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Management Data Series
No. 260
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ACKNOWLEDGEMENTS

We thank the Possum Kingdom and Dundee state fish hatcheries staffs for manpower support and delivery of samples to the lab. Bob Betsill reviewed an earlier version and provided constructive suggestions to improve this paper. Support provided by the TPWD golden alga task force is greatly appreciated. This project was funded in part by State Wildlife Grant T-14-P and Federal Aid in Sport Fish Restoration Grants F-95-D and F-96-D to the TPWD.

ABSTRACT

In previous mesocosm experiments simulating aquaculture ponds, we found that fertilization with inorganic nitrogen (N) and phosphorus (P) eliminated the toxigenic alga *Prymnesium parvum*. However, the effective inorganic fertilization regimes (three times weekly applications of 300 µg N/L plus 30 µg P/L or 117 µg N/L plus 15 µg P/L) produced high pH and un-ionized ammonia (UIA) levels that are known to reduce survival of sensitive fish. In follow-up studies, we changed the source of N from NH_4-N to NO_3-N, reduced fertilization rates, and also examined the effect of N or P fertilization alone on *P. parvum*, pH, and UIA. NO_3-N was a suitable substitute for NH_4-N, and the fertilization regimes eliminated *P. parvum* and high UIA concerns, but still produced high pH. Reduction of fertilization rates resulted in decreased control of *P. parvum* and yielded little reduction in pH. Fertilization with N or P alone was ineffective in controlling *P. parvum.* In the present study, we used hatchery ponds to examine the effects of the most effective inorganic fertilization regimes in combination with a standard cottonseed meal fertilization regime on pH, water quality, chlorophyll *a*, zooplankton, and *P. parvum.* Cottonseed meal was used to counter elevated pH caused by inorganic fertilization. The results confirmed the efficacy of inorganic fertilization in controlling *P. parvum* in ponds, but the effect was dependent on the dose of N and P applied. Ponds with the highest dose of N and P had the fastest decline in ichthyotoxicity and *P. parvum* density. As observed in previous studies, *P. parvum* cell densities increased initially after inorganic fertilization, but then declined below detectable levels. Addition of inorganic fertilizers to the standard cottonseed meal regime also improved zooplankton abundance and chlorophyll-*a* concentration. The amplitudes of diel dissolved oxygen concentration and the risk for low morning dissolved oxygen concentrations were increased in these ponds compared to ponds fertilized with cottonseed meal alone. While the combination of inorganic with organic fertilization did reduce *P. parvum* density and toxicity, pH was not reduced to an acceptable level and was substantially higher than in ponds fertilized only with the cottonseed meal. Reducing pH to safe levels for fish is still an obstacle to overcome before implementing this *P. parvum* management technique in ponds where pH sensitive fish such as *Morone* spp. fingerlings are reared. The timing of pond filling and fertilization to achieve *P. parvum* control prior to fish stocking also needs refinement under this *P. parvum* management strategy.

INTRODUCTION

The toxigenic alga *Prymnesium parvum* can cause large losses of fish in brackish aquaculture ponds unless cell densities and ichthyotoxin concentrations are eliminated or substantially reduced (Tal and Shelubsky 1952; Shilo and Shilo 1953; Kaartvedt et al. 1991; Guo et al. 1996; Larsen and Bryant 1998). The Texas Parks and Wildlife Department (TPWD) Dundee state fish hatchery (DFH) and Possum Kingdom state fish hatchery (PKFH) have managed fish production ponds under the threat of *P. parvum* toxicity since 2001. These facilities use strategies that reduce both *P. parvum* cell densities and ichthyoxicity (Barkoh et al. 2003, 2004; Barkoh and Fries 2005). These strategies include the use of ammonium sulfate $\{(NH_4)_2SO4\}$ or copper sulfate ($CuSO_4$) to reduce cell densities and potassium permanganate ($KMnO_4$) to provide short-term control of ichthyotoxicity (Barkoh and Fries 2005; Barkoh et al. 2010). However, there are significant risks or limitations associated with these approaches in ponds used to culture striped bass *Morone saxatilis* fingerlings which are an important sportfish crop of these two TPWD fish hatcheries.

In striped bass fingerling rearing ponds, the use of copper sulfate is not appropriate because first feeding fry need natural zooplankton which is partially dependant on phytoplankton bloom development. Copper sulfate is toxic to phytoplankton, zooplankton, and aquatic insects (McKnight et al.1983; Welch et al.1990; Guo et al.1996; Irwin 1997) so the pond food web can be disrupted by the same tactic used to eliminate *P. parvum*. Ammonium sulfate can be highly effective for controlling *P. parvum*, however its use in the aquaculture of striped bass requires managing pH and un-ionized ammonia (UIA) within very narrow ranges (Barkoh et al. 2003; 2004). Ammonium sulfate may fail to control *P. parvum* in early spring if temperature and pH are not high enough to increase UIA to levels ($\geq$0.14 mg/L, Barkoh et al. 2010) that lyse *P. parvum* cells. Conversely, UIA levels may become toxic to striped bass when high concentrations of ammonium sulfate are required to overcome initial low pH and then the weather warms and pH increases rapidly. Potassium permanganate oxidizes the toxin to render it harmless, but because the oxidizing fraction (MnO_4^-) is "consumed" in the process, ongoing toxin production can require frequent reapplication to maintain control. Additionally, these methods are labor intensive and costly.

An alternative to these chemical treatments is to use inorganic fertilizers to manipulate the algal assemblage to favor dominance of desirable phytoplankters. Altering algal community composition through nutrient manipulation has been suggested for managing *Prymnesium* spp. blooms (Legrand 2001; Roelke et al. 2007). Guo et al. (1996) indicated that frequent pond fertilization with manures allowed native phytoplankton to out-compete *P. parvum*, resulting in the elimination of toxic episodes in fish culture ponds. Additionally, inorganic fertilization can prevent N- or P-deficient conditions which increase the risk of toxic bloom formation and cause *P. parvum* to produce allelopathic or toxic substances that inhibit growth of other algae (Johansson and Granéli 1999, Legrand 2001, Granéli and Johansson 2003).

We have investigated fertilization with inorganic N and P as a method of *P. parvum* control since 2001 (Kurten and Smith 2005). While we have not determined whether the mechanism of *P. parvum* control is through reduction of allelopathy, competitive exclusion by

other algae, or a combination of both, we have found that inorganic fertilization is a potentially viable method to eliminate *P. parvum* blooms and ichthyotoxicity. Fertilization with N (300 µg N/L) and P (30 µg P/L) three times weekly eliminated both *P. parvum* cells and toxicity within two weeks from the start of fertilization in mesocosms (Kurten et al. 2007). However, the use of ammonium chloride as an N source and a concomitant increase in pH due to phytoplankton overstimulation created concerns for the safety of this regime if used in ponds with fish species sensitive to elevated pH and UIA concentrations (Barkoh et al. 2004). Subsequently, we identified additional research goals to refine the fertilization strategy. These included: using NO_3-N as an alternate source of N to reduce the potential for high UIA; reducing fertilization rates to prevent phytoplankton overstimulation and high pH; and adding organic fertilizers as a source of CO_2 to achieve lower pond water pH (Barkoh and Rabeni 1990; Boyd 1990).

The use of NO_3-N as an alternative to NH_4-N was effective in producing lower UIA; however, lower inorganic fertilization rates yielded slower responses and smaller reductions in *P. parvum* cell densities and ichthyotoxicity (Kurten et al. 2010). The most effective of the tested inorganic fertilization regimes still promoted pH levels that are potentially lethal to striped bass fry, and thus detrimental to striped bass fingerling production. Because high pH is still a concern, we used the most promising inorganic fertilization regimes, derived from our previous mesocosm studies, in combination with organic fertilization to determine if a regime emerges that effectively controls *P. parvum* and promotes pH levels that are suitable for the production of striped bass fingerlings.

TPWD fish hatcheries have transitioned from managing striped bass fingerling ponds for high zooplankton populations to serve as the main food source for the fish (Geiger 1983a; 1983b; Geiger et al. 1985), which is usually associated with high pH, to managing for moderate pH levels and zooplankton densities and supplementing the diet of the fish with commercial feeds beginning two weeks after stocking the fry. This approach was implemented after the recognition that high pH (>9.0) is a source of striped bass mortality (Anderson 1993; Bergerhouse 1993; Barkoh 1996). Currently, ponds are fertilized solely with cottonseed meal because it is readily available in Texas at reasonable cost. Also, its low carbon-to-nitrogen ratio results in rapid decomposition to make nutrients availability to support plankton growth (Barkoh and Rabeni 1990; Buurma et al. 1996). In this study we used cottonseed meal, although studies have indicated other plant meals may promote lower pH (e.g., Barkoh and Rabeni 1990; Barkoh et al. 2005), because it is the current standard TPWD organic fertilizer. Further, Kurten et al. (1999) reported that cottonseed meal can also reduce pH through CO_2 evolution when used in conjunction with inorganic fertilizers. We investigated two levels of our previously developed inorganic fertilization rates for *P. parvum* control in combination with the standard TPWD cottonseed meal fertilization regime for *Morone* spp. fingerling rearing ponds (Lyon et al. 2006). The objective was to determine the effects on *P. parvum* density, ichthyotoxicity, and pH. Since initial zooplankton populations are an important component of current TPWD striped bass culture strategies, we also determined the effects of these treatments on zooplankton densities and composition and phytoplankton biomass (chlorophyll *a*). Also, we conducted this study in ponds to verify our results from the mesocosm experiments.

