CHAPTER 8

Efficacy of Nitrogen:Phosphorus Ratios for Controlling *Prymnesium parvum* in Fish Culture Ponds: Summary of 2002 Experiments

GERALD KURTEN AND DENNIS G. SMITH

Abstract

The goal of this project was to determine if two specific concentrations and ratios of nitrogen and phosphorus would deter dominance and toxin production by *Prymnesium parvum* in warmwater fish culture ponds at the Dundee State Fish Hatchery. The initial objective was to establish phosphorus fertilization rates that would sustain 60 µg P/L in hatchery ponds and simultaneously determine if phosphorus fertilization alone would reduce *P. parvum* density and toxicity. An average phosphorus addition of 99 µg/L (82 – 137 µg/L) was required to achieve a target concentration of 60 µg/L. Pond temperatures averaged 13ºC and neither pond productivity nor *P. parvum* cell densities appeared to be affected by phosphorus fertilization alone. The second objective was to determine if nitrogen concentration of 300 µg/L and phosphorus concentration of 30 µg/L (N:P = 10:1; low-P) or nitrogen concentration of 300 µg/L and phosphorus concentration of 60 µg/L (N:P = 5:1; high-P) would reduce the incidence and toxicity of *P. parvum* and produce water quality conditions and food sources suitable for zooplanktivorous fish. The number of fish produced was significantly different between low-P and control ponds as well as between high-P and control ponds. Numbers of fish produced in high-P and low-P ponds were statistically similar but relatively more fish were produced in the low-P ponds. At temperatures typical of striped bass *Morone saxatilis* culture (22ºC), *P. parvum* cells appeared to be eliminated in ponds fertilized with high concentrations of phosphorus (92 µg/L).

Introduction

Recently, *Prymnesium parvum* has caused massive mortalities of brood fish, fingerlings, and fry at the Dundee and Possum Kingdom State Fish Hatcheries (DSFH and PKSFH, respectively). Control practices at these facilities have focused on killing the alga with either copper sulfate or ammonium sulfate. Unfortunately, the use of these compounds provides short-term relief and each has undesirable consequences in pond systems dependent upon zooplankton as food for small fish. Ammonium sulfate concentrations high enough to control algal blooms may yield un-ionized ammonia concentrations approaching levels toxic to fry and fingerlings. Copper sulfate has the side effect of killing desirable algae and can negatively impact zooplankton food resources.

Laboratory, pond, and reservoir research suggest that toxin production and dominance by *P. parvum* may be related to nutrient concentrations within the system. High N:P ratios combined with phosphorus limitation is suspected to be responsible for toxic blooms of *P.*

parvum in western Norway (Kaartvedt et al. 1991; Aure and Rey 1992), England (Holdway et al. 1978), and Finland (Lindholm et al. 1999). Apparently, *P. parvum* is a good scavenger of phosphorus at low concentrations and is able to acquire phosphorus from a wide array of organic compounds (McLaughlin 1958). Therefore, fish ponds with low concentrations of phosphorus may provide *P. parvum* the opportunity to dominate the phytoplankton community. However, once available phosphorus supplies are exhausted, phosphorus-limited growth appears to result in toxin production via over-synthesis of membrane intermediates (Dafni et al. 1972; Holdway et al. 1978; Shilo 1981; Johansson 2000). Toxicity coincided with phosphorus declines in control ponds at the DSFH in 2001 during studies designed to control *P. parvum* with ammonium sulfate and copper sulfate (TPWD, unpublished data). Conversely, nitrogen limitation does not appear to be a factor in toxicity of *P. parvum*. Holdway et al. (1978) reported that limiting levels of nitrogen, thiamine, or vitamin B_{12} do not result in increased toxin production. Apparently, *P. parvum* has the ability to use a wide variety of nitrogen-containing compounds including nitrates, ammonia, and amino acids (Paster 1973).

At DSFH and PKSFH, recently used pond management practices include limiting pond fertilization to applications solely of organic materials (e.g., cottonseed meal), which may supply the thiamine and B_{12} required for *P. parvum* growth (Shilo 1972). However, the N:P ratio of cottonseed meal is very high (22:1), whereas the available phosphorus is very low (Anderson 1993). Chinese aquaculturists have achieved good control of *P. parvum* in ponds containing planktivorous fish species by regular fertilization with livestock and poultry manure (Gou et al. 1996). This strategy is based upon the observation that *P. parvum* is relatively slow growing and does not compete well with other algal species when nutrients are replete, but it can tolerate extremely deplete nutrient conditions. Ponds were kept free of *P. parvum* toxicity for up to three months by fertilizing with 50-70 kg/ha/d of manure (dry weight), whereas all fish died in the control (no manure) ponds. Based upon published composition of cattle manure (Boyd 1990) and an average pond depth of one meter, this application rate would supply approximately 420 µg N/L and 98 µg P/L per day (N:P = 4.3:1) to ponds. These fertilization rates are much higher than those customarily used at TPWD hatcheries and likely would result in low dissolved oxygen in ponds.

Recent data suggest that DSFH fish culture ponds are consistently phosphorus limited. When phosphorus has been measured in hatchery ponds, concentrations generally have been at or below detectable limits (< 7 µg/L). Additionally, characteristics of Lake Diversion, the water supply for DSFH, apparently result in low solubility and high precipitation rates of phosphorus (Wetzel 1983). Boyd and Daniels (1993) reported that in brackish, high-alkalinity ponds, phosphorus must be applied frequently due to its low solubility in such waters. Phosphorus concentrations of 30-35 µg/L have been suggested as the lower limit for phytoplankton growth (Sommer 1985; Culver et al. 1993). Phosphorus concentrations in Lake Diversion have been below detectable limits in recent years (TNRCC, unpublished data), suggesting that phosphorus concentrations have been lower than that required for phytoplankton growth. However, Barkoh (1996) compared two N:P ratios with alfalfa meal against alfalfa meal fertilization alone at DSFH and demonstrated that high phosphorus concentrations were not difficult to achieve.

The practice of using organic fertilizers alone in production ponds resulted from concerns that high pH and un-ionized ammonia can adversely affect survival of moronids (Anderson 1993, Bergerhouse 1993, Barkoh 1996). High phosphorus levels could increase phytoplankton growth, if nitrogen is not limiting, resulting in high afternoon pH. Therefore, fertilization strategies aimed at controlling *P. parvum* density and toxicity should not result in phytoplankton densities that affect pond pH in an adverse manner. Inland hatcheries rearing largemouth bass *Micropterus salmoides* fingerlings typically fertilize ponds to achieve approximately 250 µg P/L and 500 µg N/L. These concentrations of inorganic nutrients typically result in good phytoplankton and zooplankton populations although resultant pH levels generally exceed those considered acceptable for striped bass *Morone saxatilis* production. Culver (1993) reported good production of percids with 30 µg P/L and 600 µg N/L. However, pH in some ponds reached 9.7 and may have influenced fish survival. Anderson (1993) used similar target nutrient concentrations in striped bass ponds. Although concentrations of about 300 µg N/L were achieved, phosphorus never exceeded 5 µg/L and ponds appeared to be phosphorus limited. Anderson (1993) thus suggested that a target concentration of 30 µg P/L might be too low in hatchery ponds with hard water supply.

The N:P ratio used by Culver (1993) was 20:1 and is similar to the ratio suggested for algal growth media (APHA 1995). However, others have stated that N:P ratios higher than 10:1 may result in phosphorus limitation (Groeger et al. 1997), although the theoretical N:P ratio for balanced phytoplankton growth is 7:1 (Cromar and Fallowfield 1997; Welch 1980). Barkoh (1996) targeted nutrient ratios of 7:1 and 15:1 to stimulate predominance of nanoplankton in ponds for striped bass production at DSFH. However, the measured N:P ratios were 11:1 and 3:1, respectively, and phosphorus concentrations were highly variable during the culture period.

The goal of this project was to determine if two specific concentrations and ratios of nitrogen and phosphorus would deter dominance and toxin production by *P. parvum* in warmwater fish culture ponds at the DSFH. The initial objective was to determine phosphorus fertilization rates that would sustain phosphorus concentrations of 60 µg/L in hatchery ponds and simultaneously determine if phosphorus fertilization alone would reduce *P. parvum* density and toxicity. The second objective was to determine if nitrogen level of 300 µg/L and phosphorus level of 30 µg/L (N:P = 10:1; high ratio, low-P) or nitrogen level of 300 µg/L and phosphorus level of 60 µg/L (N:P = 5:1; low ratio, high-P) would reduce the incidence and toxicity of *P. parvum* and produce suitable water quality conditions and food base for zooplanktivorous fish. The standard DSFH fertilization regimen using only organic fertilizers served as the control treatment. Both high-P and low-P ponds also received the same organic fertilizers as control ponds.

Materials and Methods

Objective 1.—Determine phosphorus demand and loss rates at known chlorophyll a concentrations and the effects of phosphorus fertilization on P. parvum density and toxicity, if present, and simultaneously assess phosphorus fertilization effects on water quality and zooplankton densities.

Determining appropriate phosphorus application rates requires understanding phosphorus loss rates under various scenarios. Both Anderson (1993) and Barkoh (1996) reported difficulties in achieving target phosphorus concentrations in striped bass ponds, either exceeding or never reaching target concentrations. This experiment was designed to examine phosphorus loss rates at low algal densities typical of the early fish culture period (April-May) and at high algal densities typical of late fish culture period (June-July). These data were used for fertilizer application calculations for the second objective which was to determine the affect of fertilization on *P. parvum* dominance and toxicity.

