Final Report

Evaluation of Barley Straw and Liquid Live Micro-Organisms System for Controlling *Prymnesium parvum* in Fish Hatchery Ponds

by

Aaron Barkoh, John M. Paret,
Drew C. Begley, Dale D. Lyon,
Dennis G. Smith, and J. Warren Schlechte

Texas Parks and Wildlife Department
Inland Fisheries Division
4200 Smith School Road
Austin, Texas 78744

Executive Summary

Since 2001, the toxigenic microalga *Prymnesium parvum* has caused frequent massive fish kills in several recreationally and economically important Texas reservoirs and two fish hatcheries. To effectively mitigate *P. parvum* bloom formations and related fish kills, the causes of these events must be identified and understood. Proper understanding of these natural events will require long-term research efforts. Meanwhile, the immediate concerns about impacts of *P. parvum*, including destruction of fisheries, hatchery fish losses, and economic losses to local communities and the state, necessitate the development of control techniques that can minimize the impacts until long-term solutions or prevention methods are found. Therefore, we investigated the efficacy of two natural products, barley straw Hordeum vulgare and a commercial probiotic Liquid Live Micro-Organisms System (LLMO), in controlling *P. parvum* and its ichthyotoxicity. Since these products would also be used in hatchery fish production ponds, if found effective against , their effects on factors essential for successful operation of fish hatcheries - water quality, nutrient levels, phytoplankton biomass and composition, zooplankton density and composition, and fingerling fish production - were also evaluated.

This study was conducted at the Texas Parks and Wildlife Department (TPWD) Possum Kingdom State Fish Hatchery in Palo Pinto County in 12 (0.22-0.33 ha) plastic-lined ponds in June-November 2004 (Experiment 1) and at the TPWD Dundee State Fish Hatchery in Archer County in eight (0.01-ha) plastic-lined ponds in December 2004-March 2005 (Experiment 2). In Experiment 1, barley straw was applied once at 252 kg/ha at the beginning of the study and LLMO was applied at 1 L/56,718 L of pond water biweekly. Barley straw was further tested at 280 kg/ha in Experiment 2. These barley straw treatment rates were similar to those used in other studies and the LLMO rate was provided by the supplier. In both experiments *P. parvum* blooms did occur. Neither barley straw nor LLMO, at the rates and time frames used in this study, had significant effects on *P. parvum* population density, ichthyotoxicity, or any of the other variables investigated. The results of this study did not support reports or claims that barley straw or LLMO can control *P. parvum* density and ichthyotoxicity or improve pond conditions for increased fish production.

Introduction

Prymnesium parvum, a haptophytic microalga, has caused extensive mortalities in wild and cultured fish in several parts of the world (Guo et al. 1996). In Texas, over 28 *P. parvum* blooms and related fish kills in major river systems and reservoirs have been reported since 1981 (J. Glass, Texas Parks and Wildlife Department, personal communication). Since 2001, fish kills by *P. parvum* have been more widespread and have involved more than nine economically and recreationally important reservoirs and two Texas Parks and Wildlife Department (TPWD) fish hatcheries. As of 2003, an estimated 18.3 million fishes with an economic value of over $8 million had been lost (http://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/ga).

Current strategies for controlling *P. parvum* are limited to fish hatchery ponds and small impoundments and involve destruction of the alga with chemicals such as ammonium sulfate or copper sulfate (Shilo and Shilo 1953; Smith 2005). Success at controlling *P. parvum* with these chemicals has varied (Sarig 1971) because their effectiveness is influenced by environmental conditions such as temperature and pH (Shilo and Shilo 1953; Guo et al. 1996). Additionally, these chemicals provide short-term improvements, requiring frequent applications to maintain algal control, making current strategies tedious and inefficient. Furthermore, these chemicals have undesirable side effects. Ammonium sulfate concentrations required to control *P. parvum* may produce un-ionized ammonia levels that are harmful to fish whereas copper sulfate indiscriminately kills all algae. Thus, these chemical control methods have not been used in reservoirs because of their potential negative ecological effects as well as being uneconomical for large water bodies.