MATERIALS AND METHODS

This study was conducted in twelve 0.1-ha plastic-lined ponds at the DFH, Archer County, Texas from April 2 to May 26, 2007 during the normal period of phase 1 (35-45 mm) striped bass *Morone saxatilis* and palmetto bass (female *M. saxatilis* × male *M. chrysops*) fingerling production. Ponds were filled two weeks prior to fry stocking (day -14; day 0 is day of fry stocking) with water from Lake Diversion, Archer County, Texas which has the following water quality characteristics (mg/L): total suspended solids 19; total dissolved solids 2,923; CL 1,031; SO_4 654; Br 0.7; Ca 171; Mg 51; Na 550; K 14; and Si 3. Salinity, pH, and conductivity average 3 psu, 8.2, and 4,548 µmho/cm, respectively. Four ponds were randomly assigned to each of three treatments: control (no inorganic fertilization), 300N:30P (three times weekly applications of 300 µg N/L plus 30 µg P/L), and 117N:15P (three times weekly applications of 117 µg N/L plus 15 µg P/L). For the latter two treatments, inorganic fertilization was begun one week prior to pond stocking (day -7) and continued until day 39, one day before pond draining to harvest the fish began. Potassium nitrate (KNO3; 13.5-0-46.2) and phosphoric acid (0-54-0) were the inorganic fertilizers. All study ponds received three applications of cottonseed meal: 170.3 kg/ha at pond filling on day -14, 56.8 kg/ha during the week of fry stocking, and 56.8 kg/ha in the week after fry stocking – a TPWD standard organic fertilization regimen.

P. parvum cell densities were determined on Monday and Thursday of each week from fresh unfixed samples from each pond with a hemacytometer at 400X magnification by a single experienced individual (Barkoh and Fries 2005). Ichthyotoxicity bioassays were performed each Wednesday using fathead minnow *Pimephales promelas* fry as test animals at 28°C. Individual bioassays utilized water collected from each pond and four test animals were exposed to 100 mL undiluted water, 100 mL undiluted water plus 2 mL cofactor, or water diluted by 1/5 with *P. parvum*-free water plus 2 mL cofactor. Four fish were also placed in undiluted *P. parvum*-free water for control. The cofactor solution consisted of 0.003 M 3, 3'-iminobispropylamine and 0.02 M tris buffer (pH 9.0) and functions to enhance the toxicity of the *P. parvum* ichthyotoxin, allowing the otherwise sublethal levels to be quantifiable. Mortalities of test animals were determined after 2 h, and toxicity in terms of ichthyotoxicity units (ITU) was determined as follows: mortality in undiluted water indicated a high level of toxicity (25 ITU), mortality in 1/5 water dilution plus cofactor indicated moderate toxicity (5 ITU), mortality in undiluted water plus cofactor indicated low toxicity (1 ITU), and zero mortality in all bioassays indicated no toxicity (0 ITU).

Total P (TP), orthophosphate-P (PO_4-P), total Kjeldahl N (TKN), nitrate-N (NO_3-N), ammonia-N (NH_4-N), and chlorophyll *a* (corrected for pheophytin) were measured each Wednesday after pond filling. These analyses were performed by an independent laboratory (Texas Institute for Applied Environmental Research, Tarleton State University, Stephenville) using U.S. Environmental Protection Agency-approved methods (USEPA 1983) or standard methods (APHA 1995). Methods and detection limits were as follows: TP (USEPA 365.4; 22 µg P/L), PO_4-P (USEPA 365.3; 1.7 µg P/L), TKN (USEPA 351.2; 83 µg N/L), NO_3-N (USEPA 353.2; 7 µg N/L), NH_4-N (USEPA 350.1; 19 µg N/L), and chlorophyll *a* (APHA 10200H; 1.17 µg /L). Organic P was estimated by subtracting PO_4-P from TP, organic N by subtracting NH_4-N from TKN, and inorganic N as the sum of NO_3-N and NH_4-N (APHA et al.,

1998). Morning and afternoon dissolved oxygen (DO), temperature, and pH were measured daily at about 0700 and 1500 hours with a YSI 650 MDS handheld meter fitted with a YSI 600 XL multiprobe sensor (Yellow Springs Instrument, Yellow Springs, Ohio).

Zooplankton density was estimated in all ponds once each week between 0600 and 0700 hours by an oblique 4-m tow with 5.75-cm-diameter 80-µm-mesh Wisconsin plankton net to collect samples. Samples were preserved in Lugol's solution and densities of major zooplankton groups (cladocerans, copepod nauplii, copepod adults, and rotifers) were determined from two separate 1-mL aliquots using a zooplankton counting wheel (Aquatic Eco-systems, Inc., Apopka, FL) and variable magnification on a dissecting microscope.

Data were analyzed with PROC MIXED (SAS Institute 1999), assuming repeated measures construct with ponds as subjects. We tested a variety of covariance constructs (none, variance component, compound symmetry, first order autoregressive, and spatial power structure) until we found the one that best fit the data for each variable. The Akaike's Information Criterion and the Null Model Likelihood Ratio test were used to determine the model of best fit for the data. We tested the effects of treatment, sampling date, and the treatment × sampling date interaction on each variable. When the interaction was significant, we determined significant differences in daily mean values among treatments and control by using the MULTTEST procedure in the Statistical Analysis System (SAS) and a stepdown Sidak approach to control the family-wise error rate. When mixed models failed, data were analyzed by repeated measures ANOVA (split-plot design) with the GLM procedure of SAS. Ichthyotoxicity, chlorophyll *a*, pheophytin *a*, nutrient, and zooplankton data were log (X + 1) transformed and *P. parvum* cell density data were square root (X + 0.5) transformed before analysis due to skewness and zeros in the data. Values were considered significant at *p*-values less than or equal to 0.05.

RESULTS

Water quality

A cold front reduced initial pond water temperatures from 22°C to 10°C shortly after pond filling. After day -5, water temperatures rose gradually to a peak of about 30°C on day 27 (Figure 1). During the study, pond water temperatures averaged 19.3 - 19.6°C in the morning and 21.5 - 21.6°C in the afternoon: differences were not statistically significant for afternoon but significant for morning (Table 1). Although the morning temperatures in control ponds averaged 0.2 - 0.3 °C warmer than those in the inorganically-fertilized treatments, this likely was not biologically significant. Pond temperatures varied significantly over time, but mean morning or afternoon temperatures were similar among treatment and control groups on each sampling date.

Morning and afternoon pH differed significantly among treatment and control groups and temporally (Table 1; Figure 2). Average morning and afternoon pH appeared to be a function of the amount of inorganic fertilizer added to ponds by being highest for the 300N:30P treatment, moderate for the 117:15P treatment, and lowest for the control. On numerous sampling dates, morning or afternoon pH was significantly higher in the 300N:30P

treatment than in the 117:15P treatment (Figure 2). The drop and convergence of the two trends during days 20-24 corresponded to the 5 d of overcast and rainy weather.

Dissolved oxygen averaged 8.7 - 9.4 mg/L in the morning and 9.7 - 11.2 mg/L in the afternoon among treatments and control ponds (Table 1). A temporary decline in DO concentrations during days 20-24 coincided with a 5-d episode of overcast and rainy weather (Figure 3). Morning DO dropped below 5.0 mg/L on several days in inorganically-fertilized treatments during and after the overcast weather (Figure 4) but never below 6.1 mg/L in control ponds. The lowest morning DO was in ponds receiving the highest level of fertilization (300N:30P). Morning DO levels were higher in the inorganically-fertilized treatments than in the control (Figure 4; Table 1). Both morning and afternoon DO concentrations varied significantly among treatments and control over time and on most sampling dates (Table 1; Figure 3). On most days of lower afternoon DO concentrations, values for the control were significantly lower than those of the inorganically-fertilized treatments (Figure 3).

Nutrients and Phosphorus

Nitrogen concentrations in pond waters corresponded to the levels of N fertilization. Concentrations of all N fractions were highest in the 300N:30P treatment and lowest in the control except for NH_4-N which was similar between the117N:15P treatment and the control (Table 1). Although no NH_4-N was added by inorganic fertilization, concentrations increased during the first 10 d after fry stocking before declining through the end of the study (Figure 5). NO_{2+3}-N concentrations also were significantly different between treatments and increased over time in the 300N:30P treatment (Figure 5). Concentrations of NO_{2+3}-N were significantly higher in the 300N:30P treatment than the control from day -5 until the end of the study as well as in the 117N:15P treatment from day 2 onwards. Both NH_4-N and NO_{2+3}-N were highest in the 300N:30P treatment and relatively lower in the other two treatments (Figure 5). Differences in NO_{2+3}-N and NH_4-N concentrations were not significant between the 117N:15P and control treatments. Concentrations of TKN increased over the duration of the study and corresponded to the amount of inorganic nitrogen fertilizer applied (Figure 5; Table 1). Differences in TKN were significant among treatments and over time (Table 1; Figure 5). The difference in TKN concentration between treatments 300N:30P and 117N:15P was significant only on day 9. Beginning on day 2 the TKN concentrations for the control were significantly lower than those of the other two treatments.