Three ponds of equal volume were fertilized with phosphoric acid at 60 µg P/L. Three control ponds were filled at the same time and remained unfertilized. Ponds were at full volume before fertilizer applications and, once full, received no additional water. Soluble reactive phosphorus (SRP) was measured for each experimental pond at the pond fill valve and also the day before fertilization using the stannous chloride method (APHA 1995). Chlorophyll *a* (µg/L) was measured at the same time using the filters required for the SRP determination. Chlorophyll *a* was extracted with 95% acetone and measured using a spectrophotometer (APHA 1995).

Phosphorus and chlorophyll *a* were determined at 24-h intervals following the initial fertilization until SRP concentrations fell below detectable levels on two successive intervals or after 4 days. If SRP was undetectable or lower than the target of 60 µg P/L at the first sampling interval following fertilization, an additional 60 µg P/L was applied until a minimum residual of 60 µg P/L was achieved. The amount of phosphorus required to achieve the minimum residual of 60 µg P/L was used to determine the phosphorus demand as follows:

(Total P applied – initial pond P) + (final pond P – 60) = phosphorus demand.

Phosphorus loss rates were calculated as µg P/L/h at ambient chlorophyll *a* concentrations. Regression analysis was used to develop a relationship between chlorophyll *a* and phosphorus loss.

To determine the effects phosphorus on *P. parvum*, cell counts (Appendix A) and bioassays (Appendix B) were conducted for each pond immediately prior to fertilization and twice weekly thereafter, ending one week after the last phosphorus and chlorophyll *a* sampling. All ponds also were monitored twice daily (morning and afternoon) for pH, temperature, and dissolved oxygen.

Zooplankton was sampled from each experimental pond on Monday and Thursday each week between 0600 and 0700 hours by an oblique 4-m tow with a 5.75-cm diameter 80-µm Wisconsin plankton net. Each sample was dewatered to 90 mL and densities of major zooplankton groups (i.e., cladocerans, copepod nauplii, adult copepod, and rotifers) were determined on two separate 1-mL subsamples on a plankton counting wheel under a variable-magnification dissecting microscope.

This experiment was to be repeated on the same treatment and control ponds, with minimum chlorophyll *a* concentrations near 40 µg/L (Anderson 1993) to determine P demand and loss rates at higher algal biomass. To achieve high chlorophyll *a* concentrations for this test, both control and treatment ponds were to be fertilized with P at 60 µg/L following the previous experiment if chlorophyll *a* concentrations were below 40 µg/L.

Objective 2.—Determine phosphorus and nitrogen fertilization effects on P. parvum density and toxicity, water quality, zooplankton, and striped bass production.

Nine ponds were used for this experiment, which consisted of two treatments and a control. Three control ponds were fertilized, stocked with fish, and received feed according to striped bass production guidelines (Warren 2001). Briefly, ponds were fertilized at pond filling with 280 kg/ha cottonseed meal and fertilized again at 3 days and 12 days post-stocking with 56 kg/ha cottonseed meal. Fry were stocked at 3-5 days after hatching (7 days after pond filling) and supplemental feeding was begun 14 days after stocking fry. No steps were taken to control *P. parvum* densities and toxicity in these ponds. Three ponds each served as the "high-ratio" (N:P = 10:1; 300 µg N/L, 30 µg P/L) treatment and "low-ratio" (N:P = 5:1; 300 µg N/L, 60 µg P/L) treatment, respectively. These ponds were filled, fertilized, and stocked as the control ponds. No additional water was added to ponds once they were filled.

Initial inorganic fertilization.—Ponds in high- and low-ratio treatments were fertilized immediately at pond filling to achieve 300 µg N/L with liquid urea nitrogen (URAN). Ambient nitrogen concentrations (ammonia + nitrate) were determined one day before fertilization. If nitrogen concentrations exceeded 300 µg/L, no additional nitrogen was applied; otherwise, the amount applied was equal to 300 µg/L minus ambient concentration. Likewise, on the same day, high- and low-ratio ponds were fertilized with liquid phosphoric acid. Phosphoric acid was added to achieve either the high rate (60 µg P/L; low N:P ratio ponds) or the low rate (30 µg P/L; high N:P ratio ponds). Application rates were calculated as follows:

(P demand – initial P concentration) + anticipated P loss for three days.

This calculation required that chlorophyll *a* also be determined prior to pond fertilization.

Follow-up inorganic fertilization.—Every third day following initial fertilization, both nitrogen and phosphorus were reapplied. Phosphorus was reapplied as for initial fertilization using the phosphorus demand calculation described above. Nitrogen was applied at a rate equivalent to the desired concentration (300 µg/L) minus the concentration in the pond.

Soluble reactive phosphorous, nitrate nitrogen, ammonia nitrogen, and chlorophyll *a* were measured every third day after initial pond fertilization. Morning and afternoon dissolved oxygen, temperature, and pH were measured daily. *P. parvum* cell counts and toxicity bioassays were conducted twice weekly on each pond for the duration of the culture period. Control ponds were sampled in a manner identical to fertilized ponds. Zooplankton

was sampled as described for objective one. Pond harvest and termination of the experiment occurred approximately 40 days after initial pond filling.

Analysis

Objective 1.—Phosphorus demand was calculated from the initial fertilization of each pond using the equation presented above, and then averaged to determine the application concentration required to achieve a minimum phosphorus residual of 60 µg/L. Daily phosphorus decline rate, following achievement of the 60-µg P/L target, was computed as follows:

(Initial P concentration – Final P concentration)/24 h = P loss/h.

Regression analysis was used to resolve the relationship between phosphorus loss and initial phosphorus and chlorophyll *a* concentrations. Phosphorus demand and loss rates were used to calculate the appropriate fertilization rates for objective 2. To retain target concentrations at the end of the three-day fertilization interval, the phosphorus fertilization rate was calculated as follows:

P target + P demand + P loss for 72-h interval

Because phosphorus fertilization alone could reduce *P. parvum* density and toxicity and likely has effects on water quality and pond productivity, data were examined graphically to discern trends in water quality, zooplankton, nutrient concentrations, algal biomass (chlorophyll *a*), *P. parvum* cell density, and daily net primary productivity (afternoon dissolved oxygen - morning dissolved oxygen). When differences appeared significant between treatment and control ponds, daily paired means were compared. Differences in measured variables between treatment and control were compared with repeated measures analysis of variance.

Objective 2.—The most successful fertilization strategy was judged as that treatment with the lowest incidence of toxicity and *P. parvum* cell density, which were evaluated as for objective 1. Additionally, treatment means or daily means were evaluated based on production of appropriate zooplankton for striped bass feeding and pH, un-ionized ammonia and dissolved oxygen concentrations for suitable striped bass survival (Barkoh 1996; Warren 2001). Zooplankton and water quality also were evaluated as in objective 1. Survival, growth, production rate (kg/ha/d), and net fish biomass (harvest biomass-stocking biomass) were compared among the two treatments and the control by analysis of variance. Survival was log (X+1) transformed prior to analysis. Significance for all tests was set at $P \leq 0.05$.

Results and Discussion

Objective 1

Phosphorus demand and decline.—Two applications of 60-µg/L phosphorus were required to achieve target concentrations of at least 60 µg P/L. Phosphorus concentrations

averaged 86 µg/L the morning after the second addition. The calculated application rate to achieve 60 µg/L was 88 µg P/L. No additional phosphorus was applied for the next five days. Ninety-six hours after target concentrations of phosphorus were measured, concentrations declined to an average of 60 µg P/L (Figure 1). Average phosphorus loss rate was 0.03 µg/L/h (0.65 µg/L/d).

Chlorophyll *a* concentrations averaged 19.1 µg/L during the phosphorus application period and increased to 23.0 µg/L in fertilized ponds 5 days later when the experiment ended. Changes in chlorophyll *a* concentrations were not significantly different between beginning and end of sampling or between treated (fertilized) and control (untreated) ponds. Similarly, there were no statistically significant differences among water quality variables between treated and control ponds. Morning and afternoon pond temperatures averaged 13 and 16ºC, respectively. Morning and afternoon dissolved oxygen averaged 10.7 and 12.0 mg/L, respectively, and morning and afternoon pH both averaged 8.2. Apparently, productivity was not measurably stimulated during the 5-day period at these ambient temperatures because the indicators of pond productivity (algal biomass, pH, and afternoon dissolved oxygen) did not differ between fertilized and unfertilized ponds.

A second series of phosphorus applications was made to the three unfertilized ponds to determine phosphorus demand. Chlorophyll *a* concentrations averaged 22.0 µg/L. Calculated phosphorus required to achieve 60 µg P/L averaged 110 µg P/L. The study was not continued as planned to examine phosphorus decline rates at higher algal biomass due to labor constraints. The 12 applications of phosphorus during the first and second application series and the ratio of applied phosphorus to measured phosphorus was used to calculate the amount of phosphorus required to achieve 60 µg P/L. The mean additional phosphorus required was 99 µg /L (range: 82 – 137 µg/L).

P effects on P. parvum toxicity and densities.—P. parvum cell densities on the morning of the first phosphorus application averaged 13,000 cells/mL (10,000-15,000 cells/mL) and bioassays indicated that both fertilized and unfertilized ponds were toxic to fathead minnow fry. On the morning after the second application of phosphorus when target concentrations had been achieved, cell densities were significantly different ($P = 0.029$) between treatment and control ponds, averaging 12,333 cells/mL for P-fertilized ponds and 6,667 cells/mL for unfertilized ponds (Figure 2). Bioassays at the same time were inconclusive because more fish died in diluted pond water than in undiluted pond water. At the termination of the experiment, all fathead minnow fry died in both diluted and undiluted pond water for both P-fertilized and unfertilized ponds. *P. parvum* cell densities were statistically similar. These results seem to indicate that phosphorus fertilization alone had no short-term effect on toxicity or cell density. The difference in cell densities detected the morning after the second phosphorus application may have been a result of an error associated with the sampling technique rather than actual differences, since cell densities were similar between treatments on dates before and after that sampling date.

