Evaluation of Two Ammonium Sulfate Application Strategies to Control *Prymnesium parvum* in Striped Bass Fingerling Production Ponds

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Management Data Series
No. 266
2011

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ACKNOWLEDGMENTS

ABSTRACT

Since *Prymnesium parvum* became endemic in the source water for Texas Parks and Wildlife Department Dundee and Possum Kingdom state fish hatcheries in 2001, standard pond management protocols have been changed in order to control this toxic alga and maintain fish production. Both hatcheries control *P. parvum* by applying ammonium sulfate with target un-ionized ammonia nitrogen levels (UIA-N) of 0.16 – 0.25 mg N/L when ponds contain fish. However, one hatchery applies ammonium sulfate only when *P. parvum* is present (therapeutic or T) and the other maintains UIA-N levels of 0.16 – 0.25 mg N/L throughout the production period (prophylactic or P). We compared these two strategies for efficacy in controlling *P. parvum* and their effects on water quality, zooplankton, and striped bass fingerling production in plastic-lined ponds. *P. parvum* occurred more frequently and in higher densities in T than in P ponds. Conversely, dissolved oxygen and ammonia concentrations were significantly higher in P ponds. Water temperature, pH, zooplankton density, and fish production (harvest density, production, survival, total length, and weight) were statistically similar between strategies. These results suggest that the P strategy provided better control of *P. parvum*; however, both strategies supported acceptable water quality and zooplankton populations for successful fish production.

INTRODUCTION

Prymnesium parvum, commonly called golden alga, is a haptophyte protist (Green and Leadbeater 1994). Toxins produced by this microalga have an ichthyotoxic effect (Shilo 1981; Igarashi et al. 1998) that can cause massive fish kills (Guo et al. 1996; Lindholm et al. 1999; Barkoh et al. 2003). This alga has been reported from various parts of the world including 18 US states (Sager et al. 2008; TPWD unpublished). In Texas, it has been associated with fish kills in inland waters since 1985 (James and De La Cruz 1989). These Texas fish kills have been limited to areas where the water is brackish (2-4 ppt.). Since 2001, *P. parvum* has also been problematic and responsible for extensive fish kills at two Texas Parks and Wildlife Department (TPWD) inland fish hatcheries, the Possum Kingdom State Fish Hatchery (PKFH) near Graford and the Dundee State Fish Hatchery (DFH) near Electra. To curb its adverse effects on fish populations and potential spread in Texas, effective means of controlling this alga without jeopardizing favorable environments for fish and other organisms are needed.

Currently, the preferred chemical for controlling *P. parvum* in small impoundments is ammonium sulfate, $(NH_4)_2SO_4$ (Sarig 1971; Barkoh et al. 2003). However, results of *P. parvum* control using ammonium sulfate had until recently been inconsistent. Barkoh et al. (2004; 2010) improved ammonium sulfate treatment results by basing treatment rates on un-ionized ammonia nitrogen (UIA-N) and defining a range of 0.14-0.25 mg N/L as effective concentrations for controlling this alga in fingerling *Morone* spp. culture ponds. These effective concentrations have served as the framework for the development and implementation of *P. parvum* control strategies at PKFH and DFH (Smith 2005a; Lyon et al. 2005).