Phosphorus concentration also was a function of phosphorus input through fertilization (Figure 6; Table 1). Concentrations of TP were significantly different over time and among treatments and increased over the course of the study, being higher in the inorganically-fertilized treatments than in the control (Table 1; Figure 6). From day 2 through16, the TP concentrations for 300N:30P were significantly higher than those of the control. On the last three sampling dates, concentrations of TP were significantly higher in the 300N:30P treatment than in the 117N:30P treatment or the control. Concentrations of PO_4-P varied significantly among treatments and temporally (Table 1; Figure 6). Prior to day 30, PO_4-P were near the detection limit of the analytical method. However, on days 23 - 37 the PO_4-P concentrations in the 300N:30P treatment were higher above the detection limit and significantly different from those of the other two treatments.

The organic plus inorganic fractions of both N and P closely mirrored the amounts of inorganic fertilizers applied (Figure 7). However, in the 300N:30P treatment a large fraction of inorganic nitrogen (2 mg N/L) was not converted to organic nitrogen and remained as NO_3-N (TKN; Figure 5). Conversely, most of the NO_3-N added to the 117N:15P treatment was converted to organic nitrogen because little NO_3-N was measured during the study. Inorganic P remained very low for all treatments and ultimately reached 40 µg/L for the 300N:30P treatment. This was only slightly more than a single 30 µg/L P dose of the 300N:30P treatment. Unlike N, almost all of the P applied was converted to organic P because final TP concentrations were near fertilizer application totals (Figure 7).

Phytoplankton and toxicity

Chlorophyll *a* concentration significantly differed among treatments and over time (Table 1), with the trends corresponding to fertilizer treatment doses (Table 1; Figure 8). From day 2 until the end of the study, chlorophyll *a* was significantly different between the control and the inorganically-fertilized treatments. On days 9 and 37 chlorophyll *a* concentrations were highest for the 300N:30P treatment, followed by the 117N:15P treatment, and lowest for the control (Figure 8). Pheophytin *a* concentration trends were similar to those of chlorophyll *a*, being highest in the treatment receiving the most inorganic fertilizer and lowest for the control (Figure 8). However, differences were never statistically significant between the 300N:30P and 117N:15P treatments on any sampling date. Pheophytin *a* concentrations for the control differed significantly from those of the 300N:30P treatment on days 9 and 37 and from those of the 117N:15P treatment on days 30 and 37.

Overall mean *P. parvum* cell density was highest in the 300N:30P treatment, followed by the 117N:15P, and then the control (Table 1). Cell densities varied significantly among treatments and the control and temporally (Table 1). Cell densities in the 300N:30P and 117N:15P treatments began to rise dramatically around day 0, peaking on days 10 and 5, respectively before declining (Figure 9). *P. parvum* densities were significantly higher in inorganically-fertilized treatments than in the control on days 3, 7, and 10 and in the 300N:30P treatment than in the 117N:15P treatment on days 7, 10, and 21. *P. parvum* was undetectable in the 300N:30P and 117N:15P treatments by day 21 and 28, respectively (Figure 9) but persisted at low cell densities in the control treatment through the end of the study.

Toxicity was present in all ponds from the day pond filling began (day -15) until after the target day for stocking moronid fry (day 0) for fingerling production (Figure 9). Ichthyotoxicity declined in a dose-response fashion to the level of inorganic fertilization. The degree of ichthyotoxicity was inversely related to average cell density and was lowest in the treatment receiving the highest inorganic fertilization (Table 1). Differences in toxicity were significant over time and among treatments and the control (Table 1). The 300N:30P treatment was nontoxic and significantly different from the other two treatments beginning on day 9, about 15 days before the control became nontoxic. The 117N:15P treatment became nontoxic and significantly different from the control on day 16, about 10 days before the control ponds became nontoxic. On the last two sampling dates, ichthyotoxicity was detected in the control treatment while both inorganically-fertilized treatment ponds remained nontoxic.

Zooplankton

The zooplankton population mainly consisted of rotifers, copepod nauplii, and adult copepods in descending order of abundance, and the differences in densities among treatments were not statistically significant, except for rotifers (Table 1). Mean density of rotifers was significantly lower in control ponds compared to the inorganically-fertilized treatment ponds. Densities in both inorganically-fertilized treatments were statistically similar. Zooplankton densities were low on the day of fry stocking (day 0) but increased dramatically after day 10 (Figure 10). Zooplankton peaked in 117N:15P treatment ponds by day 18 and in the control and 300N:30P treatment ponds by day 25. The initiation of zooplankton density peaks approximately coincided with the onset of chlorophyll *a* concentrations declines in inorganically-fertilized treatment ponds. The typical zooplankton succession (dominance trend: rotifers, copepod nauplii, adult copepods and cladocerans in that order) was not evident in this study. Copepods and rotifers co-occurred in statistically similar densities throughout most the study period with rotifers dominating the population between days 15 and 25. Cladocerans appeared during the last two sampling days. Total zooplankton density did not significantly differ among treatment and control groups.

DISCUSSION

P. parvum ichthyotoxicity became negligible approximately 10-15 days earlier in inorganically-fertilized ponds than in control ponds, and cell densities were eliminated from inorganically-fertilized ponds but not from control ponds. These results are consistent with those of our previous research in mesocosms (Kurten et al. 2007; 2010). The initial *P. parvum* response to inorganic fertilization was an increase in cell densities that was greater than the densities in the control. This increase in cell densities was greatest for ponds that received the most N and P. Following the initial increase in cell density, there was a reduction in ichthyotoxicity supporting the view that appropriate concentrations of nutrients (N and P) can reduce the propensity for *P. parvum* to produce toxins (Johansson and Granéli 1999, Legrand 2001, Granéli and Johansson 2003). Ichthyotoxicity was eliminated most rapidly in ponds receiving the most N and P where pH was also highest. Valenti et al. (2009) found that *P. parvum* toxins are likely more toxic at high pH, but we observed that toxicity was lowest for treatments achieving the highest pH. This suggests that nutrients addition likely reduced toxin production and with negligible toxin levels in pond waters pH was no factor in toxicity. Neither *P. parvum* nor toxicity was completely eliminated from ponds that did not receive inorganic fertilization. Even though the ponds that did not receive N and P ultimately experienced a decline in *P. parvum* densities to very low levels, an increase in toxicity towards the end of the simulated culture period would have rendered the ponds toxic near the time of fish harvest. These results confirm those of our mesocosm studies and support reports (e.g., Guo et al. 1996; Kurten et al. 2007; 2010) that the appropriate fertilization regime can control *P. parvum*.

The measured organic and residual inorganic nutrient fractions revealed the fate of the added inorganic fertilizers. Nearly 2 mg NO_3-N/L remained at the end of the experiment in ponds fertilized at the higher rates (300N:30P) while little inorganic P remained in either

inorganically-fertilized treatment. We would not recommend an overall reduction in N application rates because warmer water temperatures might require higher initial N concentrations to eliminate *P. parvum*. However, a reduction of N application rate later in the fertilization regimen might be warranted to reduce the potential waste of N and its release into receiving waters when ponds are drained to harvest fish.

In inorganically-fertilized ponds, chlorophyll *a* increased while *P. parvum* densities declined, in spite of grazing pressure from increasing zooplankton density. These trends correlated with the level of inorganic fertilization, suggesting that inorganic fertilization reduced the propensity of *P. parvum* to produce toxic substances and thereby eliminated its allelopathic or toxic effect on other algae (Granéli and Johansson 2003), or that *P. parvum* is not an effective competitor when nutrients are replete, or some combination of both. In the first case, further research may result in the development of a fertilization regime that would supply adequate nutrients to prevent stress in *P. parvum* and thereby prevent or minimize the production of ichthyotoxins. In the latter case, timing would be critical to insure that elimination of *P. parvum* and toxicity precedes stocking of striped bass fry into treated ponds.

Total zooplankton density did not significantly differ among treatments and control groups; however, the 30-52% higher densities in inorganically-fertilized treatments compared to the control could be of biological significance in supporting fish production in hatchery ponds where zooplankton is the primary source of food for fish. In addition, inorganic fertilization supported significantly higher rotifer densities of 43-60% more than the control. Rotifers are preferred first food of many larval fish including *Morone* spp. (Misra and Phelps 1992; Pfeiffer and Ludwig 2007; Ludwig and Lochmann 2007), and zooplankton of appropriate types and quantities are essential for successful production of fingerlings from planktivorous fry (Geiger 1983b; Geiger et al. 1985). Thus, these results reveal that the inorganic fertilization rates used in this study would enhance zooplankton density and composition in ways that benefits *Morone* spp. fingerling production.