Objective 2

Ponds were started filling on 17 April 2002, first fertilized on 18 April 2002, and stocked with striped bass fry on 25 April 2002. All ponds were stocked at a density of 500,000 fish/ha. All ponds were fertilized with cottonseed meal following the standard regimen used at the DSFH for striped bass production. Inorganic fertilizers were added twice weekly to high-P and low-P ponds. Pond temperatures did not differ between treatment and control ponds and averaged 22ºC in the morning and 24ºC in the afternoon.

P effects on P. parvum toxicity and densities.—Bioassays were complicated by factors that rendered the data difficult to interpret. On one date striped bass were used rather than fathead minnows. On three sampling dates controls were completed without co-factor additions and in 15 samples on various sampling dates more fish died in diluted pond water samples than in undiluted samples. Therefore, the toxicity of pond waters could not be reliably determined. However, among undiluted pond water, a significant difference in test animal mortalities occurred between control ponds and P-fertilized ponds. Overall, more fish died in bioassays using control pond water than P-fertilized pond water (Figure 3). No fish died in bioassays with undiluted pond water after 7 May 2002 in either P-fertilization treatment, and in none of the bioassays did all test animals die. In control ponds, test animals died in bioassays through 13 May 2002. Unlike P-fertilized ponds, numerous bioassays of control pond water resulted in mortality of all test animals. On 22 April 2002 (3 days before fish stocking) all control ponds had complete mortality among all test animals, whereas on the same date in both high-P and low-P ponds no test animals died in bioassays using undiluted pond water.

There were no significant differences in overall mean *P. parvum* densities among treatments and the control. However, in high-P ponds no *P. parvum* cells were found from 20 days after initial fertilization through pond harvest (Figure 4). The trend in cell density in low-P and control ponds was a gradual decline through the study period with a moderate peak in cell density during the third week after fish stocking.

Water quality and nutrients.—Analysis of nitrate-nitrogen samples was unreliable and as a result total nitrogen concentrations were not determined. Ammonia-nitrogen concentrations averaged 24 µg/L in the low-P ponds and 32 µg/L in the high-P ponds (Figure 5). The difference in ammonia-nitrogen concentration between high- and low-P treatments was not statistically significant. Although no inorganic nitrogen was added to control ponds, ammonia concentrations averaged 63 µg/L and were significantly higher in the control than in both high-P and low-P ponds.

Because total nitrogen concentrations were not determined, the objective of maintaining specific target N:P ratios was not achieved. However, elevated phosphorus concentrations were maintained in P-fertilized ponds and low levels of phosphorus were observed in unfertilized ponds allowing the examination of phosphorus fertilization effects on water quality and cell density. Due to an error in calculating fertilization rates, phosphorus concentrations were almost double the target levels (Figure 6). Phosphorus concentrations averaged 67.7 µg/L in the low-P ponds, 91.9 µg/L in the high-P ponds, and

2.9 µg/L in the control ponds. These concentrations were significantly different between treatments and among treatments and the control.

Trends in algal growth were similar between high-P and low-P ponds. Chlorophyll *a* concentrations increased after pond fertilization and peaked six to seven weeks later (Figure 7). High-P and low-P ponds reached chlorophyll *a* maxima of around 100 µg/L by 30 days after first fertilization. Control ponds achieved chlorophyll *a* maxima of 70 µg/L. High-P and low-P ponds did not differ significantly in chlorophyll *a* however, both had significantly higher chlorophyll *a* concentrations than the control ponds. Chlorophyll *a* averaged 54.9 µg/L, 52 µg/L, and 32 µg/L in high-P, low-P, and control ponds, respectively.

Average morning and afternoon pH values differed significantly among treatments and control. Both morning and afternoon pH were highest for high-P ponds (Figure 8 and Figure 9), intermediate for low-P ponds and lowest for control ponds. Morning and afternoon pH appeared to be a function of phosphorus concentration. In both high-P and low-P ponds, pH values exceeded those recommended by Warren (2001) for the production of fingerling striped bass and their hybrids.

No differences were detected in mean morning dissolved oxygen among treatments and control ponds (Figure 10 and 12). However, near time of harvest, low morning dissolved oxygen concentrations were observed in all ponds. From 24 May 2002 through harvest, most ponds experienced 3-5-day episodes of repeated morning dissolved oxygen concentrations between 4 and 5 mg/L. One high-P pond had five mornings with dissolved oxygen concentrations of less than 4 mg/L (range 0.6-3.4 mg/L). Afternoon dissolved oxygen was significantly different between low-P ponds and control ponds (Figure 11), but no differences were found between high-P and low-P ponds or between high-P ponds and control ponds. Pond temperatures were similar among treatments and the control (Figure 12).

Zooplankton.—Densities were similar among treatments and control ponds for most zooplankton groups. Rotifers, copepod nauplii, and total zooplankton densities were statistically similar among treatments and control (Figure 13). For cladocerans, densities in high-P and control ponds were statistically different. High-P ponds averaged 209 cladocerans/L while control ponds averaged 43 cladocerans/L (Figures 13 and 14). For adult copepod, densities were statistically different among treatments and control ponds (Figure 15). Low-P ponds averaged 492 adult copepod /L while high-P ponds averaged 289 organisms/L and control ponds averaged 171 organism/L. For all treatments, zooplankton densities appeared to be adequate to support fish growth, and food limitation did not appear to be likely despite differences in densities of some zooplankton groups.

Fish production.—The most fish was produced in low-P ponds (Table 1). The number of fish produced was significantly different between low-P and control ponds ($P < 0.05$). Numbers of fish produced in high-P and low-P ponds were not statistically different. Near the end of the study two control ponds (pond 12 and 14) had observable mortality on 31 May 2002 and 1 June 2002. These mortalities were not due to low dissolved oxygen since morning concentrations were near 6 mg/L in both ponds. Such mortality is typical of previous mortality episodes observed in Dundee striped bass culture ponds, which has been

attributed to a wide variety of causes, but most recently *P. parvum*. No such mortality events were noted in P-fertilized ponds. It is likely, however, that low morning dissolved oxygen of 0.63 mg/L observed in one high-P pond may have resulted in fish mortality.

Conclusions

Phosphorus decline at cool pond temperatures was estimated to be 0.03 µg/L/h. Phosphorus decline rates were not determined at varied chlorophyll *a* concentrations nor at different temperatures. Under the conditions of this study an average phosphorus addition of 99 µg/L was required to achieve a target concentration of 60 µg/L.

Bioassays were not very helpful in determining phosphorus effects on *P. parvum* because of inconsistent results and variable bioassay methods. Bioassay methods should be standardized to avoid this problem in the future.

Cell densities did not appear to be affected by phosphorus fertilization in the short term at low temperatures (13ºC). However, at temperatures typical of striped bass culture (22ºC) *P. parvum* cells appeared to be eliminated in ponds fertilized with high levels of phosphorus (92 µg/L).

Under the culture conditions during objective 2, target phosphorus rates were grossly exceeded and total nitrogen concentrations were not determined so N:P ratios were unknown. No differences were found in indicators of productivity (dissolved oxygen, pH and chlorophyll *a*) between fertilization rates. However, productivity of control ponds differed significantly from that of P-fertilized ponds. It is likely that phosphorus was in excess of algal needs in both treatments and it is also possible that nitrogen limitation affected algal dynamics since nitrogen concentrations were lower in P-fertilized ponds than control ponds. Both phosphorus fertilization rates may be unsafe for striped bass production due to resulting elevated pH.

The most important indicator of efficacy of any fish culture pond treatment is fish production. The standard DSFH fertilization regime (control ponds) resulted in complete mortality of fish in two ponds near the end of the culture period. However, bioassays did not confirm that this mortality was due to *P. parvum* toxicity. If mortality was due to *P. parvum* it occurred at low cell densities. One control pond had cell densities of 1,000/mL and cells were not found in the other two control ponds. Algal cell counts may be inadequate to accurately warn of impending fish kills if mortality was due to *P. parvum*. Low dissolved oxygen in P-fertilized ponds likely resulted in fish mortality, which may have obscured differences in fish production between treatments. It is possible that fertilization rates more in line with those targeted in the original proposed rates may have less affect on pH and dissolved oxygen while providing a reduction in *P. parvum* toxicity. However, this can only be adequately determined by repeating the experiment.

Overall, this study provided some compelling evidence that phosphorus fertilization was beneficial in reducing *P. parvum* densities and toxicity in striped bass production ponds; however, more work is required to better validate these findings and to refine inorganic

fertilization strategies. The following problems, modifications and recommendations need to be addressed before the study is repeated:

1. The ability to accurately measure nitrate and ammonia concentrations needs to be insured through appropriate training and quality control measures.
2. Bioassays should be standardized with respect to test organisms, use of cofactor, and criteria used to determine if the test should be repeated.
3. Fertilization rates should be accurately calculated and verified via entry into the FHS system.
4. Phase 1 should be completed between now and initiation of phase 2.
5. Nutrient ratios and concentrations may need to be reconsidered or the number of treatments expanded based on results from the high-P treatments.
6. Methods for assessing *P. parvum* cell densities may need to be refined.