Barley straw Hordeum vulgare is a natural product reported to provide relatively long-term (6-8 months) algal control with no known adverse effects on humans, waterfowl, fish and invertebrates (Gibson et al. 1990; Welch et al. 1990; Newman 1997; Lynch 2002). Barley straw is reported to provide selective control of planktonic and filamentous algae including chlorophytes (Welch et al. 1990), cyanobacteria (Newman and Barrett 1993), diatoms (Barrett et al. 1996), desmids (Martin and Ridge 1999), and chrysophytes (Butler 1999) without inhibiting growth of macrophytes (Welch et al. 1990). This selective control property makes barley straw a potentially suitable product for targeted control of harmful algae, such as *P. parvum*.

The process of algal control by decomposing barley straw, however, is not well understood. A few hypotheses have been put forth to attempt to describe the mechanism of the anti-algal activity of barley straw (e.g., Gibson et al. 1990; Pillinger et al. 1994). However, the most plausible hypothesis is that as barley straw decomposes, lignins are released into the water and if adequate oxygen exists the lignins are oxidized into humic substances. In the presence of sunlight and oxygen these humic substances ultimate form hydrogen peroxide that inhibits algal growth (Newman 1997). Several researchers (e.g. Newman and Barnett 1993, Pillinger et al. 1994), however, believe that the algal-inhibiting activity may be caused be a complex of compounds rather that a single compound. The activity of barley straw is described as algistatic (prevents growth of new algae) rather than algicidal (kills existing algae) (Gibson et al. 1990; Newman and Barrett 1993; Lembi 2002).

Another product that the supplier, H2O Sales and Services, Inc., claims has the potential to control *P. parvum* density and ichthyotoxicity is the Liquid Live Micro-Organisms System (LLMO) manufactured by General Environmental Science Corporation. The LLMO is marketed for its ability to reduce nitrogen (N) and phosphorus (P) levels and consequently improve water clarity in ponds. Lynch et al. (2003) claimed that LLMO improved water clarity (Secchi disk visibility) by 133-156% and reduced chlorophyll a, orthophosphate (PO4-P), and total P levels by 43.5, 83, and 65%, respectively in 4-5 months. The effect of LLMO on nitrogen levels or N:P ratios was not addressed in their study, and we are unaware of any published results of the effect of LLMO on N:P ratios under controlled conditions. However, we reasoned that because high N:P ratios appear to promote *P. parvum* blooms (Holdway et al. 1978; Kaartvedt et al. 1991; Lindholm et al. 1999), if use of LLMO can achieve low N:P ratios it may control *P. parvum* blooms and ichthyotoxicity. Additionally, Shilo and Aschner (1953) reported that the toxin was rapidly inactivated by the ubiquitous bacterial species Bacillus subtilis and Proteus vulgaris, which may have similar characteristics as some of the species (Bacillus, Pseudomonas, Nitrosomonas, Nitrobacter, Cellulomonas, Aerobacter and Rhodopseudomonas) in LLMO (Yung-Tse et al. 1986). The LLMO contains three species of Bacillus including B. subtilis that Shilo and Aschner (1953) found to inactivate the toxin. Therefore, we decided to evaluate the effects of LLMO on *P. parvum* blooms and ichthyotoxicity. The objectives of this study were to (1) determine if decomposing barley straw or LLMO inhibits *P. parvum* population growth and ichthyotoxicity and (2) determine effects of decomposing barley straw or LLMO on water quality, nutrient levels, phytoplankton, zooplankton, and fish production.

Methods

Experiment 1

Treatments

Experiment 1 of this study was conducted at the TPWD Possum Kingdom State Fish Hatchery, Palo Pinto County, in 12 (0.22-0.33 ha) plastic-lined ponds in June-November 2004. Four ponds were randomly assigned to each of two treatments (barley straw or LLMO) or control (no treatment). The barley straw was loosely packed in burlap sacks (97 x 168 cm) so that water can move freely through it and applied at 252 kg/ha (Lembi 2002) in 3-5 sacks per pond. The sacks were secured to cables (2, 12-gauge twisted wire) that were stretched across the widths of the ponds. Each sack was weighted down by a pair of cinder blocks at two corners, and a pair of floats was attached to the opposite corners to provide buoyancy. This set-up allowed the sacks to suspend horizontally in the photic zone, about 15 cm from the pond water surface when waterlogged, where adequate oxygen and sunlight are available for aerobic decomposition of the straw (Newman 1997). The sacks were placed at depths of > 0.9 m to ensure they did not sit on the pond bottoms where mud rapidly absorbs the algistatic material released by decomposing barley straw (Newman 1997). Filling of ponds started on June 9, 2004 with water from Possum Kingdom Reservoir, and sacks were under the water surface in 4-5 days after ponds were full. Characteristics of the source water were: alkalinity, 78 mg/L as CaCO3; hardness, 418 mg/L as CaCO3; and chlorides, 800 mg/L.