While these results are promising because *P. parvum* was eliminated and zooplankton populations were enhanced, the treatments did not alleviate the issue of high pH as a source of mortality in *Morone* spp. fingerling aquaculture. Even at the lower inorganic fertilization rates, pH exceeded the upper threshold of 9.0 for successful production of *Morone* fingerlings (Anderson 1993; Barkoh 1998). Since lower doses of inorganic fertilizers appear to delay control of *P. parvum*, especially when initial cold weather delays phytoplankton population growth (Kurten et al. 2010), further improvement in pH control under the highest fertilization rates (300N:30P), which appears to provide consistent *P. parvum* control, is needed. The amount of cottonseed meal used in these ponds apparently did not provide enough CO_2 to effectively lower pH. Additional cottonseed meal application would likely increase the DO demand and produce lower DO minima to the detriment of fingerling fish production. Dissolved oxygen, chlorophyll *a*, and pH trends were similar, suggesting the observed elevated pH was mostly a function of CO_2 removal by phytoplankton for photosynthesis. Boyd (1990) provided a method that uses aluminum sulfate to reduce carbonate alkalinity and thereby reduce pH to 8.34. The 300N:30P treatment in conjunction with the aluminum sulfate method, or wheat shorts which Barkoh and Rabeni (1990) found to promote lower pH than cottonseed

meal, should be investigated for resolving the issue of high pH associated with using inorganic fertilization.

Another challenge to effective use of inorganic fertilization to control *P. parvum* in striped bass culture ponds is time to effect control. In our first mesocosm experiments (Kurten et al. 2007) *P. parvum* density and toxicity were controlled within 15 d, a period similar to the pre-stocking interval for production of *Morone* spp. fingerlings in ponds. In the present study, *P. parvum* densities and toxicity were not substantially reduced in 14-15 d or until 10 d after the target time for stocking fry into ponds. Water temperatures were relatively warm ($\geq$ 20 C) and stable during the first 13 d of the mesocosm study while they were substantially reduced below 20 C by late cold fronts during the same period in the present study. These cold temperatures also delayed production of zooplankton in ponds by the time of fry stocking. To minimize the adverse effects of cold weather on the efficacy of inorganic fertilization in controlling *P. parvum*, facilities located in areas that experience late cold fronts should increase the 2-week pre-stocking interval for *Morone* spp. fingerling aquaculture.

The adverse effects of *P. parvum* toxicity on fish production and the increasing cost of control will endure as long as the alga persists in the lakes that supply waters to the affected fish hatcheries. Currently, there are no solutions to the *P. parvum* problems in lakes and other large impoundments because the methods developed for hatchery ponds are either not feasible in terms of labor, materials, and costs or limited by pollution concerns and regulation (Sager et al. 2007; 2008). The results of the present and previous studies (Kurten *et al*. 2007; 2010) suggest that management strategies that would prevent inorganic nutrients from becoming limiting could prevent *P. parvum* bloom formation. Throughout our series of experiments to control *P. parvum* by nutrient manipulation, we have consistently observed high TKN concentrations associated with low inorganic N and P concentrations in the water supply from Lake Diversion. This suggests the lake is rich in organic nutrients and poor in inorganic nutrients, at least during certain periods of the year. This condition allows *P. parvum* to out-compete other algae that depend solely on inorganic nutrients and form dense blooms because it can thrive on various organic sources of N and P (Estep and McIntyre 1989; Johansson and Graneli 1999; Tillmann 2003; Michaloudi *et al*. 2009). We recommend future research investigate the link between nutrients and *P. parvum* population dynamics in the affected lakes. If our theory that "excessive organic nutrients and limiting inorganic nutrients contribute to *P. parvum* bloom formation" is proven, ecosystem management strategies could be implemented to reduce organic N inputs into the watersheds to deplete the niche for *P. parvum.*

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Table 1.—*P* values for model effects of repeated measures analysis of variance and mean (standard error) values of parameters measured in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Values for organic and inorganic N and P were derived and not analyzed using analysis of variance.

	Model Effects			Treatment mean (standard error)					
Parameter	Treatment	Day	Day X Treatment	300N:30P		117N:15P		Control	
Morning temperature (°C)	< 0.0001	< 0.0001	0.9388	19.4	(0.31)	19.3	(0.32)	19.6	(0.32)
Afternoon temperature (°C)	0.7216	< 0.0001	0.9946	21.6	(0.34)	21.5	(0.35)	21.6	(0.34)
Morning dissolved oxygen (mg/L)	0.0002	< 0.0001	< 0.0001	8.9	(0.15)	9.4	(0.15)	8.7	(0.07)
Afternoon dissolved oxygen (mg/L)	< 0.0001	< 0.0001	< 0.0001	11.2	(0.17)	11.2	(0.17)	9.7	(0.07)
Morning pH	< 0.0001	< 0.0001	< 0.0001	9.3	(0.05)	9.2	(0.05)	8.6	(0.02)
Afternoon pH	< 0.0001	< 0.0001	< 0.0001	9.5	(0.06)	9.4	(0.05)	8.7	(0.02)
Chlorophyll *a* (µg/L)	< 0.0001	< 0.0006	< 0.0001	98.9	(12.58)	71.3	(7.25)	31.4	(2.18)
Pheophytin a (µg/L)	< 0.0001	< 0.0006	0.0108	16.1	(3.24)	11.2	(2.55)	3.1	(0.56)
NH_4-N (mg/L)	0.0115	< 0.0001	0.0382	0.050	(0.0135)	0.013	(0.0043)	0.014	(0.0051)
NO_{2+3}-N (mg/L)	< 0.0001	< 0.0001	< 0.0001	1.122	(0.1492)	0.100	(0.0262)	0.005	(0.0048)
Total Kjeldahl N (mg/L)	< 0.0001	< 0.0001	0.0002	2.372	(0.2507)	1.731	(0.1887)	0.921	(0.0954)
Total P (mg/L)	< 0.0001	< 0.0001	< 0.0001	0.354	(0.0333)	0.239	(0.0173)	0.142	(0.0110)
PO_4-P (mg/L)	0.0092	0.0530	0.0134	0.017	(0.0043)	0.004	(0.0013)	0.003	(0.0060)
Organic N (mg/L)	.	.	.	2.322	(0.2464)	1.718	(0.1877)	0.907	(0.0948)
Organic P (mg/L)	.	.	.	0.337	(0.0313)	0.235	(0.0174)	0.139	(0.0111)
Inorganic N (mg/L)	.	.	.	1.173	(0.1531)	0.113	(0.0167)	0.019	(0.0066)
P. parvum density (cells/mL)	0.0010	< 0.0001	< 0.0001	11,003	(1,720.0)	8,177	(1,133.3)	5,917	(6,213.7)
Ichthyotoxicity units	< 0.0001	< 0.0001	< 0.0001	9.5	(2.16)	12.6	(2.23)	17.4	(2.02)
Cladocerans/L	0.9544	< 0.0001	0.9753	5	(3.5)	4	(2.8)	3	(2.1)
Adult copepods/L	0.4610	< 0.0001	0.0248	113	(25.7)	109	(28.7)	75	(16.5)
Copepod nauplii/L	0.4857	< 0.0001	0.4029	211	(65.6)	327	(113.7)	251	(70.9)
Rotifers/L	0.0069	< 0.0001	0.0268	1,018	(409.9)	1,138	(413.9)	711	(249.6)
Total zooplankton/L	0.4857	< 0.0001	0.0702	1,346	(443.5)	1,579	(463.5)	1,036	(282.3)