TABLE 1.—Striped bass fingerling production in ponds fertilized with high or low levels of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

Treatment (N:P ratio)	N:P rates (µg/L)	Pond	Fish stocked	Fish harvested	Weight harvested (kg)	Harvest length (mm)	Survival (%)
Low P (10:1)	300 : 30	7	50,156	6,792	6.85	44.3	0.14
Low P (10:1)	300 : 30	15	50,184	1,392	0.68	36.0	0.03
Low P (10:1)	300 : 30	16	50,184	10,270	7.17	41.6	0.20
High P (5:1)	300 : 60	9	50,000	4,317	5.22	47.2	0.09
High P (5:1)	300 : 60	13	50,184	0	0.00	0.00	0.00
High P (5:1)	300 : 60	67	52,693	0	0.00	0.00	0.00
Control	0	10	50,184	0	0.00	0.00	0.00
Control	0	12	50,184	0	0.00	0.00	0.00
Control	0	14	50,184	167	0.00	39.2	0.00

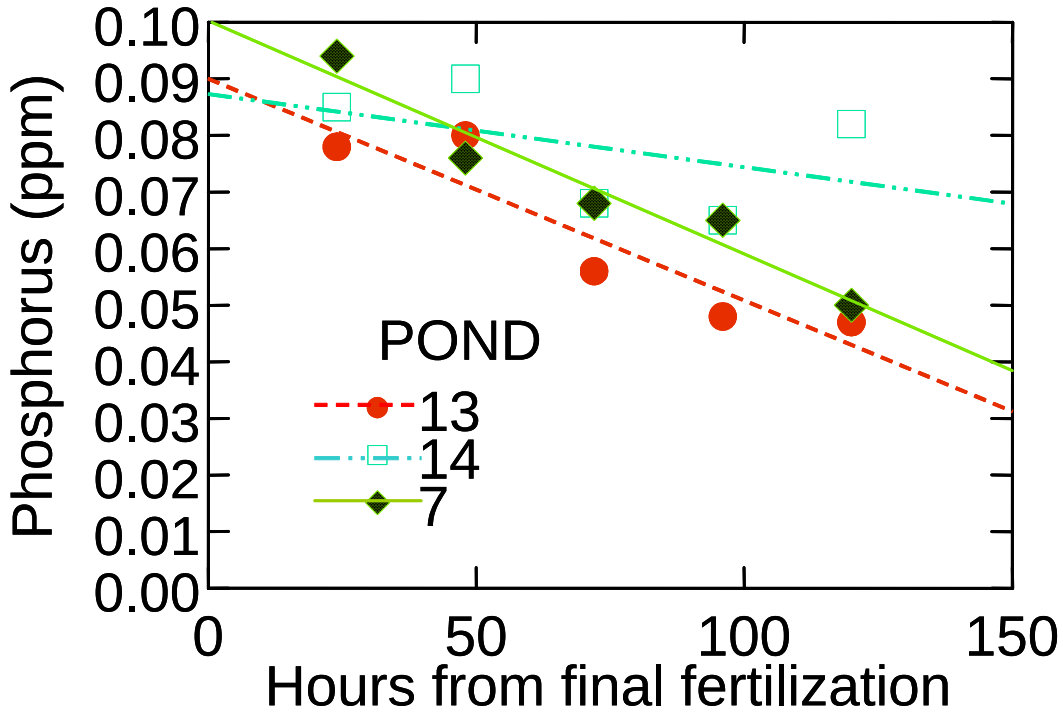

FIGURE 1.—Phosphorus decline rates in ponds fertilized with two 60-µg/L applications of phosphorus at the Dundee State Fish Hatchery in Spring 2002.

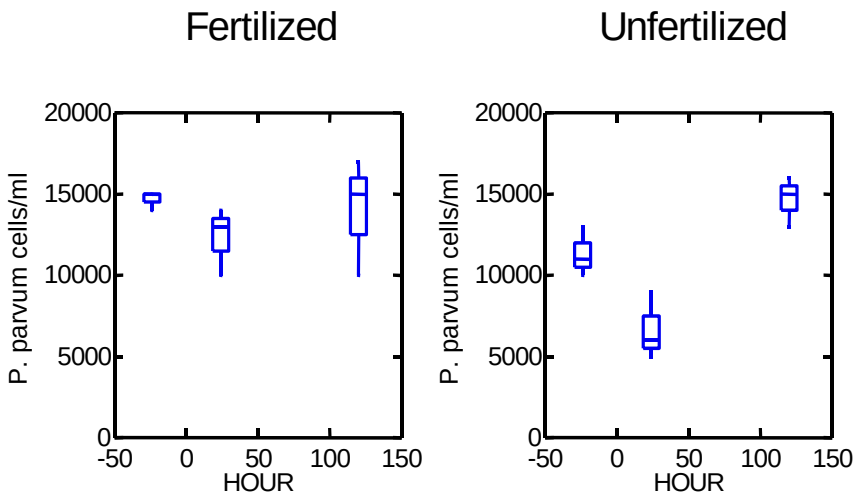

FIGURE 2.—*Prymnesium parvum* cell densities in ponds fertilized with 60 µg P/L or unfertilized with phosphorus at the Dundee State Fish Hatchery in Spring 2002.

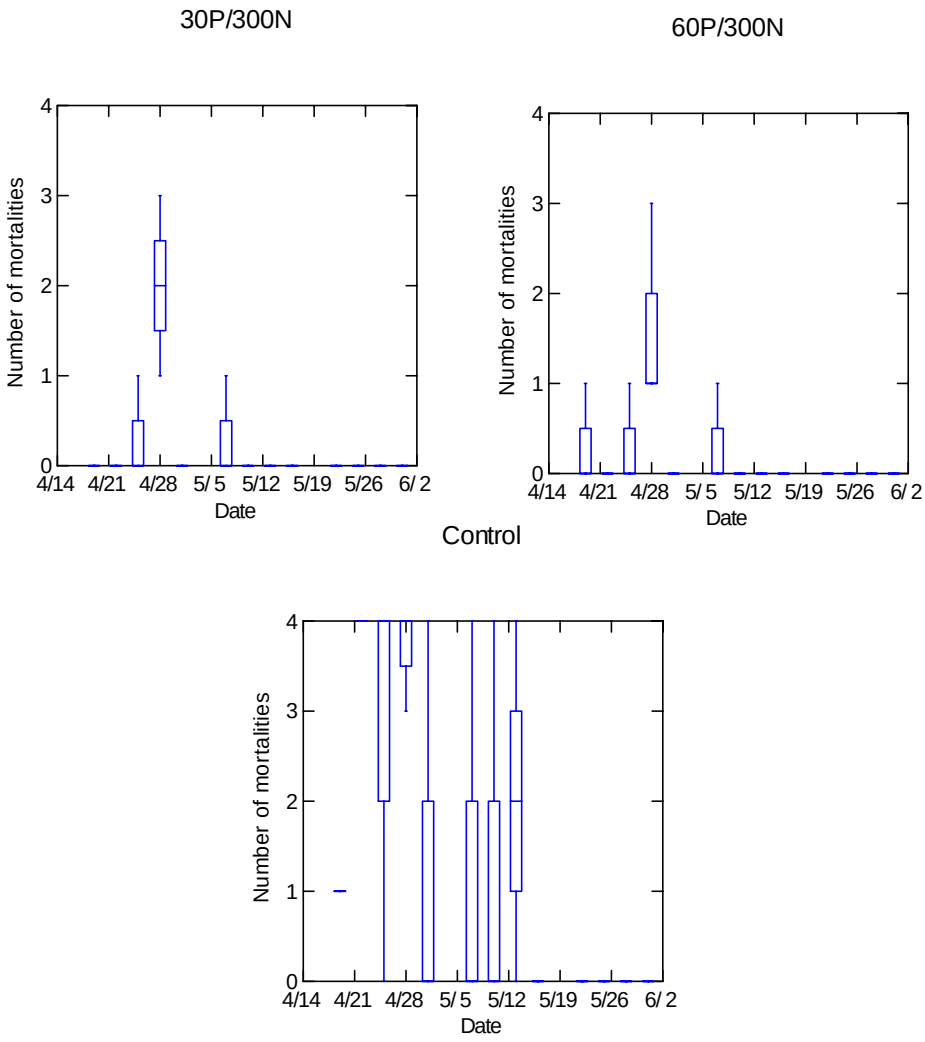

FIGURE 3.—Mortality among test fish in bioassays with undiluted water from ponds fertilized with high or low levels of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

