follow-up treatments were triggered on two occasions. On 27August 2001, a bioassay indicated that three ponds contained ichthyotoxin: two ponds were ammonium sulfate treatment and one was a copper sulfate treatment. Mean cell density in the ammonium sulfate treatment ponds was 3,333 cells/mL at that time but decreased to 667 cells/mL one day after treatment. Cells were not detected in the copper treatment pond containing the ichthyotoxin either on August 27 or 28. Bioassays were performed again on August 28 and no test fish died in water from any of the treated ponds. Bioassays and cell enumerations conducted on 17 September 2001 indicated that one of the ammonium sulfate treated ponds had ichthyotoxin and a cell density of 48,000 cells/mL. All ponds were treated again, and cell counts and bioassays conducted the following day revealed neither cells nor toxicity in any of the treated ponds.

In one of the control ponds, *P. parvum* cells were first detected on 6 August 2001 and again on 27 August 2001. Thereafter, *P. parvum* persisted in the control ponds and steadily increased in density, reaching a peak mean of 66,000 cells/mL on 18 September 2001, and then gradually declined. Cells remained in the control ponds up to the final cell counts

performed one week prior to fish harvest. There were no positive bioassays in water from the control ponds until August 27. Beginning on that date, all but five of the 27 bioassays conducted with water from the control ponds indicated the ichthyotoxin was present.

On 28 August 2001, fish in one of the untreated control ponds appeared stressed. Mortalities were noticed the following day and continued through 31 August 2001. No live fish were collected from this pond during a weekly seine sampling conducted on 4 September 2001. Signs of stress and mortality were noticed in the other two control ponds on 4 September 2001 and continued to worsen throughout the week. No live fish were collected from any of the control ponds during weekly seine sampling conducted on 10 September 2001 or thereafter, and no fish were harvested from any of the control ponds.

When follow-up treatments were applied, the same amount of chemical as the initial treatment was used. Pond water levels declined from evaporation, and actual pond volumes were not determined, which resulted in higher application rates for the follow-up treatments. Doses higher than the target are evidenced in Figure 1, which illustrates progressively higher total ammonia levels on the days after treatments as the pond volumes were reduced by evaporation over time.

Table 1 summarizes cell enumeration, bioassays, nutrient analysis, and water quality measurements. Mean cell density was four-fold higher in the ammonium sulfate treatment ponds than in the copper sulfate treatment ponds, however the difference was not statistically significant. Conversely, cell densities in the treated ponds were significantly lower than that of control ponds, indicating that both treatments were effective at reducing *P. parvum* populations. The results of bioassays showed a similar trend, implying that reducing cell densities also diminished toxin levels.

As expected, total ammonia was significantly higher in the ammonium sulfate treatment ponds than in the copper sulfate treatment or control ponds. Other studies have shown that the total ammonia concentrations in earthen ponds following applications of ammonium sulfate were reduced by half in 24 hours (Shilo and Shilo 1953, Sarig 1971). During this study, total ammonia concentrations declined by approximately 50% per week in the plastic-lined ponds (Figure 1). Although more frequent sampling would better show the rate of decline, ammonia was more persistent in our plastic-lined ponds than has been reported for earthen ponds. Phosphorous levels were low and similar among treatment and control groups. Afternoon temperatures were similar among treatments whereas morning temperature was significantly lower in control ponds. However, the small temperature difference was likely not biologically significant. Morning dissolved oxygen levels did not significantly differ among treatment and control groups; while afternoon dissolved oxygen level was highest in ammonium sulfate treatment ponds, followed by the control, then copper sulfate ponds. Similarly, the greatest diel shift in mean dissolved oxygen level was found in the ammonium sulfate ponds while the least was found in the copper sulfate treatment ponds, probably a reflection of the differences in phytoplankton standing crops among treatment and control groups.

Mean morning and afternoon pH readings were significantly different among treatments and control ponds (Table 1). Both morning and afternoon mean pH levels were significantly lower in copper sulfate ponds that in the ammonium sulfate treatment or the control ponds. Ammonium sulfate treated ponds also had the greatest diel shift in pH values, followed by the control, then copper sulfate ponds. The diel shifts in dissolved oxygen and pH levels are indicators of primary productivity with larger shifts indicating greater primary productivity. Thus, primary productivity was highest in the ammonium sulfate treatment ponds that were richer in nitrogen. The copper sulfate treatment ponds had the least amounts of primary productivity and probably had the lowest mean phytoplankton standing crop (Boyd 1990).

Initial numbers, weights, and mean lengths of fish stocked were not significantly different among treatments and control (Table 2). Mean production days (75.3) were identical for all treatments. Harvest information varied significantly among treatment and control groups. Ammonium sulfate treatment ponds had significantly higher fish biomass, survival, and mean length than copper sulfate treatment ponds. No fish survived in the control ponds. Fish in one of the ammonium sulfate treated ponds suffered some mortality during harvest due to high sediment loading and low dissolved oxygen levels. The loss was estimated at 25 kg of fish and excluded from data analysis. If these fish had not been lost, harvest differences between treatments would have been even more pronounced. Ponds treated with copper sulfate had a net loss of biomass from the time of stocking to harvest, even though the mean length at harvest was almost 20 mm greater than the length at stocking, due to the low mean survival rate in these ponds.