Both PKFH and DFH *P. parvum* control strategies employ an initial ammonium sulfate application of 10 mg/L to achieve target UIA-N concentrations equal to or greater than 0.2 mg N/L to eradicate or at least significantly reduce densities of the alga from ponds before fish stocking. These UIA-N concentrations are allowed to decline to levels striped bass *Morone saxatilis* fry can tolerate (Barkoh et al. 2004) before fry stocking. Sublethal levels of ammonia are achieved by allowing adequate time for treatment concentrations to decline naturally (Smith 2005b) or by dilution of pond water with *P. parvum*-free fresh lake water, if necessary. After fry stocking, the DFH applications of ammonium sulfate target and maintain UIA-N levels of 0.16-0.25 mg N/L throughout a fish production cycle. This DFH strategy, referred to as prophylactic (P), was adopted because Lake Diversion, the source of water for the hatchery, has been chronically infested with *P. parvum.* With the DFH strategy, less emphasis is placed on *P. parvum* density monitoring. Instead, ammonia levels are monitored and maintained at target UIA-N levels known to control the alga by at least maintaining populations low enough to prevent ichthyotoxicity. The potential problems with this strategy include high UIA-N levels that also can kill fish (Tomasso 1994; Kurten et al. 2010) or reduced fish growth (Boyd 1990). Also, the frequent re-application of ammonium sulfate can over-stimulate primary productivity to promote high pH that can become lethal to fish (Anderson 1993;

Barkoh 1996; Kurten et al. 2007). The PKFH strategy targets the same UIA-N range of 0.16-0.25 mg/L but only applies ammonium sulfate treatments when *P. parvum* is present in pond water. Besides a single initial application of ammonium sulfate, a pond without *P. parvum* cells receives no follow-up treatments and ammonia levels are allowed to diminish. The PKFH therapeutic (T) strategy was adopted because *P. parvum* blooms in Possum Kingdom Lake, the source of water for the PKFH, are less frequent and less persistent. The potential problem with the T strategy is untimely detection of the presence of the alga or bloom that can result in fish mortality before corrective actions are taken.

Overall, each fish hatchery has successfully implemented its golden alga control strategy to the extent that fish culture has been possible despite the continued threat of ichthyotoxicity. However, the more often occasional loss of fish to golden alga toxicity at PKFH has necessitated the need to compare the two strategies to determine which one is more reliable in preventing fish kills due to golden alga blooms. The objectives of this study were to compare the effectiveness of the P and T strategies in controlling *P. parvum* in ponds and their effects on water quality, zooplankton, and phase 1 (35-40 mm total length) striped bass production.

MATERIALS AND METHODS

Study Location and Design

This study was conducted at PKFH, Palo Pinto County, Texas and used 14 striped bass fingerling production ponds and two sources of water available to the hatchery. The study design was a 2 × 2 factorial consisting of the following treatments: PKFH therapeutic strategy (T) and deep water (D) (TD), DFH prophylactic strategy (P) and D (PD), T and shallow water (S) (TS), and PS. Treatments were randomly assigned to ponds, resulting in four replicate ponds each for TD and PD and three replicate ponds each for TS and PS. The TD and TS ponds were managed as follows: an initial treatment of 10 mg/L ammonium sulfate (approximately 0.2 mg/L UIA-N) one day after pond filling was initiated, three-times weekly monitoring of *P. parvum* and ammonia concentration, and application of ammonium sulfate when *P. parvum* cells were present in water samples. The subsequent target UIA-N concentrations were 0.16-0.25 mg N/L. The PD and PS ponds received an initial ammonium sulfate treatment of 10 mg/L one day after pond filling was started, three-times weekly monitoring of ammonia levels, and application of treatments when UIA-N was less than or equal to 0.15 mg N/L to maintain UIA-N levels of 0.16-0.25 mg N/L throughout the study. For the purposes of this study, *P. parvum* densities also were monitored three-times weekly. For both strategies the ammonium sulfate application rates required to achieve the desired UIA-N concentrations were calculated from temperature, pH, total dissolved solids, and total ammonia nitrogen (TAN) data (Colt 2001).

Pond Management

Pond filling began 15 d before the anticipated date of stocking striped bass fry into production ponds. Because of the water intake pipes plumbing configuration, it was

not possible to fill at the same time ponds assigned to deep-water and shallow-water treatments. Thus, deep- and shallow-water ponds were started filling 15 and 12 d before fry stocking, respectively or completely filled approximately 3 d apart. Ponds were fertilized with cottonseed meal (CSM) to stimulate production of plankton to support fish production. Initial CSM applications of 284 kg/ha each were made to deep- and shallow-water ponds 13 and 9 d pre-stocking, respectively. Additional CSM applications of 57 kg/ha each were made to all ponds on days 5 and 2 pre-stocking and 2 and 6 post-stocking. The CSM was applied by hand into filling ponds or dispersed along the upwind sides of full ponds.