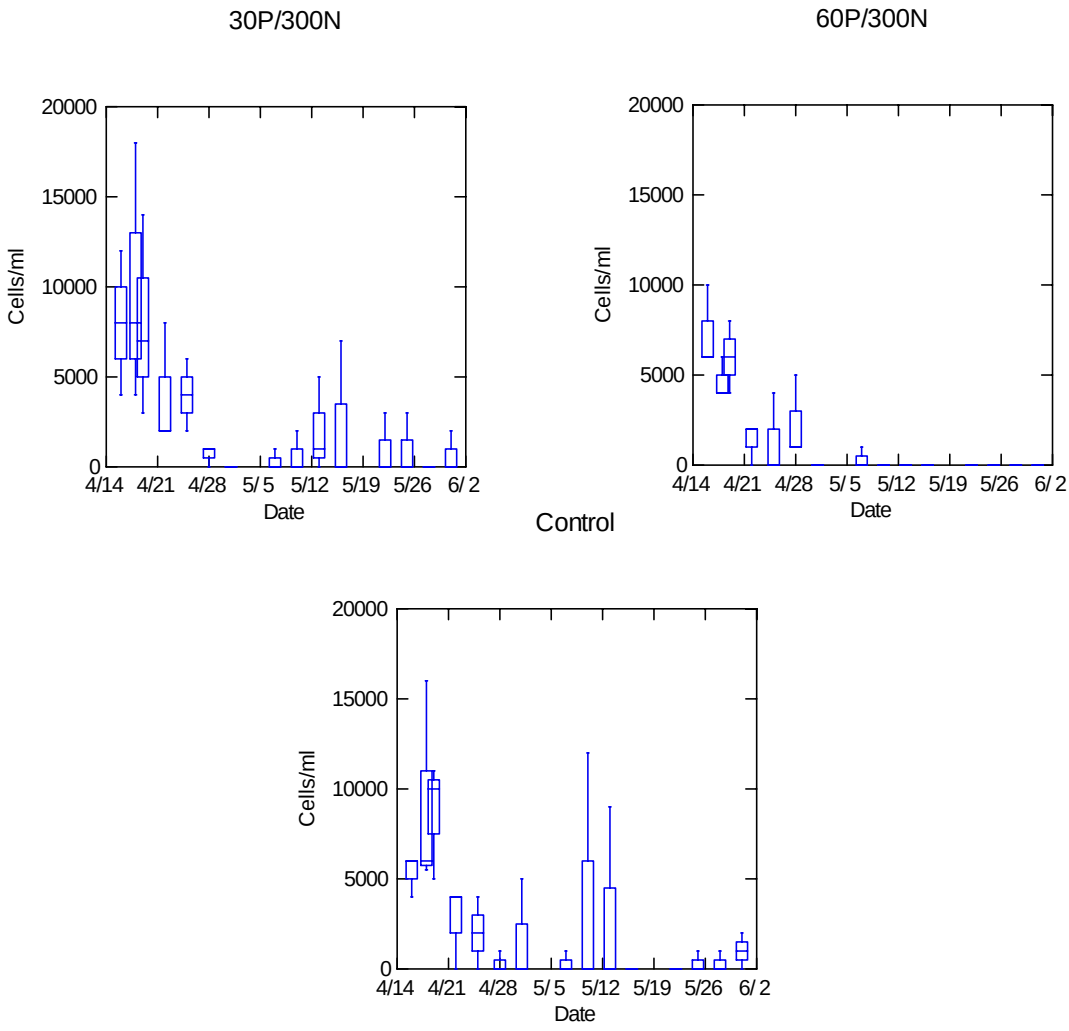

FIGURE 4.—*Prymnesium parvum* cell densities in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

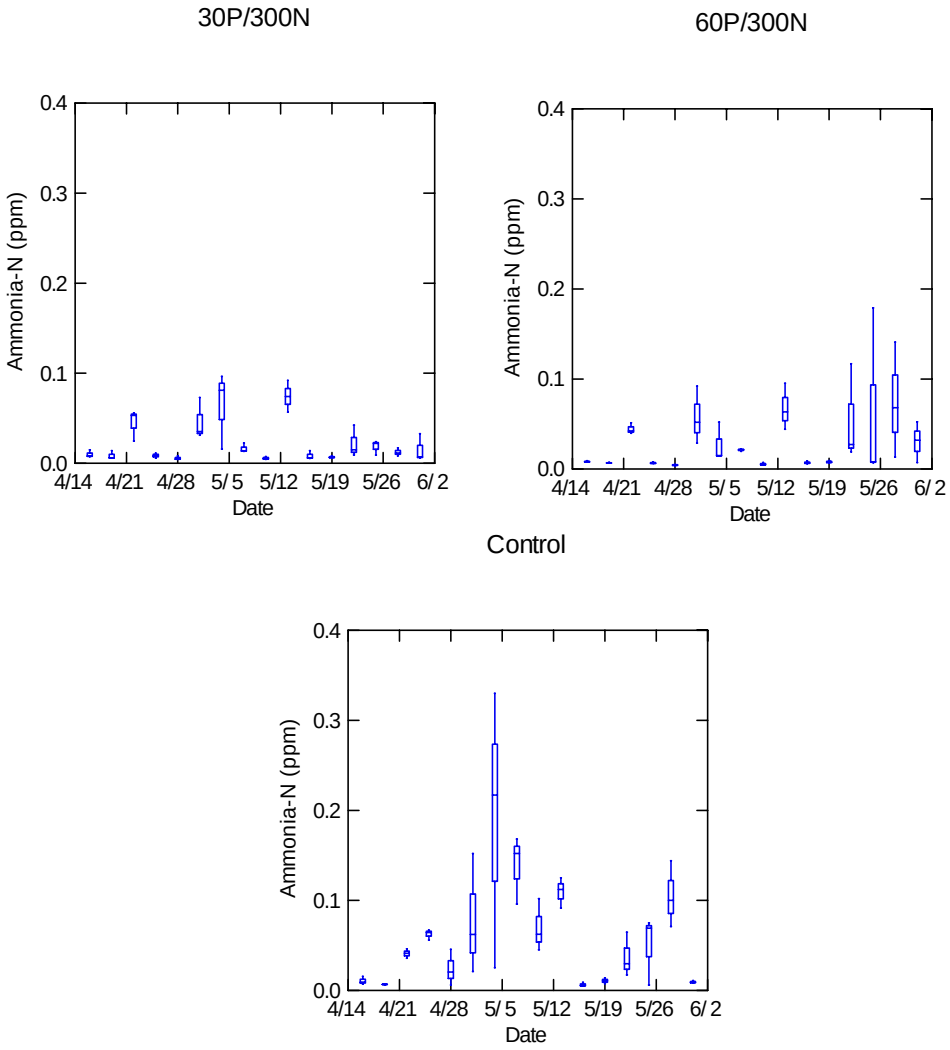

FIGURE 5.—Ammonia nitrogen concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