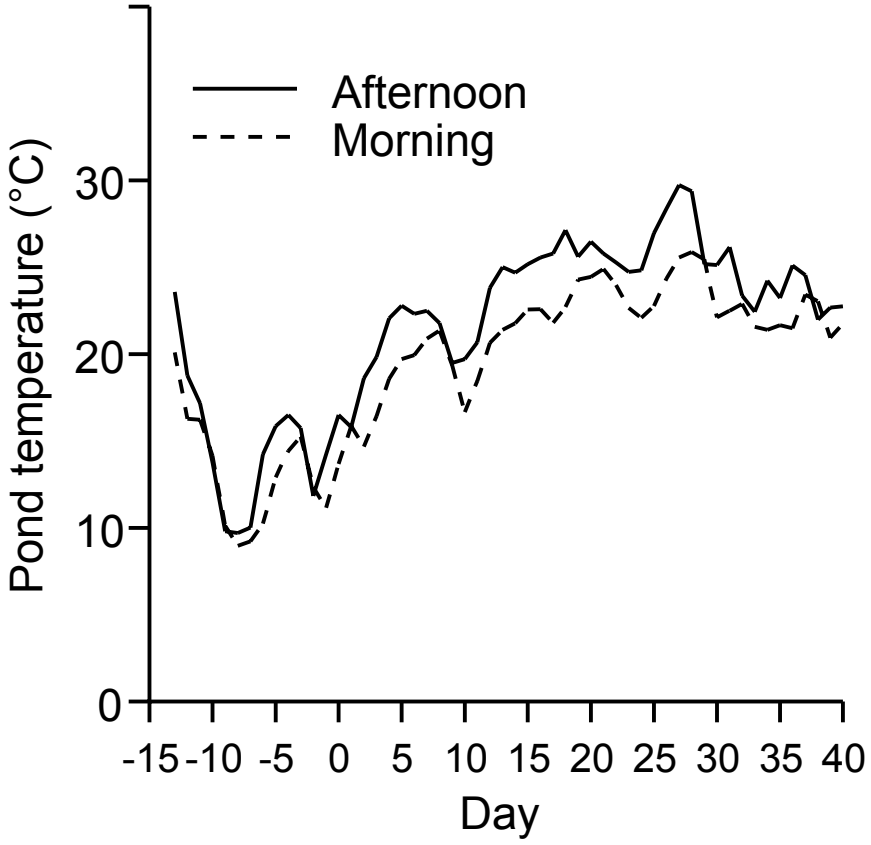

Figure 1.—Trends of pooled morning and afternoon water temperatures in ponds fertilized with cottonseed meal only or cottonseed meal plus inorganic fertilizers at the Dundee state fish hatchery in spring 2007. No statistical differences ($P > 0.05$) in temperature existed among treatments.

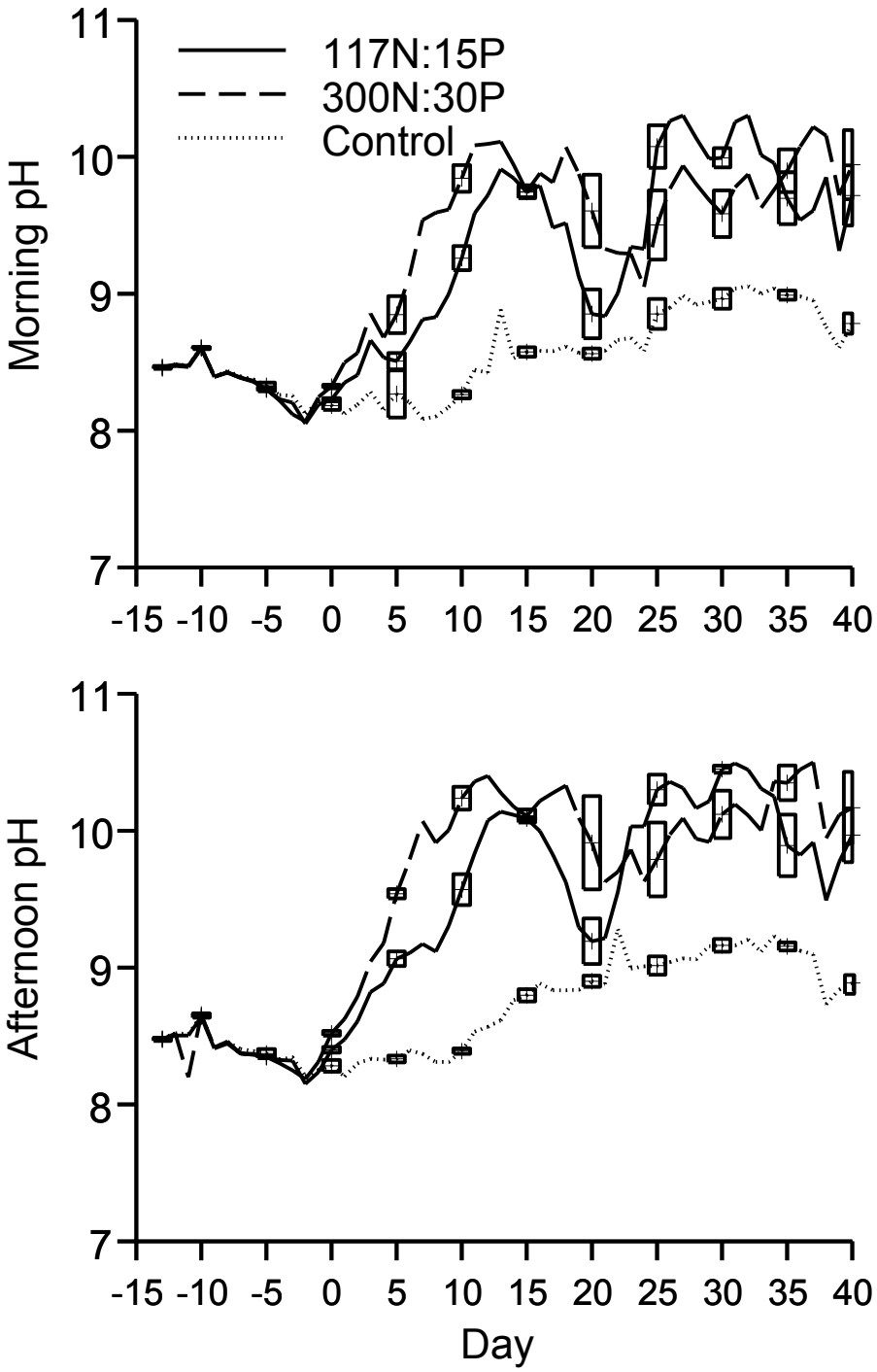

Figure 2.—Trends of morning and afternoon pH in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Boxes are standard errors at 5-d intervals for clarity.

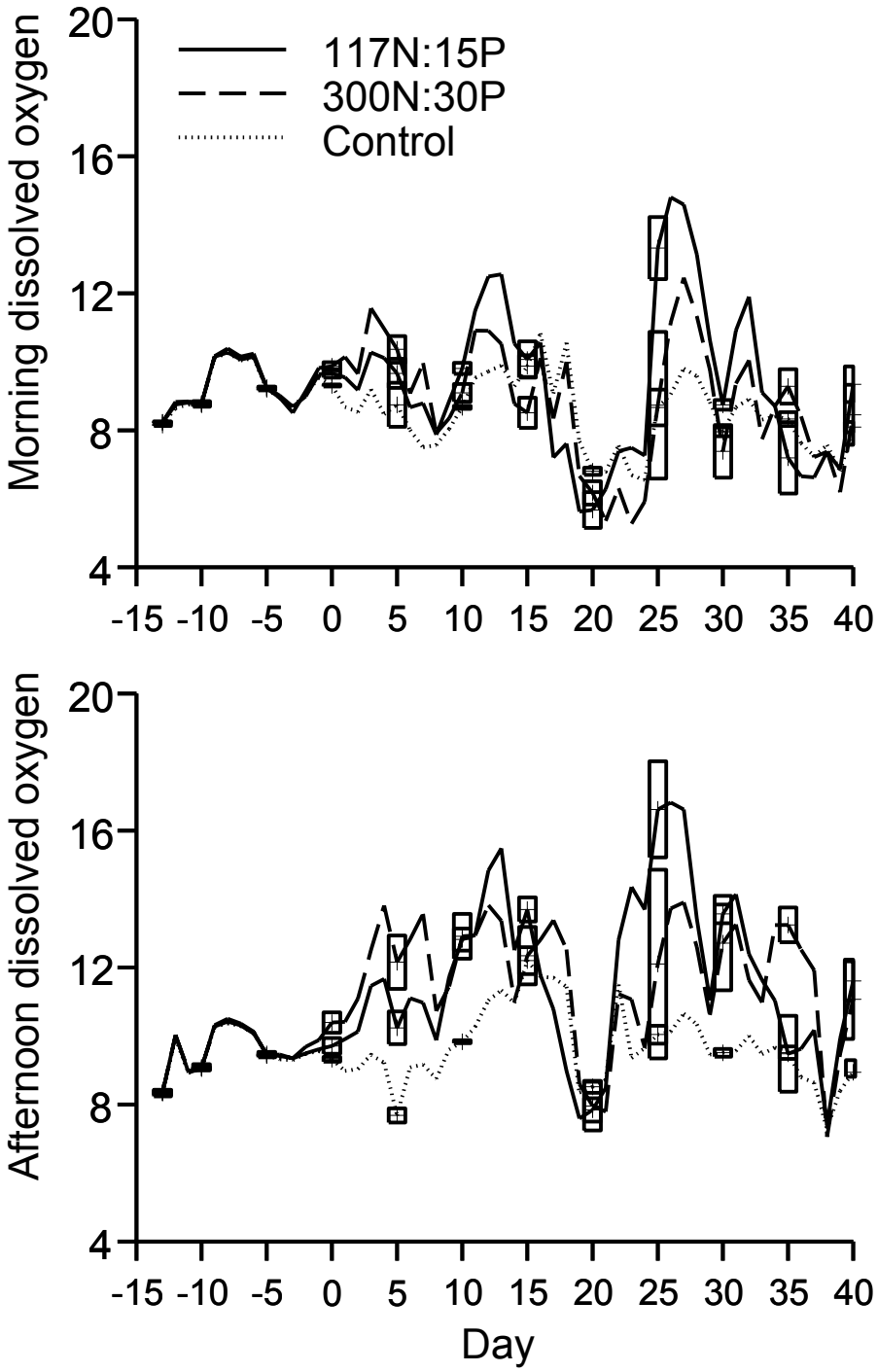

Figure 3.—Trends of morning and afternoon dissolved oxygen (mg/L) in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Boxes are standard errors at 5-d intervals for clarity.