The LLMO consisted of LLMO N-1, LLMO S-1, Liquid Activator, and Powdered N Activator which were mixed together in a 284-L tank, incubated, and applied to ponds according the supplier's instructions. The LLMO was spread along the perimeter of the ponds at two-week intervals (i.e., after each 14-day incubation period) at an average rate of about 1 L/56,718 L of pond water. The LLMO application period was June 22-October 26.

Fish Production

Ponds were stocked with swim-up fry koi carp, a strain of the common carp Cyprinus carpio, on June 23 at rates of 174,300-210,900/ha and reared for 4.5 months. Ponds were not fertilized but fish were offered a commercial diet, consisting of a salmon ration starter and #1 granules (50% protein), followed by #4 crumbles (32% protein). After 4.5 months of production, ponds were drained completely to harvest the fish. Production variables [yield (number/ha), growth (mm), production (kg/ha) and survival] were calculated from fish sampling, fish harvest, and pond size data.

Cell Density, Toxin, and Ichthyotoxicity

Prymnesuim parvum density and toxicity in pond water were measured twice weekly. The cell density estimates and toxicity bioassays were performed on the same day for each pond using standard TPWD fish hatchery procedures (Southard and Fries 2005). Cell densities were estimated using a hemacytometer and microscope at 40X magnification to examine 1-mL aliquots of fresh unfixed pond water samples. Bioassays were performed using fathead minnow *Pimephales promelas* fry as test fish at 28°C. Each bioassay used water collected from a pond and processed into the following: 100 mL undiluted water, 100 mL undiluted water plus 2 mL cofactor, and 100 mL water diluted by 1/5 with P. parvum-free water plus 2 mL cofactor. *P. parvum*-free water (100 mL) was used as control. The cofactor solution consisted of 0.003 M 3, 3'-iminobispropylamine and 0.02 M tris buffer (pH 9.0) and functioned to enhanced toxicity of the *P. parvum* ichthyotoxin and thereby allow otherwise sublethal levels to be quantifiable. Mortality of test fish (4 per assay) was determined after 2 hours exposure to assays, and the degree of toxicity was estimated in ichthyotoxicity units (ITU) as follows: zero mortality indicated no toxicity (0 ITU); mortality in undiluted water plus cofactor indicated low toxicity (1 ITU); mortality in the one-fifth water dilution plus cofactor indicated moderate toxicity (5 ITU), and mortality in undiluted water indicated a high level of toxicity (25 ITU). Water samples were also analyzed for toxin concentrations biweekly using a ThermoFinnigan LCQ Advantage Liquid Chromatography Mass Spectrometry that utilizes Electro Spray Ionization. The multi-charged species of the toxin (prymnesin 1 and prymnesin 2) were detected. Because no *P. parvum* standard is commercially available, the toxin was quantified with respect an internal standard (P. Hamlett, TPWD Environmental Contaminants Lab, San Marcos, Texas, personal communication).

Nutrients and Water Quality

Water quality measurements and water samples were taken from 25-30 cm below the pond water surface. Water temperature, pH, and dissolved oxygen were measured twice daily (before sunrise and at 1400-1500 hours), beginning 5 days after pond filling began, using a YSI 650 MDS handheld meter fitted with a YSI 600 XL multiprobe sensor (Yellow Springs Instruments, Yellow Springs, Ohio). Total N, ammonia-N, and total P were measured biweekly beginning June 22 by an independent laboratory (Texas Institute for Applied Environmental Research,

Tarleton State University, Stephenville, Texas) using USEPA-approved protocols (USEPA 1983). Nitrate-N and PO4-P also were measured once every two weeks at the TPWD Fish Health and Genetics laboratory using procedures in standard methods (APHA 1999).