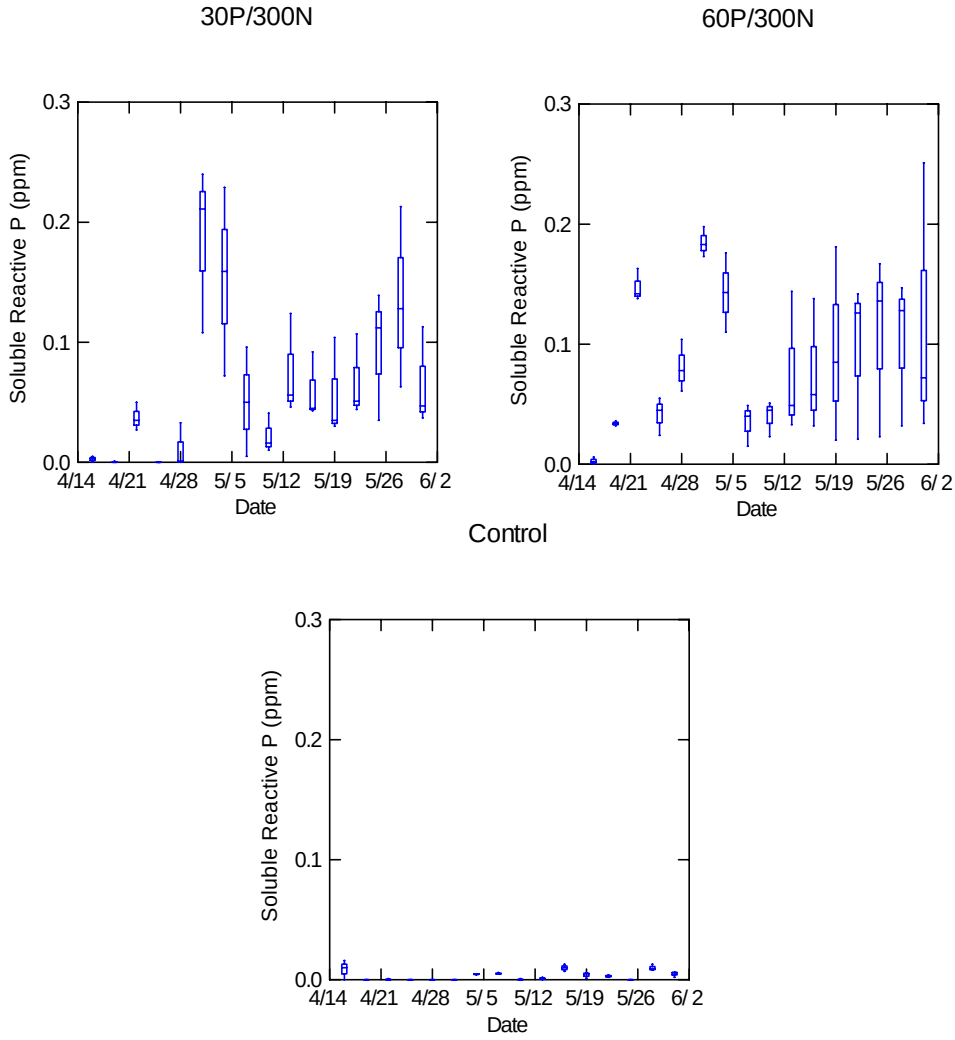

FIGURE 6.—Phosphorus concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

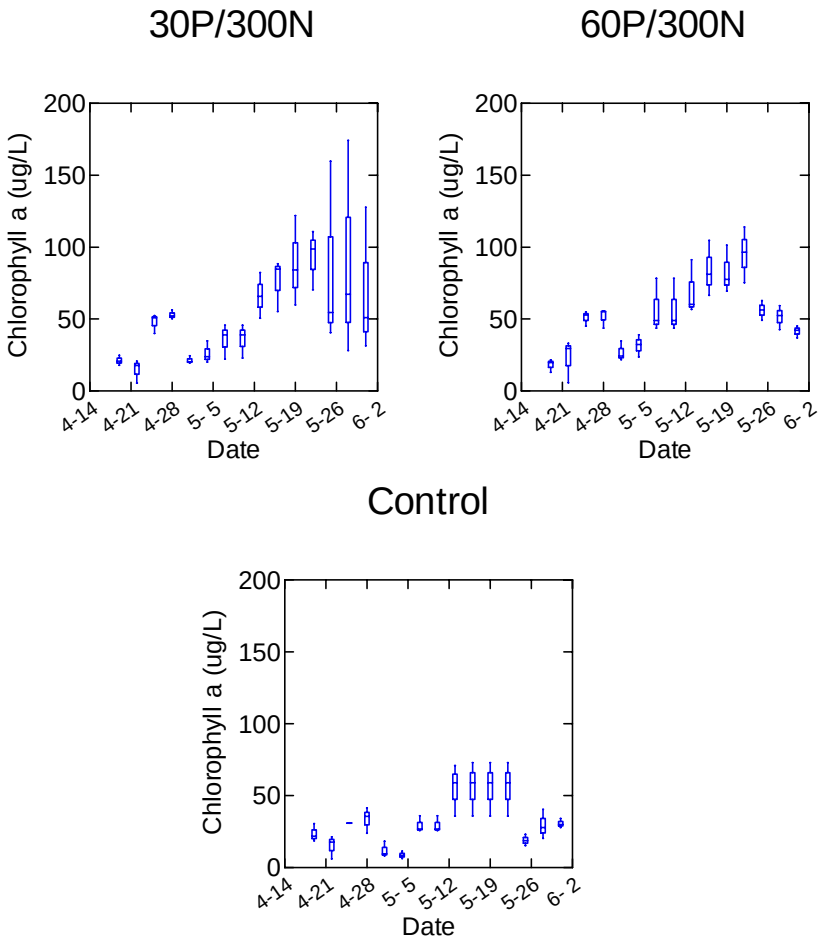

FIGURE 7.—Chlorophyll *a* concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

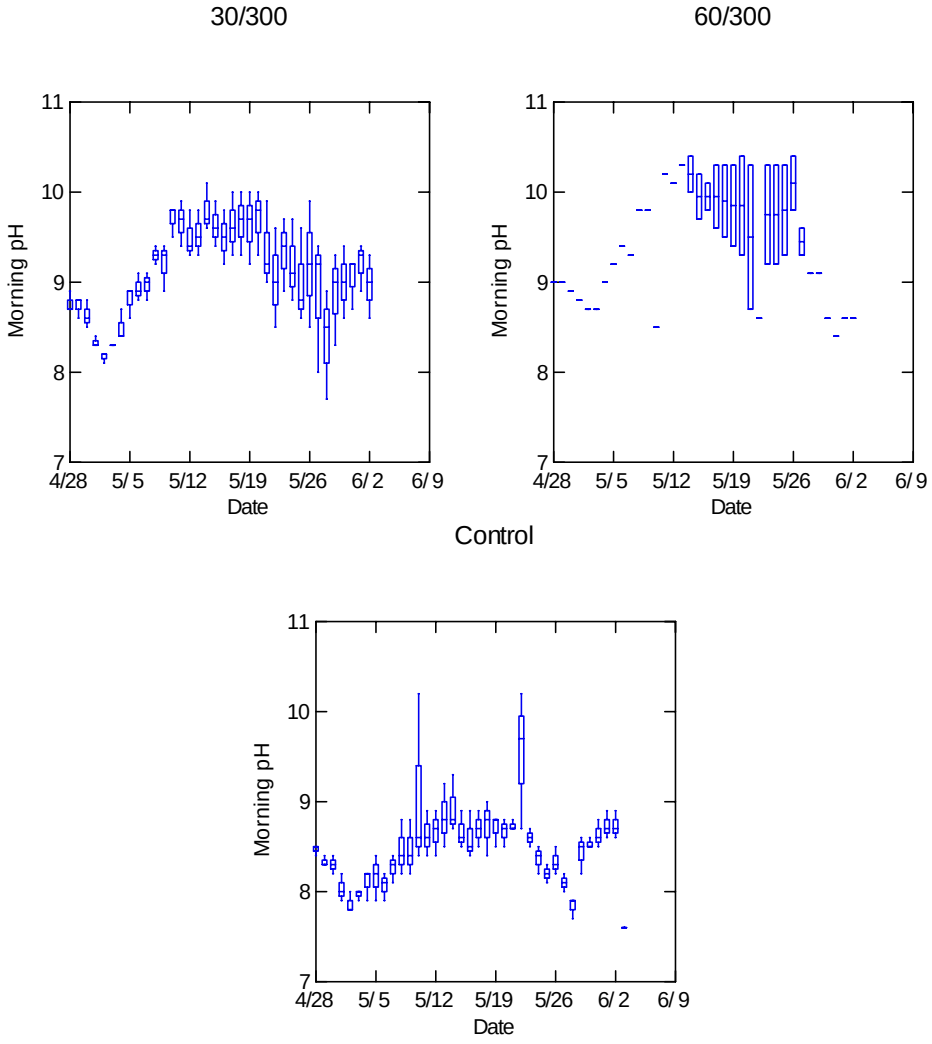

FIGURE 8.—Morning pH values in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

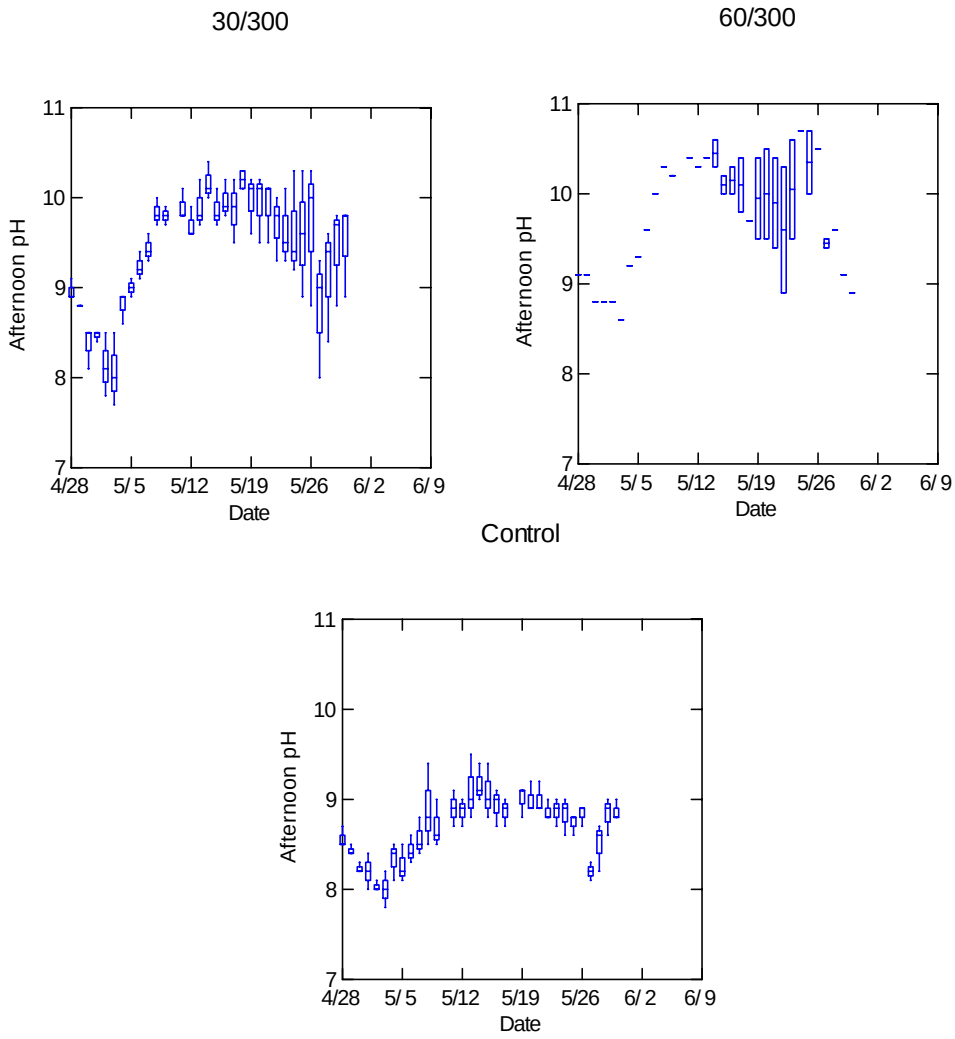

FIGURE 9.—Afternoon pH values in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

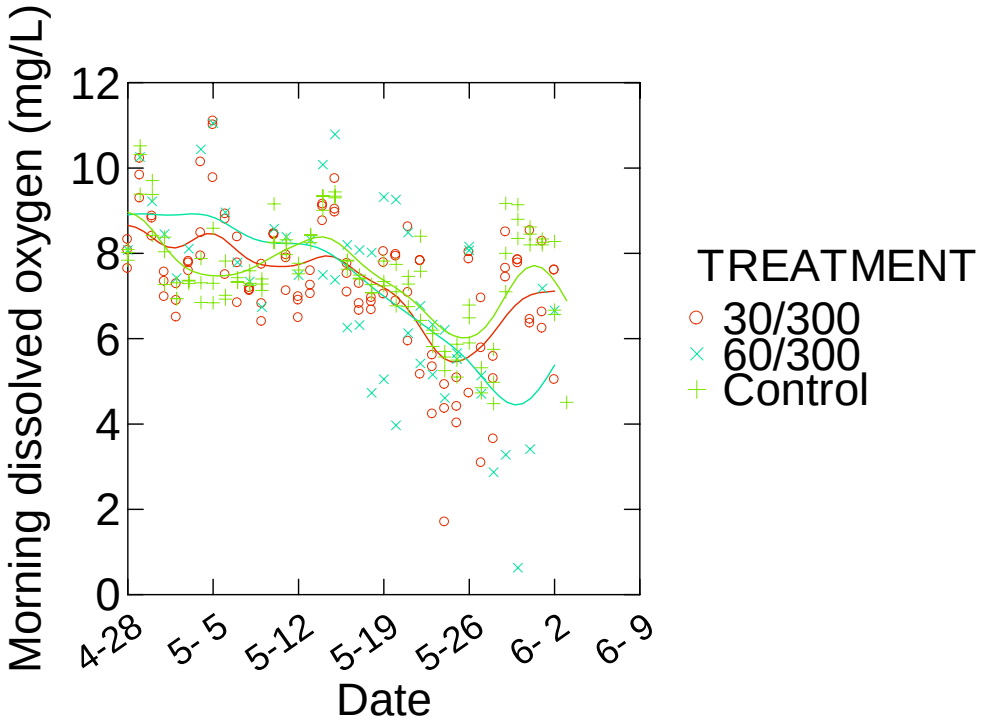

FIGURE 10.—Morning dissolved oxygen concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