Water Quality and Plankton

Pond water quality was managed by dilution with fresh lake water, if necessary and by water circulation using Ramco aerators (20 cm diameter × 45 cm tall; RAMCO Sales, Inc., Cushing, OK) to maintain a pH range of 8.5-9.2 (Lyon et al. 2006). This pH range was selected because it allows effective ammonium sulfate treatment of *P. parvum* (Shilo and Shilo 1953) without significant detrimental effect on the production of phase 1 striped bass particularly when pH >8.5 are avoided before these fish are 14 d old (Brewer and Rees 1990; Bergerhouse 1993; Anderson 1993). Pond water quality was monitored twice daily at 0600 and 1500 hours for temperature, pH, and dissolved oxygen (DO) using a YSI MDS 650 data logger fitted with a 600 XL sonde. Total ammonia nitrogen was measured using a Denver Instruments Model 250 ISE meter and Fisher Accumet ammonia probe. *P. parvum* cell counts were conducted three times weekly using a standard TPWD protocol (D. G. Smith, Dundee State Fish Hatchery, Electra, Texas, personnel communication). Water samples for total ammonia nitrogen measurements or cell counts were collected from each pond in the early mornings from depths of 50-100 mm.

Zooplankton populations were examined every 3-4 d beginning 7 d pre-stocking and ending 14 d post-stocking of fry when offering of commercial feed (Rangen Inc., Angleton, Texas) to the fish was started (Warren 2001). Zooplankton samples were taken using 80-micron Wisconsin plankton net with an 11.43-cm-diameter opening. Tows were initiated at 0600 hours and completed for all ponds prior to sunrise. Zooplankters were identified into rotifers, copepod nauplii, copepod adults, and cladocerans groups (Pennak 1978) and enumerated under a dissecting microscope at 2- to 3-X magnification. Densities were reported as number of organisms/L.

Fish Production

Striped bass fry (4-5 d old) were stocked into all ponds on April 19. The stocking densities for the four treatments were 566,706-601,660 fry/ha. Harvest of fingerlings began when the average total length was 38 mm. Fish production data were collected at the time of fish harvest using standard protocols (Lyon et al. 2006). Briefly, three random samples of approximately100 fish each from each pond were weighed and subsamples of 30 fish each were individually measured for total lengths. All the fish from each pond were weighed. These data were used to calculate values for the following fish production variables: harvest density (fish/ha), production (kg/ha), survival

(% return), fish weight (g/fish), and average total length (TL). Production days were recorded for all ponds.

Data Analysis

All data, except those of fish production and *P. parvum* frequency-of-occurrence, were analyzed with PROC MIXED (SAS Institute Inc. 2002). Because data were collected repeatedly from each pond, we modeled the data assuming a 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 provided the best fit to the data for each response 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 (Littell et al. 1996). We tested the effects of treatment, sampling date, and treatment × sampling date interaction on each variable. When treatment x sampling date interaction was significant, pairwise multiple comparisons were performed with PROC MULTTEST and a stepdown Sidak approach to control the familywise error rate. *P. parvum* cell density and zooplankton data were $\log_{10}(x + 1)$-transformed before analysis due to skewness and zeros in the data. The fish production data were analyzed by two-way analysis of variance using the general linear models (GLM) procedure of the Statistical Analysis System (SAS Institute Inc. 2002). Duncan's Multiple Range test was used to differentiate significant differences among treatments. The *P. parvum* frequency-of-occurrence data were analyzed using a Chi-square test. For all analyses, differences were considered significant at *p*-values less than or equal to 0.05.