Plankton

Phytoplankton identification and enumeration were performed by a commercial laboratory (PhycoTech, Inc., St. Joseph, Michigan) on samples collected before application of LLMO treatments, 3 months after treatments began, and just before termination of the study. Water samples (250 mL each) were preserved with approximately 1% glutaraldehyde and shipped by overnight delivery. Phytoplankters were identified to the species level where possible, enumerated, and grouped into eight taxonomic divisions and a miscellaneous group using an Olympus (BX51) compound microscope equipped with Brightfield optics at 400X magnification and a proprietary software (Aquatic Sample Analysis System). Phytoplankton abundance was expressed as natural units/mL or cells/mL.

Chlorophyll a and Secchi disk visibility were measured once weekly. Water samples for chlorophyll a were taken at 0900-1000 hours and the chlorophyll a measured by the whole water, nonfiltered sample extraction and fluorometry method (APHA 1999). For each water sample, 1.5 mL was mixed with 8.5 mL of 100% acetone in a screw-capped vial and stored in a light-tight container at 4°C for analysis within 10 days. Secchi disk visibility readings were taken during chlorophyll a sampling using a 20-cm diameter black and white metal plate (Boyd 1990).

Zooplankton density and population structure were monitored once 3-5 days before application of treatments, then weekly thereafter. Zooplankton samples were taken before sunrise with an oblique tow of 80- m-mesh Wisconsin plankton net from one station at the deepest part of each pond and preserved with Lugol's solution for later analysis. Two 1-mL aliquots of the preserved samples were separately placed on a plankton counting wheel and viewed under a dissecting microscope at variable magnification. Zooplankters were identified according to Pennak (1978) and enumerated into four categories: rotifers, cladocerans, copepod nauplii, and adult copepods. Densities were expressed as organisms per liter.

Experiment 2

Treatments

Experiment 2 was conducted at the TPWD Dundee State Fish Hatchery in Archer County in eight (0.01-ha) plastic-lined ponds in December 2004-March 2005, and it focused on *P. parvum* control by barley straw. Ponds were randomly assigned to either the barley straw treatment or the untreated control group. The barley straw was handled and deployed as described for Experiment 1 but at 280 kg/ha (Lembi 2002) in 2 sacks per pond. Ponds were filled December 13-15 with water from Diversion Lake, and all sacks were submerged approximately 6 days after pond filling ended. The final locations of the sacks were in the photic zone approximately 15 cm from the water surface and ≥9 m from the pond bottoms. Characteristics of the source water were: alkalinity, 78 mg/L as CaCO3; hardness, 734 mg/L as CaCO3; and chlorides, 725 mg/L.

Cell Density, Toxin, Ichthyotoxicity, and Water Quality
Prymnesium parvum density, toxin level, ichthyotoxicity, chlorophyll a, and Secchi disk visibility were measured on either December 16 or 17, then weekly from January 12 to March 16. Water temperature, pH, and dissolved oxygen were measured daily from December 16 to March 16. Methods were the same as those described for Experiment 1. Hydrogen peroxide was measured weekly from January12 to March 16 using a HACH test kit (Model HYP-1).

Data Analysis
Because exploratory analysis revealed no treatment effects on water temperature, dissolved oxygen and pH, the data for each variable were pooled and presented graphically to show trends. The cell density, ichthyotoxicity, toxin concentration, nutrients, chlorophyll a, Secchi disk visibility, and zooplankton data were analyzed using PROC MIXED (SAS Institute Inc. 2002), assuming repeated measures construct with ponds as subjects. We tested a variety of covariance constructs (e.g., compound symmetric structure, first order autoregressive structure and spatial power structure) until we found the one that provided the best fit to 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. When a treatment-by-sampling-date interaction was significant, the SLICE option was used to determine significant differences in daily mean values among groups. For those sampling dates with significant differences among groups, pairwise multiple comparisons were performed using PROC MULTTEST and a stepdown Sidak approach to control the familywise error rate. The phytoplankton data were analyzed using GLM MANOVA whereas the fish production (yield, growth, production and survival) data were compared among treatment and control groups using one-way ANOVA. Appropriate data transformation was performed before statistical analysis. Differences were considered significant at $P < 0.05$.