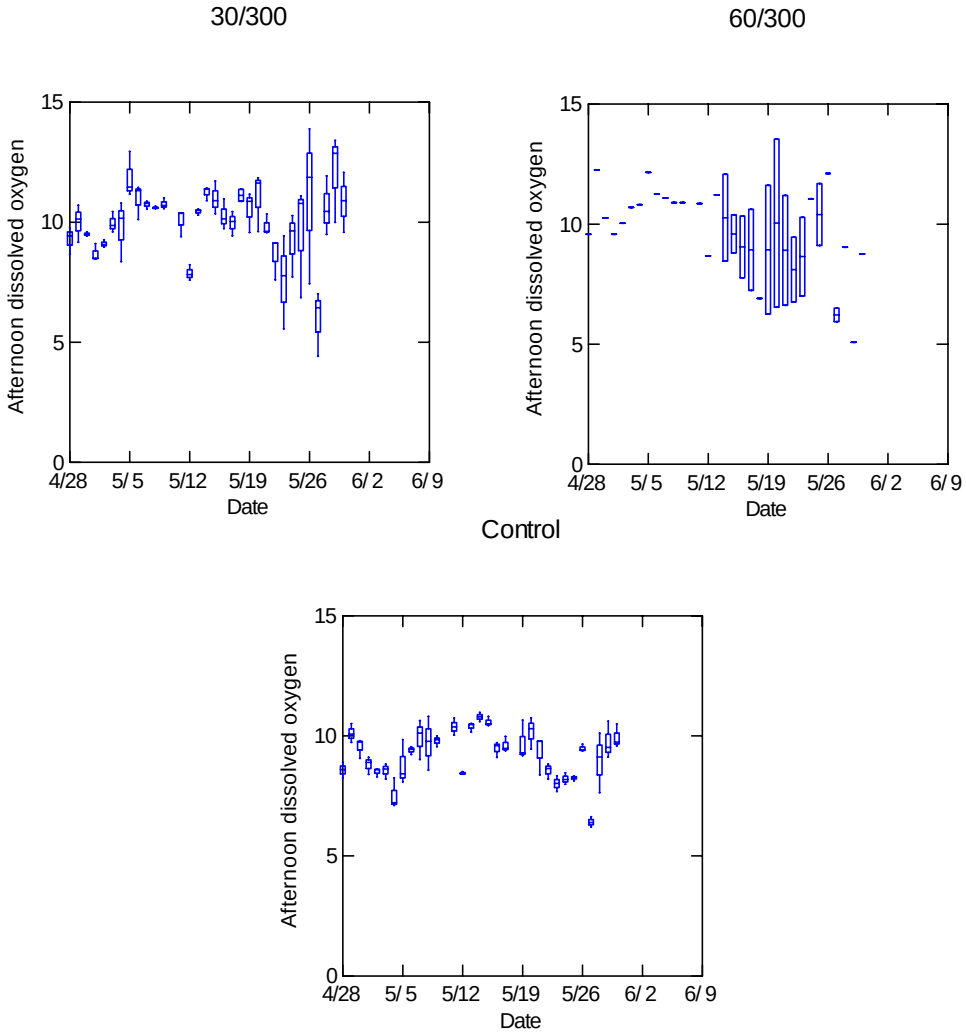

FIGURE 11.—Afternoon dissolved oxygen concentrations in ponds fertilized with high or low levels of phosphorus or no phosphorus at the Dundee State Fish Hatchery in Spring 2002.

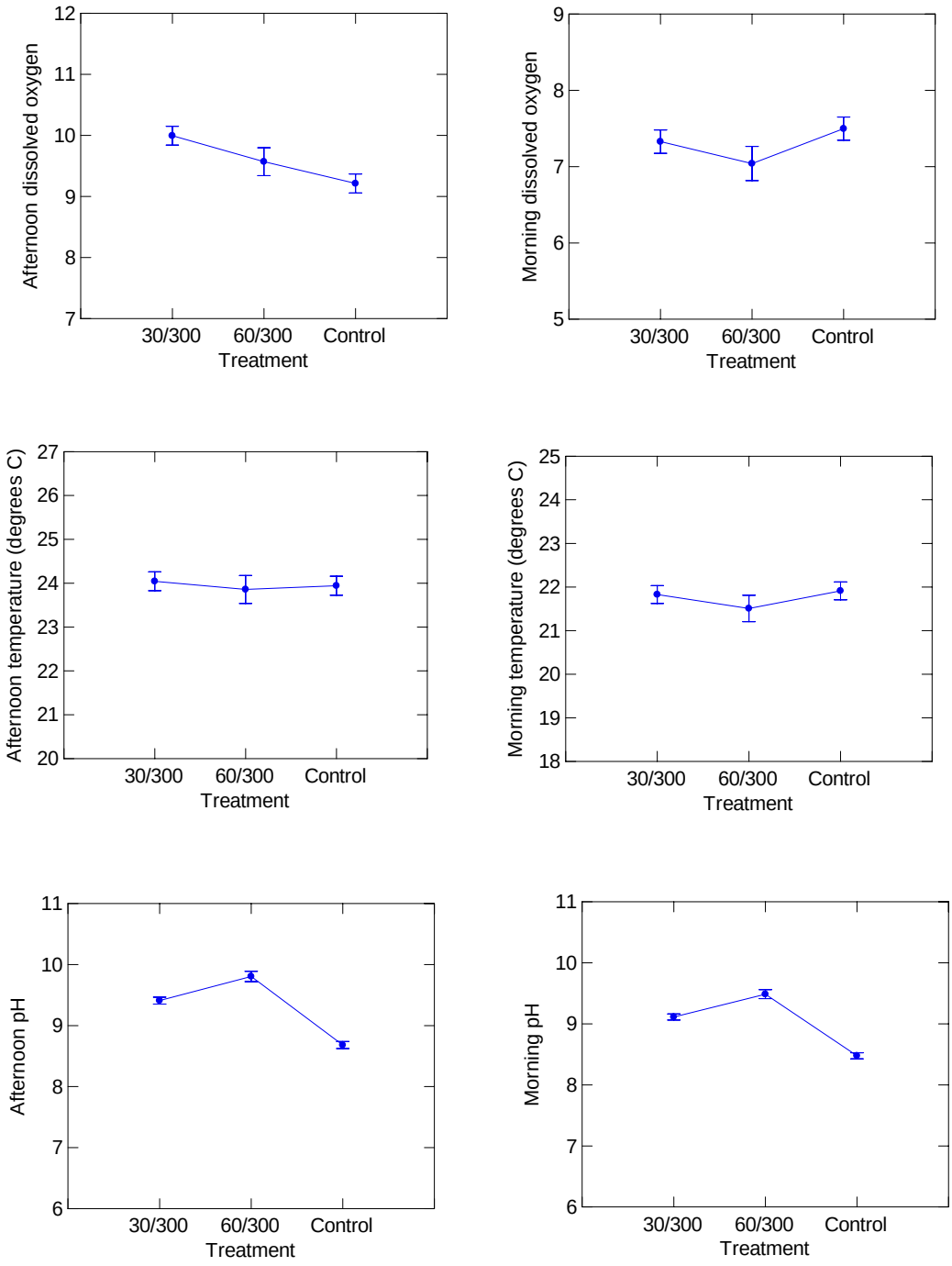

FIGURE 12.—Least squared means for water quality parameters in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