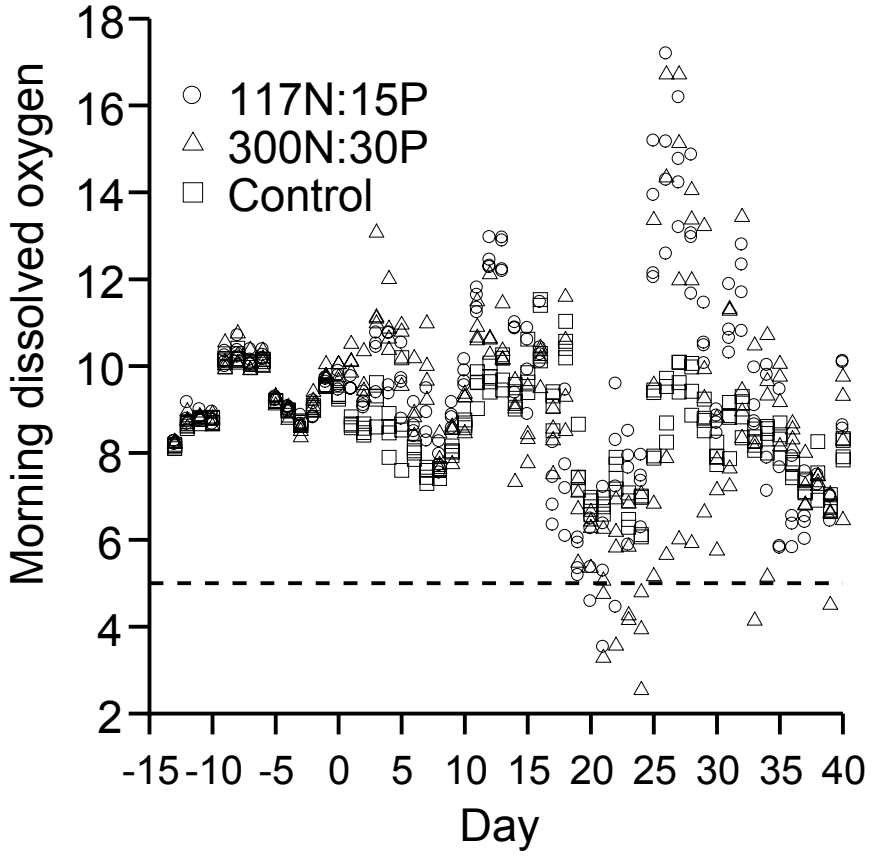

Figure 4.—Distribution of morning dissolved oxygen (mg/L) values from ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. The horizontal dashed line is 5.0 mg/L dissolved oxygen.

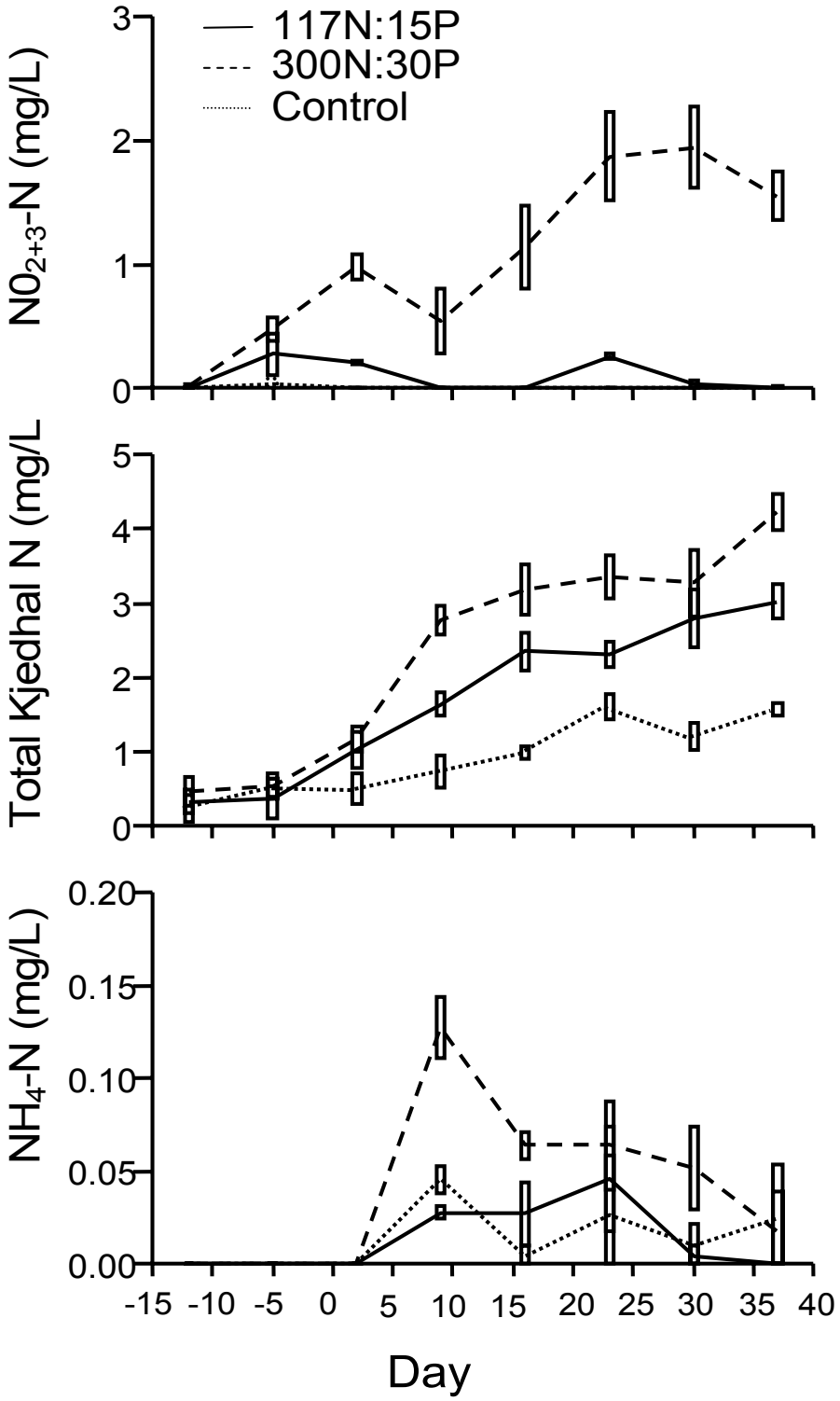

Figure 5.—Trends of nitrogen concentrations in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Boxes are standard errors.