RESULTS AND DISCUSSION

Ammonia

Ammonium sulfate applications totaled 18 (151.3 kg) for T ponds and 53 (198.4 kg) for P ponds. Consequently, TAN and UIA-N concentrations were significantly higher ($P \leq 0.05$) in P ponds than in T ponds on most sampling days (Figure 1). Both *P. parvum* control strategies promoted the highest TAN and UIA-N concentrations shortly after the initial ammonium sulfate applications. These high ammonia levels were mitigated by dilution of pond water with fresh lake water just before fry stocking and that explains the steep declines in ammonia concentrations by April 18. During the post-stocking period, UIA-N was maintained below the upper threshold of 0.25 mg N/L for controlling of *P. parvum* (Barkoh et al. 2004) on most sampling days in all treatment ponds. On two sampling days (May 2 and 17) mean UIA-N values in P ponds exceeded the 0.25-mg N/L upper threshold. However, because these high UIA-N levels were of short duration no significant adverse effect in terms of fish kills or reduced fish growth (Russo and Thurston 1991) was observed in these ponds. Also, UIA-N levels did not seem to have affected fish production as discussed below. Conversely, the UIA-N values in T ponds were always within safe limits for the production of *Morone* spp. fingerlings (Oppenborn and Goudie 1993) after the initial spike.

Water Quality

Water quality variables were mixed in terms of their suitability for the production of phase 1 striped bass. Dissolved oxygen and water temperature were within ranges considered suitable for the production of these fingerlings (Table 1; Harrell et al. 1990) whereas pH and ammonia levels seemed less than ideal for their production (Anderson 1993; Barkoh 1996). Morning water temperature was significantly lower in PD ponds than in the other three treatment ponds. However, for a temperature range of 21.80-22.40^{o}C among treatments, the difference was probably not biologically significant. Morning water temperature was unaffected by source of water or *P. parvum* control strategy. Conversely, morning DO was related to *P. parvum* control strategy and was significantly higher in P than in T ponds. This could be due to a difference in photosynthesis that resulted from a greater phytoplankton biomass in P ponds than in T ponds. The more frequent application and larger overall quantity of ammonium sulfate used in P ponds appear to have promoted higher phytoplankton biomass in P ponds than in T ponds. Source of water had no effect on DO level. In contrast to DO, afternoon pH was not influenced by *P. parvum* control strategy but rather by source of water. Afternoon pH was statistically higher in D ponds than in S ponds. However, all treatments achieved mean afternoon pH levels that potentially can be detrimental to the production of phase 1 *Morone* fingerlings (Barkoh 1996; Brewer and Rees 1990), especially if these pH levels were achieved before the fish were 14 d old (Anderson 1993).

Prymnesium parvum

P. parvum occurred at significantly higher frequencies and densities in T ponds than in P ponds (Table 1; Figure 2). Examination of water samples from the TD and PD ponds on 24 and 22 occasions, respectively for the presence *P. parvum* cells revealed that this alga was present in 13.09% of the samples from the TD ponds and absent from the PD samples. Likewise, TS and PS ponds were sampled 21 and 23 times, respectively and *P. parvum* cells were present in 14.29 % of the samples from TS and 0.03% of the samples from PS ponds. *P. parvum* control strategy regulated the recurrence of *P. parvum* whereas source of water did not have a similar effect. The higher density of cells in T ponds compared to P ponds also was an indication that the P strategy was more effective in controlling *P. parvum* blooms. Overall, cell densities were dramatically lower (0-550 cells/mL) in the study ponds compared to the density (30,000 cells/mL) in the source water used to fill the ponds. This shows that the target UIA-N concentrations of 0.16-0.25 mg N/L were effective in eradicating *P. parvum* from some ponds and drastically reducing densities in other ponds. Future research should investigate the cause of these disparate results and provide information on the maximum UIA-N concentration that would consistently eradicate *P. parvum* from hatchery ponds.