During weekly seine sampling, fish collected from the ammonium sulfate treated ponds appeared to have a prevalence of gill hyperplasia, with the gill filaments actually extending beyond the operculum. This observation was confirmed by laboratory examination and although no direct cause could be determined, the hyperplasia could have been the result of exposure to high ammonia concentrations (Thurston et al. 1984). Another possible explanation is that the fish could have mistakenly ingested ammonium sulfate granules as food particles since both feed and ammonium sulfate were distributed with the same machinery. However, despite the hyperplasia, survival from these ponds was comparatively high (58.3%) and biomass production was well above the historic average of 53% survival and for DSFH koi fingerlings reared in plastic-lined ponds.

Summary

Complete fish mortality in the control ponds dictate that some form of control must be employed to prevent fish losses when *P. parvum* is present in ponds. Treatment with ammonium sulfate or copper sulfate was effective in reducing *P. parvum* density and toxicity. Both appeared to work within 24 hours after treatment. Although *P. parvum* cells persisted in some ponds the day after treatment, none of the treated ponds were toxic. Due to large differences in harvested biomass, survival, and mean total length of fish, ammonium sulfate appears to be much better than copper sulfate for controlling *P. parvum* in plastic-lined ponds for rearing koi carp when temperature and pH are high enough to make such

treatments feasible. The ammonia concentration applied to plastic-lined ponds was reduced to about 50% in approximately one week.

TABLE 1.—Mean values of water quality variables, nutrient levels, cell density, and toxicity in plastic-lined ponds for rearing koi carp fingerlings treated to control *Prymnesium parvum* with 9.5 mg/L ammonium sulfate or 2 mg/L copper sulfate, or untreated control. Values in a row bearing the same letter are not significantly different ($P > 0.05$). Standard deviations are in parentheses.

	Treatment		
Variable	$(NH_4)_2SO_4$	$CuSO_4 \cdot 5H_2O$	Control
Cell density (no./mL)	1,867 z (7,266)	400 z (1,175)	16,089 y (25,023)
Toxicity (bioassay % mortality)			
Whole water	6.1 z (22.2)	2.9 z (11.7)	50.0 y (49.0)
1:5 dilution	5.6 z (22.0)	2.4 z (11.4)	35.3 y (47.2)
Total ammonia (mg/L)	1.06 z (1.05)	0.05 y (0.10)	0.07 y (0.09)
Phosphorous (mg/L)	0.004 z (0.004)	0.003 z (0.004)	0.004 z (0.006)
Temperature (^oC)			
Morning	24.0 z (3.9)	23.8 z (3.9)	24.3 y (3.7)
Afternoon	26.0 z (3.4)	25.9 z (3.8)	26.4 z (3.6)
Dissolved oxygen (mg/L)			
Morning	6.7 z (0.9)	7.1 z (0.8)	7.0 z (1.0)
Afternoon	9.3 z (1.0)	8.4 y (1.0)	8.9 x (0.9)
pH			
Morning	8.69 z (0.37)	8.28 y (0.24)	8.75 x (0.31)
Afternoon	8.92 z (0.36)	8.34 y (0.23)	8.88 x (0.32)

TABLE 2.—Mean ± SD values of harvest variables for koi carp fingerlings reared in plastic-lined ponds and treated to control *Prymnesium parvum* with 9.5 mg/L ammonium sulfate or 2 mg/L copper sulfate, or untreated control. Values in a row bearing the same letter are not significantly different ($P > 0.05$). Standard deviations are in parentheses.

	Treatment		
Harvest variable	$(NH_4)_2SO_4$	$CuSO_4 \cdot 5H_2O$	Control
Number	58,958 z (15,255)	23,800 y (4,804)	0 x
Weight (kg)	299.8 z (52.2)	30.7 y (4.0)	0 x
Length (mm)	71.3 z (3.6)	46.7 y (2.0)	0 x
Survival (%)	58.3 z (15.0)	23.3 y (4.5)	0 x

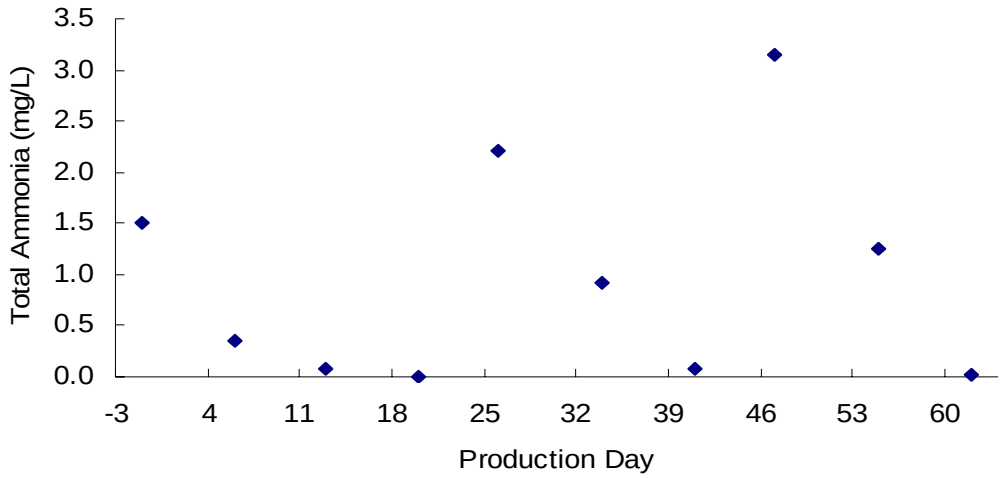

FIGURE 1.—Mean total ammonia levels in ammonium sulfate treatment ponds. Initial treatments were applied two days prior to fish stocking (production day = 0). Follow-up treatments were applied on days 25 and 46 after stocking.