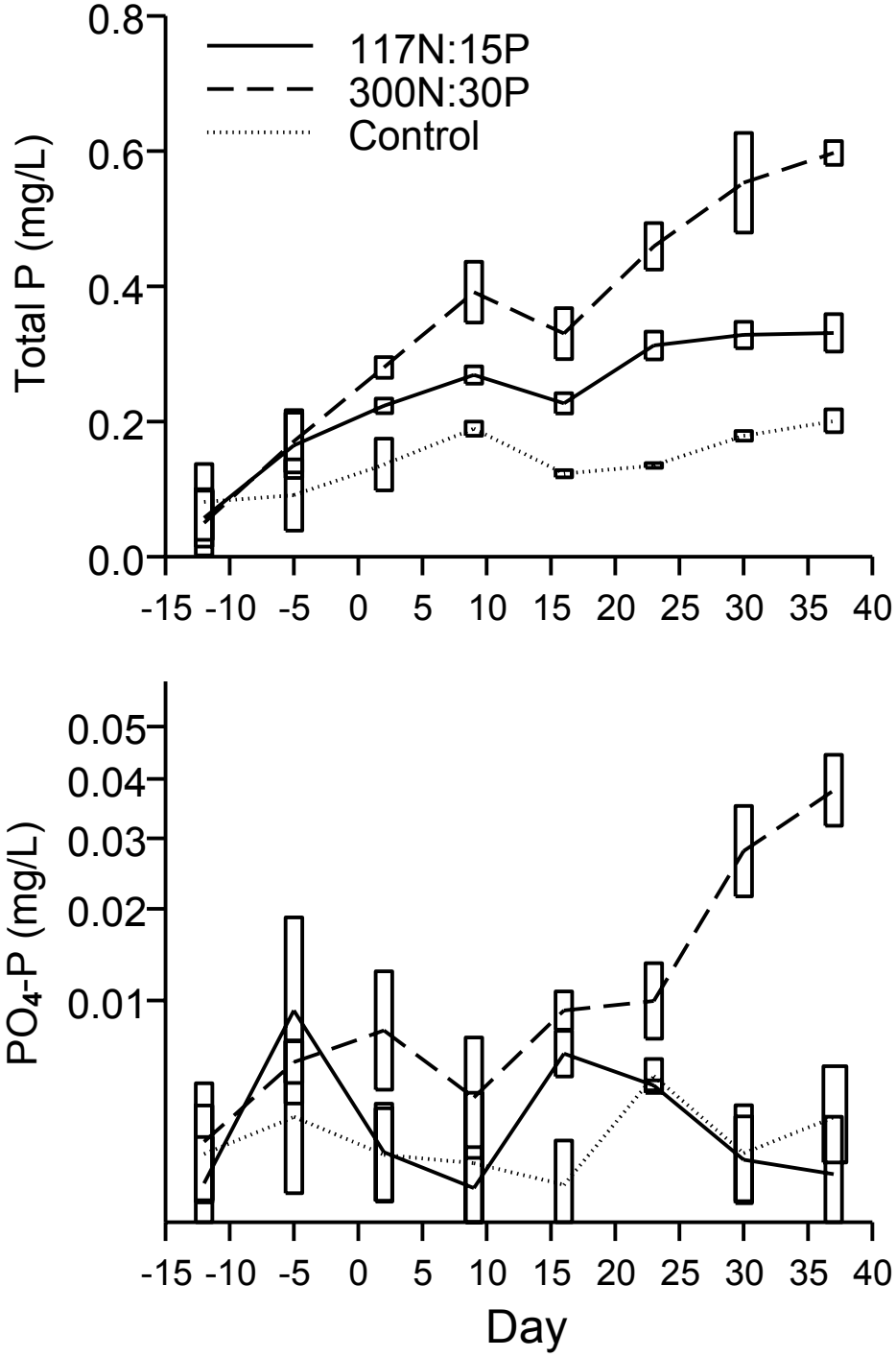

Figure 6.—Trends of phosphorus concentrations in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Boxes are standard errors.

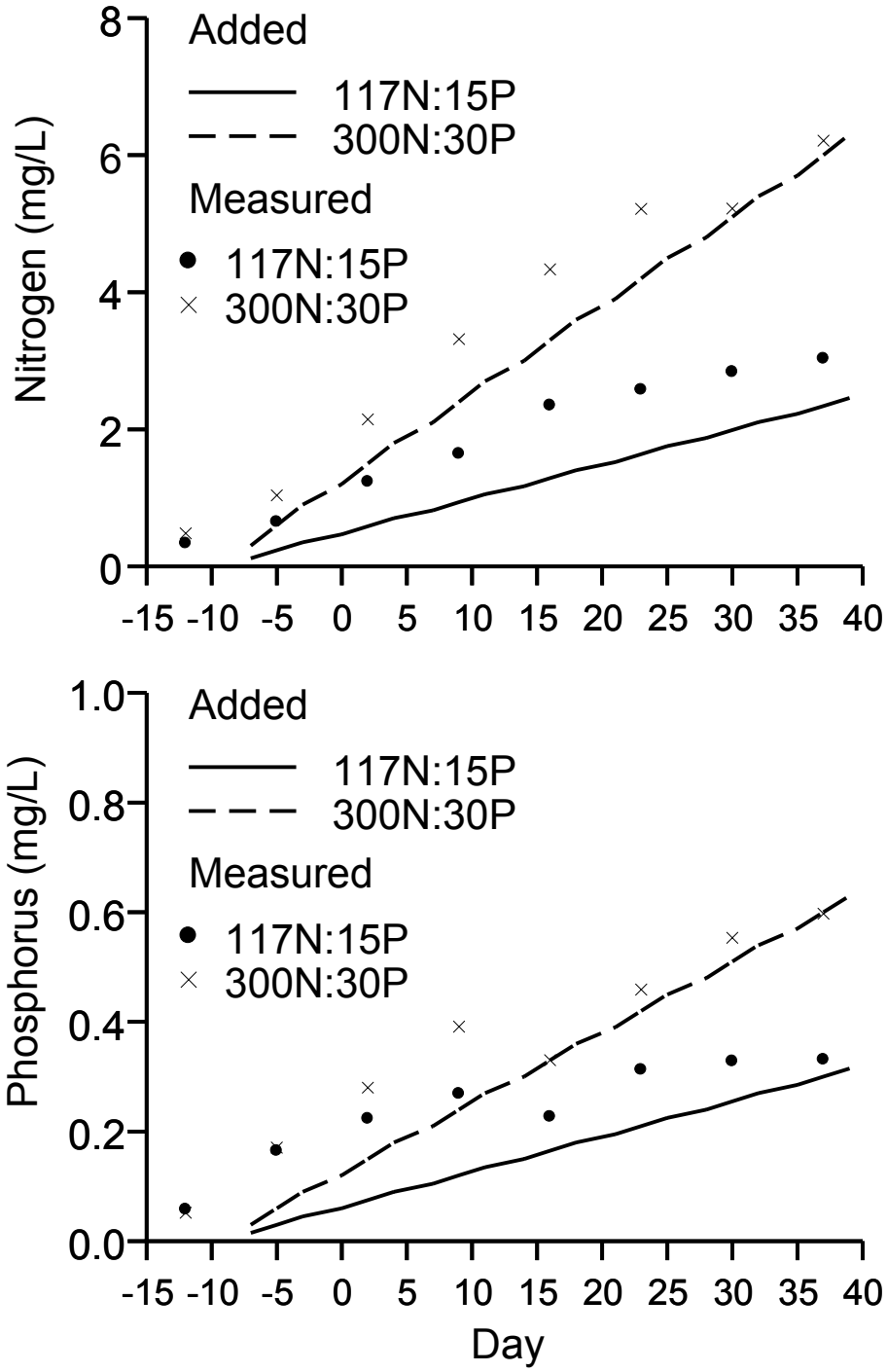

Figure 7.—Relationship between inorganic nitrogen or phosphorus added as fertilizer and concentrations measured in ponds fertilized with cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P) or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007.

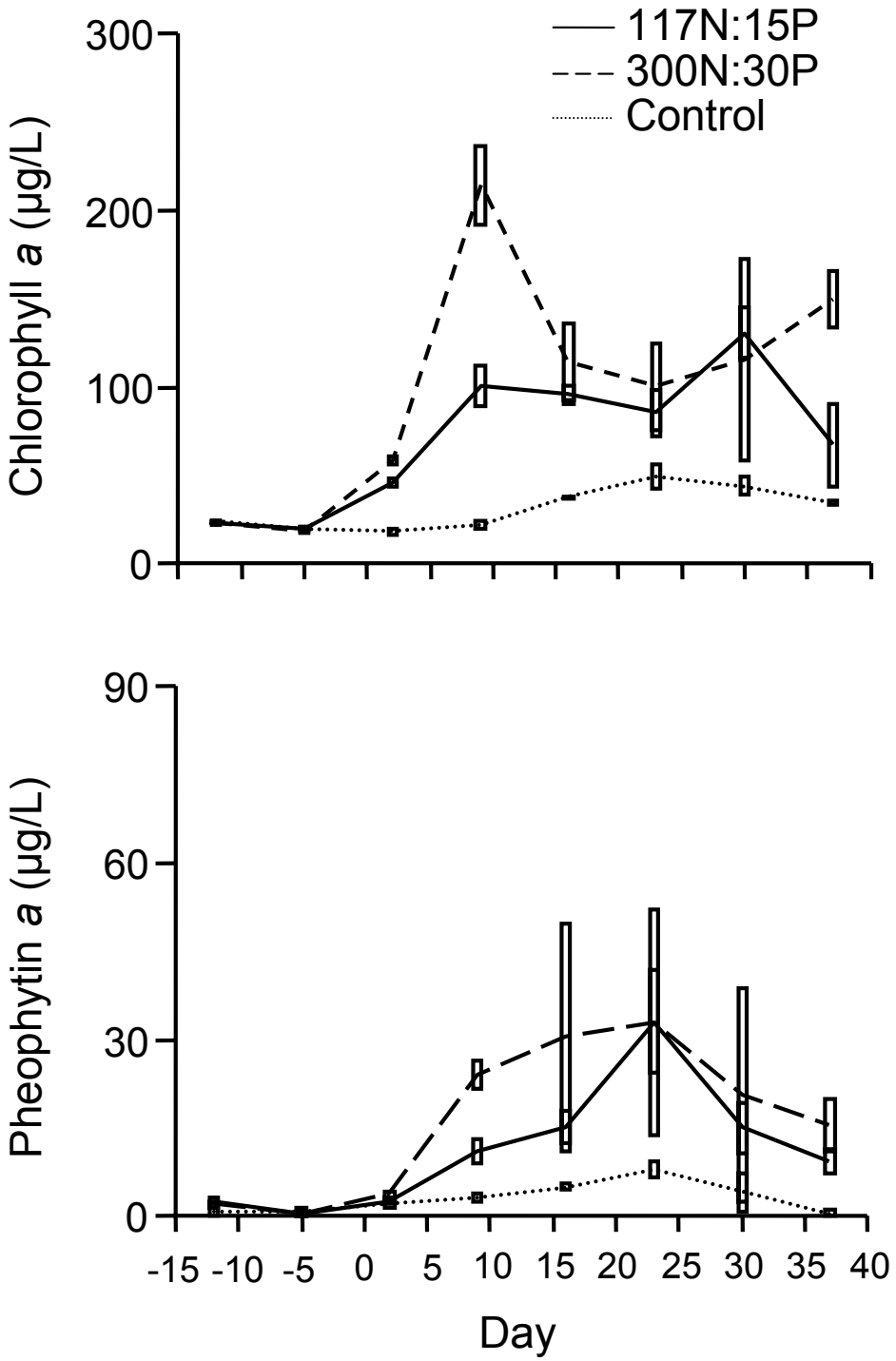

Figure 8.—Trends of chlorophyll *a* and pheophytin *a* concentrations in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Boxes are standard errors.