P. parvum-related ichthyotoxicity was not observed in any pond during this study probably because *P. parvum* densities were kept to near zero in P ponds and very low in T ponds (Figure 2). Although there appears to be no documented relationship between *P. parvum* cell density and toxicity expression, most reported *P. parvum* toxicity events have been associated with cell densities far greater than those observed

in this study. Shilo and Aschner (1953) characterized a *P. parvum* density of 50,000 cells/mL low and yet it was associated with extreme toxicity. The two *P. parvum* control strategies compared in this study adequately controlled *P. parvum* densities and thereby prevented ichthyotoxicity to allow successful production of striped bass fingerlings.

Zooplankton

Mean densities of rotifers, copepod nauplii, and adult copepods did not statistically differ among treatments. The observed lack of difference in copepod nauplii density among treatments may be due to the high variability in the data (Table 1). Density of copepod nauplii was consistently higher by more than 36% in T ponds than in P ponds, and that is biologically significant. It appears *P. parvum* control strategy had an effect on these zooplankters. The lower densities of copepod nauplii in P ponds may be due to the high ammonia levels in these ponds. Because copepod nauplii were the only zooplankton category with consistently lower densities in P ponds suggests that they were the most vulnerable to the high ammonia levels. Previous studies have reported differential susceptibility of zooplankters to ammonia toxicity (e.g., Arauzo and Valladolid 2003). Cladocerans were significantly more abundant in D ponds than in S ponds (Table 1). One plausible explanation is the distribution of zooplankton in Possum Kingdom Lake, the source of water to the hatchery. Probably during the period of pond filling with water from the lake, cladocerans were in deeper water compared to the other categories of zooplankton. Because of the different factors that control vertical migration, zooplankters can show differential spatial distribution (George 1983; Lagadeuc et al. 1997; Sekino and Yamamura 1999). Thus, the D ponds were seeded with adequate cladocerans to establish denser populations than the S ponds. If this suggestion were true, it would also suggest that rotifers and copepod adults, unlike cladocerans, were more evenly distribution between the deep and shallow waters of the lake. Another explanation might be the age of the zooplankton communities in the D and S ponds. Because the D ponds were filled before the S ponds, 3 d apart, the zooplankton communities of the D ponds may have been more established or mature than those of the S ponds during the course of the study (Allan 1976; Morris and Mischke 1999). Though zooplankton communities can be affected by *P. parvum* toxicity (Brooks et al. 2010; Sarig 1971), we do not consider toxicity a factor in explaining the observed differences in the zooplankton communities of the treatments because none of the ponds experienced ichthyotoxicity. Source of water had no effect on copepod nauplii density but appears to have had a significant effect on density of cladocerans.

Fish Production

Fish production was similar among treatments since none of the measured production variables differed statistically among treatments (Table 2). Nonetheless, the fish survival results appear to suggest a practical benefit in favor of D ponds. Fish survival averaged 12% higher in D ponds than in S ponds, and it correlated positively with cladocerans density and negatively with fish stocking density. However, because fish size at harvest was not significantly different, differential feeding on cladocerans is likely not the reason for the observed differences, *albeit* not statistically significant but practically significant, in the survival values. On the other hand, the potential effect of

fish stocking density on fingerling striped bass survival in plastic-lined ponds requires further study since this relationship is not well documented. Striped bass culturists have routinely related stocking rates to production (kg/ha) or yield (number/ha) rather than to fish survival (Brewer and Rees 1990). In contrast to what appears to be source water effect on fish survival, *P. parvum* control strategy did not have any effect on fish survival or any other production variable. The frequent, higher UIA-N levels achieved under the P strategy did not seem to have affected striped bass production. Perhaps, the duration of these high UIA-N levels were not long enough to adversely affect the fish or water chemistry may have ameliorated an otherwise ammonia toxicity effect on the fish (Meade 1985). Calcium and salinity that increase tolerance of fish to ammonia toxicity (Tomasso et al. 1980; Weirich et al. 1993) were high in the study waters. Similarly, the more frequent occurrence of higher densities of *P. parvum* in T ponds than in P ponds, although precarious, did not affect fish production. Apparently, *P. parvum* densities were kept low enough to prevent ichthyotoxicity expression in any of the ponds.