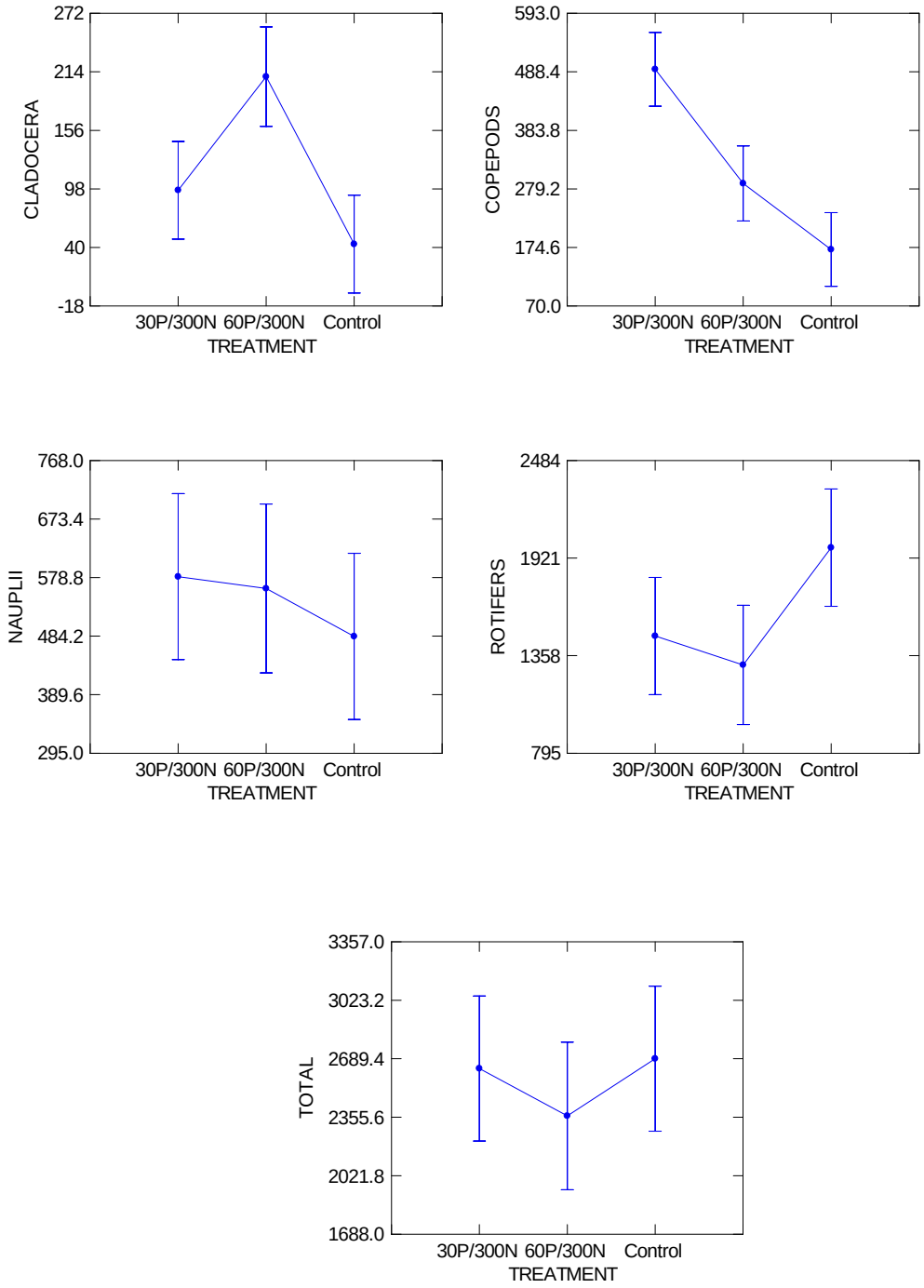

FIGURE 13.—Least squared means of zooplankton densities in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

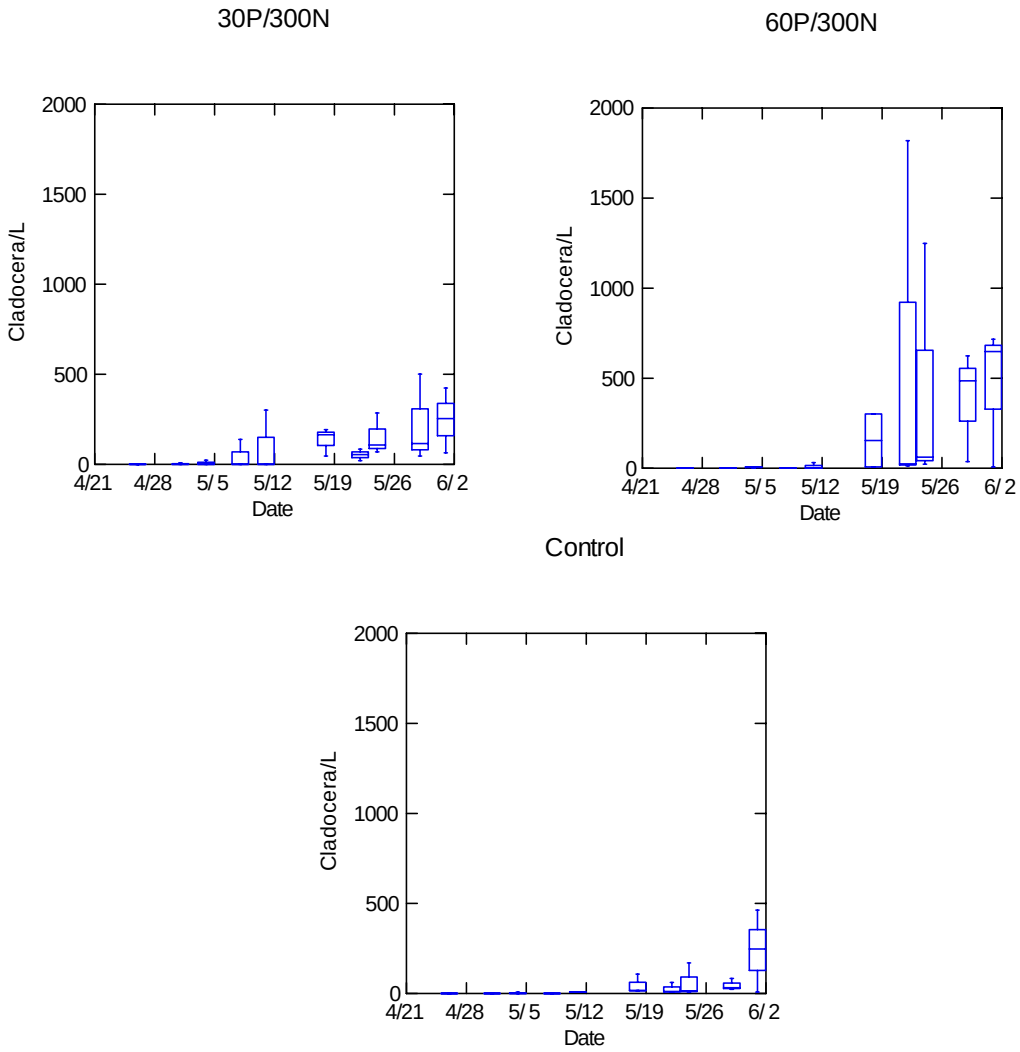

FIGURE 14.—Cladoceran densities in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in Spring 2002.

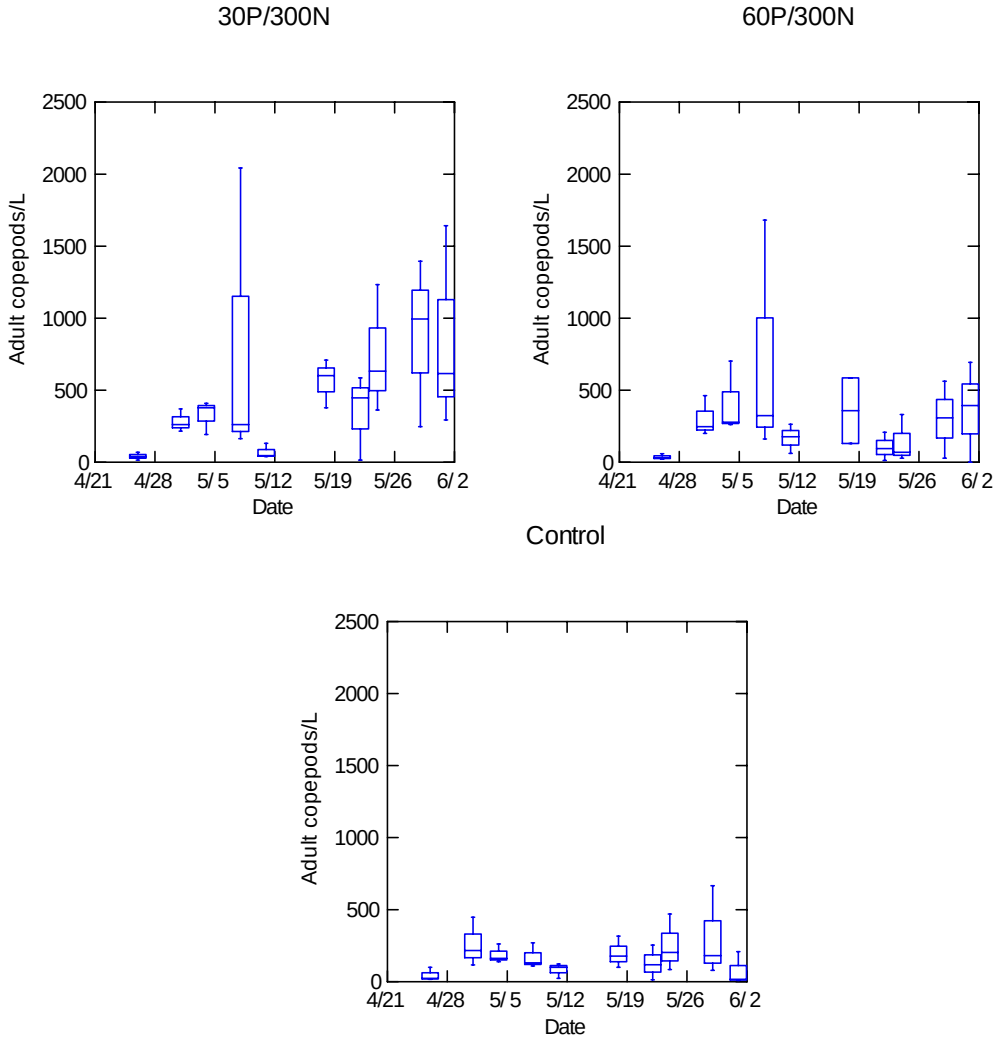

FIGURE 15.—Adult copepod densities in ponds fertilized with two concentrations of phosphorus or no phosphorus (control) at the Dundee State Fish Hatchery in spring 2002.