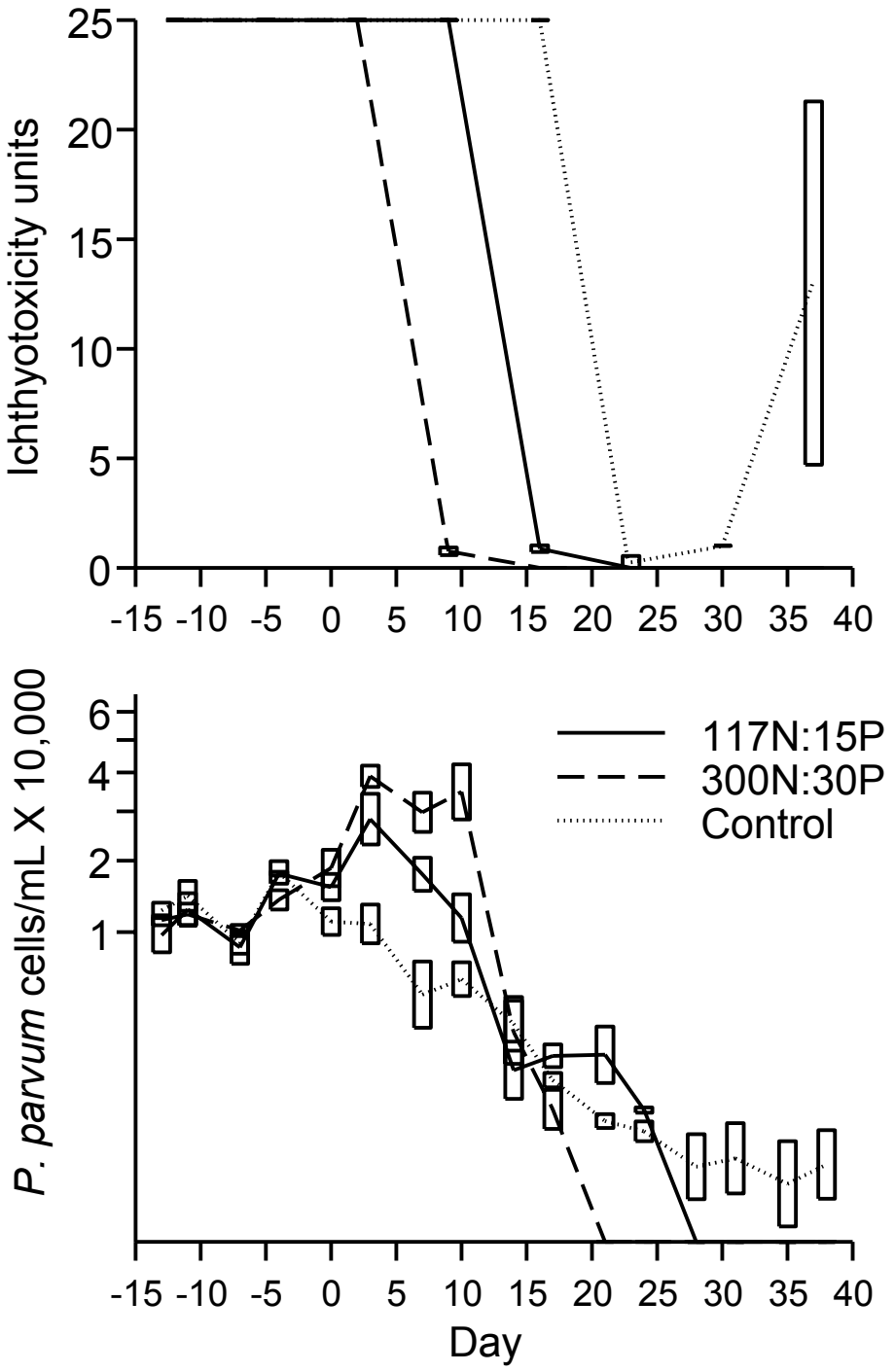

Figure 9.—Ichthyotoxicity and *P. parvum* cell densities in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Boxes are standard errors.

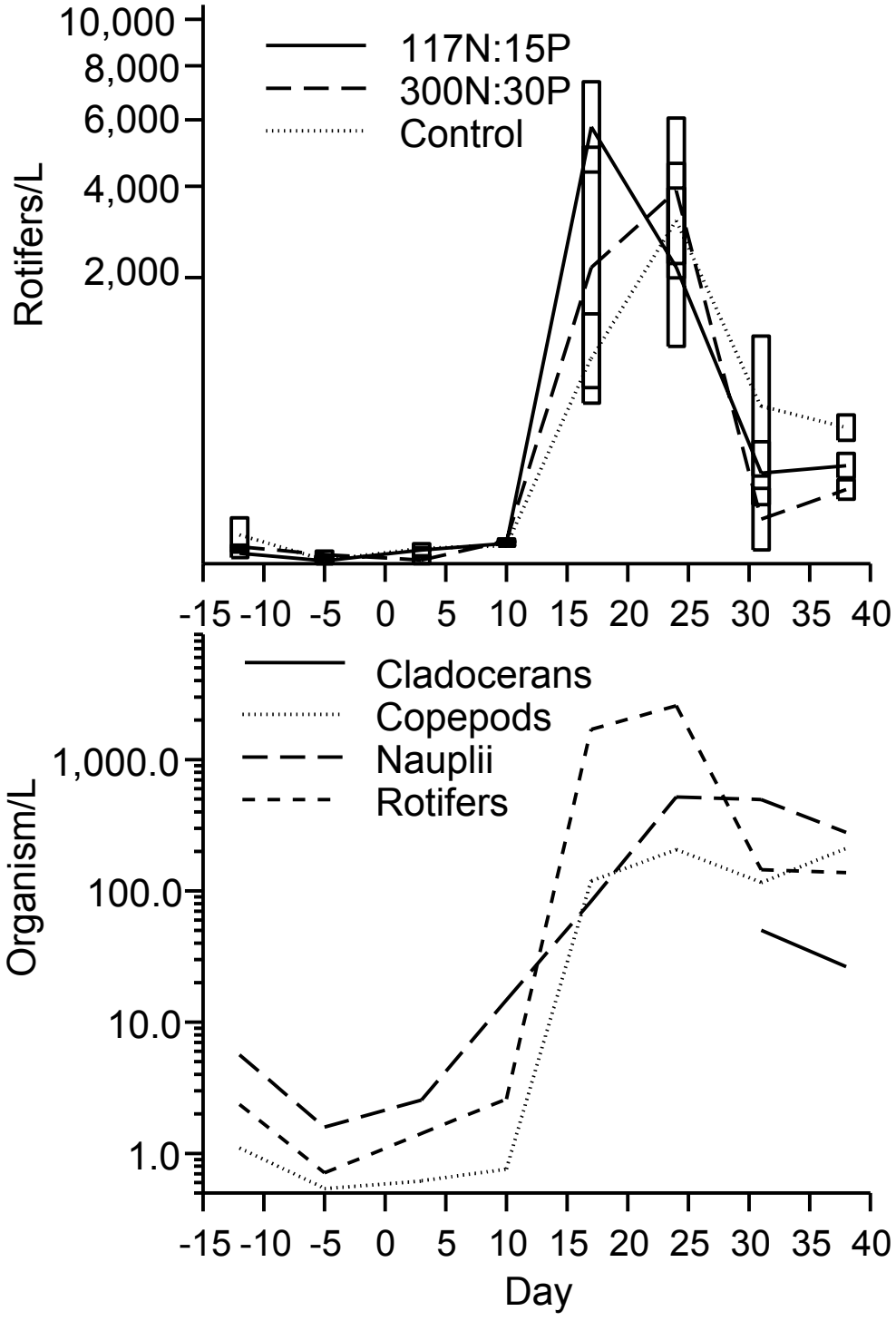

Figure 10.—Density trends of zooplankters in ponds fertilized with cottonseed meal only (Control), cottonseed meal plus 300 µg N/L and 30 µg P/L three times weekly (300N:30P), or cottonseed meal plus 117 µg N/L and 15 µg P/L three times weekly (117N:15P) at the Dundee state fish hatchery in spring 2007. Boxes are standard errors.

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