CONCLUSIONS

Both *P. parvum* control strategies were effective in creating conditions that were suitable for the successful production of phase 1 striped bass. For both strategies water quality and fish food resources were essentially similar, and the fish production variables were equal to or better than those reported elsewhere for state and federal hatcheries (e.g., Brewer and Rees 1990). Thus, we cannot recommend one strategy over the other based solely on the results of this study. However, because of the persistence of *P. parvum* in the T ponds despite keen attention and continuous management action throughout the study, it seems the margin of safety in preventing fish kill was better for the P strategy than for the T strategy. Other factors that may influence the decision to choose either strategy are the level of expertise required for monitoring ammonia levels versus presence of *P. parvum* and the manpower requirements for monitoring and treatment applications. In short, the potential cost of losing fish as well as the economic cost of implementing the strategies must be considered in the choice of either *P. parvum* control strategy. Based on the margin of safety in preventing fish kill and the ease of ammonia monitoring compared to the expertise needed for *P. parvum* identification and enumeration of live samples, we conclude that the P strategy is a better choice, especially for individuals less familiar with this alga.

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TABLE 1.—Mean ± SD values of water quality, *P. parvum,* and zooplankton variables in ponds filled with deep (D) or shallow (S) reservoir water and subjected to a therapeutic (T) or prophylactic (P) *P. parvum* control strategy at the Possum Kingdom State Fish Hatchery. Values bearing the same letter within a row are not significantly different (*P* > 0.05).

Variable	TD	PD	TS	PS
Water quality				
Water temperature (°C)	22.30 ± 3.10 z	21.80 ± 3.10 y	22.40 ± 2.50 z	22.30 ± 2.50 z
Dissolved oxygen (mg/L)	7.76 ± 1.75 z	8.10 ± 1.65 y	7.72 ± 1.39 z	8.05 ± 1.84 y
Afternoon pH	9.16 ± 0.42 z	9.19 ± 0.41 z	9.10 ± 0.39 y	9.08 ± 0.41 y
P. parvum				
Density (cells/mL)	22.90 z	0.00 y	34.50 z	6.00 y
Occurrence (%)	13.09 z	0.00 y	14.29 z	0.03 y
N	24	22	21	23
Zooplankton				
Rotifers/L	211.6 ± 336.6 z	198.0 ± 322.1 z	233.7 ± 390.7 z	154.3 ± 222.4 z
Copepod nauplii/L	112.2 ± 139.6 z	82.0 ± 88.0 z	130.6 ± 523.1 z	82.8 ± 96.9z
Copepod adults/L	60.4 ± 123.5 z	57.2 ± 84.8 z	58.9 ± 68.8 z	83.9 ± 120.3 z
Cladocerans/L	144.4 ± 200.1 y	117.5 ± 133.3 y	63.0 ± 89.7 z	71.4 ± 156.7 z

TABLE 2.—Mean ± SD values of fish production variables in ponds filled with deep (D) or shallow (S) reservoir water and subjected to a therapeutic (T) or prophylactic (P) *P. parvum* control strategy at the Possum Kingdom State Fish Hatchery. For each variable there are no significant differences among treatment means ($P > 0.05$).

Variable	TD	PD	TS	PS
Days in production	45 ± 3.7	41 ± 3.9	40 ± 3.5	43 ± 1.5
Stock density (fish/ha)	566,706 ± 4,856	567,810 ± 4,009	601,660 ± 36,309	598,888 ± 9,156
Harvest Density (fish/ha)	300,009 ± 195,098	308,817 ± 182,686	239,887± 96,267	259,533± 28,176
Production (kg/ha)	254 ± 72.3	246 ± 55.5	237 ± 94.8	266 ± 52.2
Survival (%)	53 ± 4.9	54 ± 31.6	40 ± 17.8	43 ± 4.7
Average fish weight (g/fish)	1.0 ± 0.4	0.9 ± 0.4	1.0 ± 0.1	1.0 ± 0.2
Average fish length (TL)	43.3 ± 4.8	44.6 ± 1.0	44.4 ± 1.4	44.6 ± 1.0
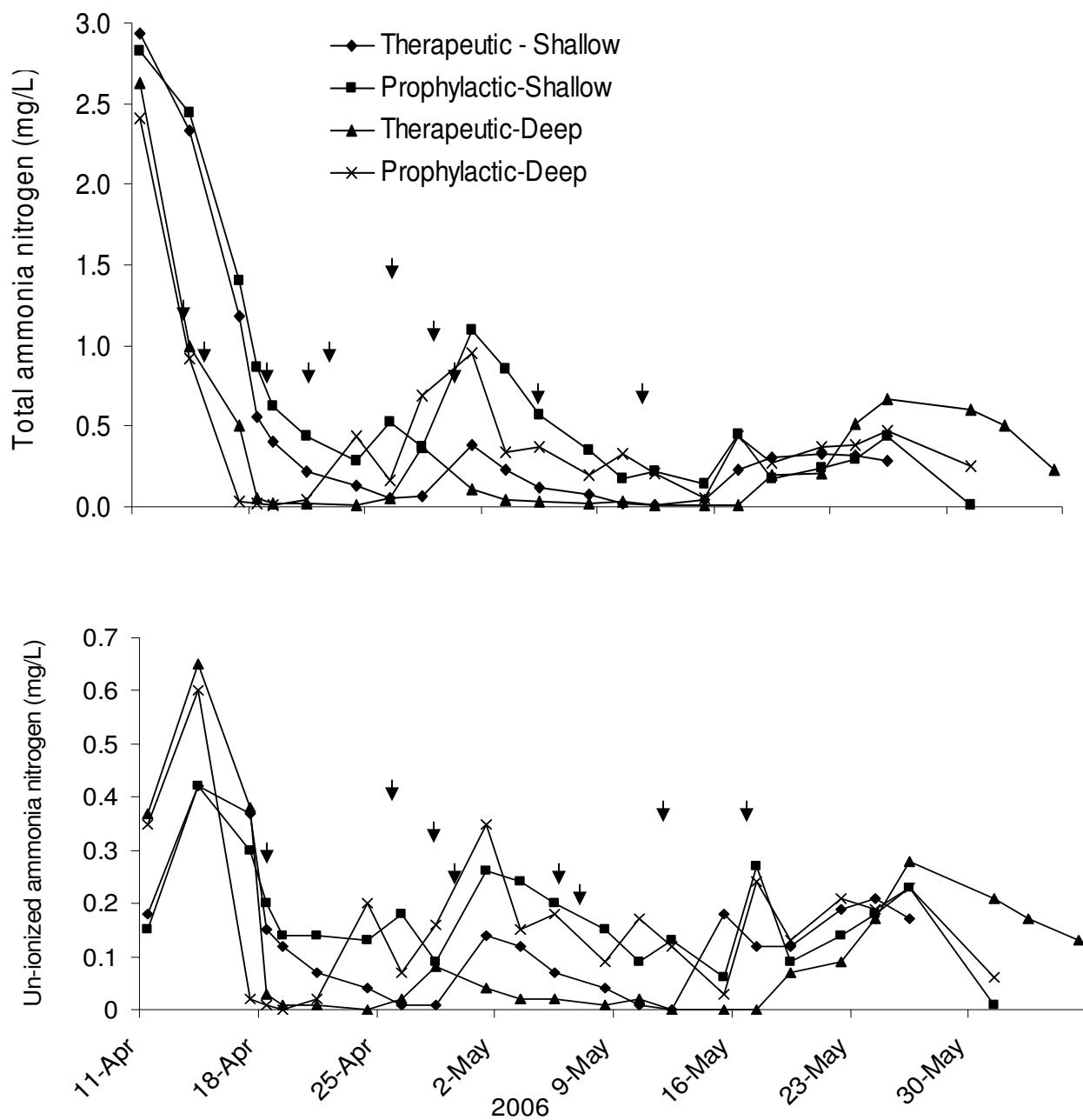
FIGURE 1.—Mean daily total ammonia nitrogen and un-ionized ammonia nitrogen concentrations in ponds subjected to a therapeutic or prophylactic *P. parvum* control strategy at the Possum Kingdom State Fish Hatchery. Total ammonia nitrogen and un-ionized ammonia nitrogen concentrations are significantly different ($P \leq 0.05$) on sample days indicated by arrows.
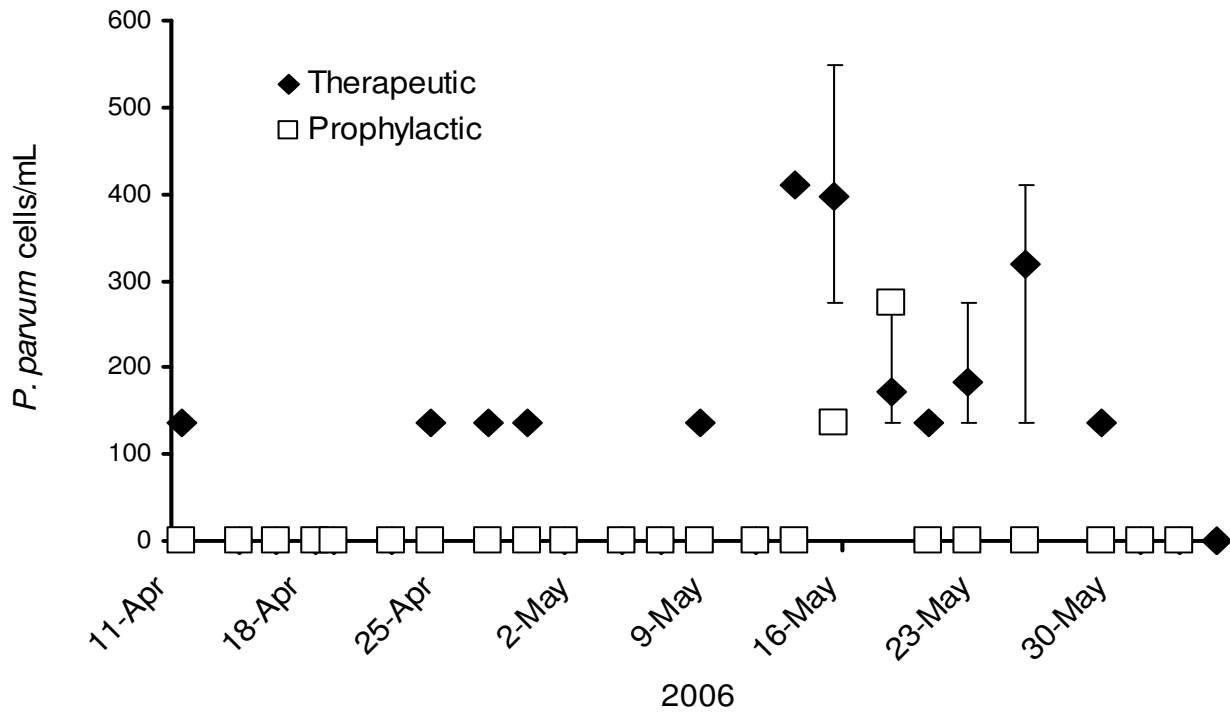
FIGURE 2.—Mean *P. parvum* cell densities in ponds subjected to a therapeutic or prophylactic *P. parvum* control strategy at the Possum Kingdom State Fish Hatchery. Vertical lines represent ranges where cells were present in more than one replicate pond.