Results

Experiment 1

Cell Density, Toxin, Ichthyotoxicity, Nutrients, and Water Quality
Prymnesium parvum was present in all ponds at the beginning of the study; however, densities began to significantly increase after the first week of September (Figure 1). Throughout the experiment, mean cell densities did not significantly differ among treatment and control groups. Similarly, mean toxin and ichthyotoxicity levels did not significantly differ among treatments and control groups, although mean levels significantly differed through time (Figure 1). The toxin was measurable in treatments and control ponds throughout the study, with peaks in mid-September and mid-October. Conversely, ichthyotoxicity was not detectable until after the first week in September, then increased sharply to a maximum of approximately 18.8 ITU in barley straw ponds before stabilizing at 12.5 ITU in all groups from October 21 to the end of the study.

Throughout the experiment, mean total P and total N values did not significantly differ due to treatment effects; however, both increased steadily through time (Figure 2). Mean ammonia-N also increased gradually during most of the study period, and then peaked in late September, followed by steep declines. The mean ammonia-N was significantly lower in the barley straw

ponds than in control ponds on August 2, but the concentration in LLMO ponds did not differ significantly from that of barley straw or the control. Orthophosphate-P was always below the detectable limit (0.05 mg/L) whereas nitrate-N averaged 0.31-0.32 mg/L for all groups during the first two weeks before becoming undetectable ($\leq$ 0.05 mg/L). Mean daily water temperature declined gradually over time with values ranging from 18.1 to 32.3oC and mean daily pH increased from 8.2 to 9.8 then declined below 9.0 (Figure 3). Mean daily dissolved oxygen concentration was extremely variable over time and ranged from 4.9 to 10.4 mg/L. Secchi disk visibility was never significantly different because of treatment effects; however, it did decline sharply in early July then varied between 60 and 80 cm for most of the study period (Figures 4).

Plankton

Mean chlorophyll-a concentration did not significantly differ among treatment and control groups, and despite the considerable variability in chlorophyll a, the general trend was an increase over time (Figure 4). Phytoplankton density generally was lowest in June, higher in November, and highest in September. Mean densities of each taxomic division did not significantly differ among treatments and control groups. However, mean densities of the Bacillariophyta, Cyanophyta, Chrysophyta, and Haptophyta significantly differed through time (Figure 5). Bacillariophytes were significantly lower in September and November than in June; chrysophytes were significantly higher in November than in June and September; and haptophytes were significantly higher in September and November than in June. The cyanophytes dominated the phytoplankton community throughout the study, with densities greater in September and November than in June. Conversely, the chlorophytes did not exhibit seasonal differences in density among treatments and control groups, although there was a slightly steady increase in density in barley straw ponds over time.

Mean densities of rotifers, copepod nauplii, adult copepods, and cladocerans did not significantly differ among treatments and control groups, although densities significantly varied through time (Figure 6). Copepod nauplii peaked first in late June, followed by adult copepod in early July, and cladocerans in early-mid July; then all three groups declined thereafter, with cladocerans disappearing by the end of September. Rotifers peaked in early July and remained high through early September before gradually declining. The zooplankton community was dominated by rotifers, followed by copepod nauplii, adult copepods, and cladocerans.

Fish Production

Most of the fish in the study ponds died from ichthyotoxicity in September. These mortalities were preceded by the fish exhibiting characteristics typical of ichthyotoxicity such as crowding in the shallow waters, piping, weakness, and attempting to jump out of the ponds. Fish production was poor across treatments and control groups, and there were no significant differences among groups. Mean survival ranged from 6.1 to 28.4%, production from 30.6 to 48.0 kg/ha, and harvest length from 21.1 to 33.6 mm (Table 1).

Experiment 2

Cell Density, Toxin Levels, Ichthyotoxicity, and Water Quality

Mean cell density, toxin concentration, and ichthyotoxicity did not significantly differ between treatment and control groups, although there were significantly different mean values through time (Figure 7). Similarly, mean water temperature, dissolved oxygen, pH, chlorophyll a, and Secchi disk visibility did not significantly differ on any sampling date due to treatment effect, but there were significantly different mean values through time (Figures 8 and 9). Mean hydrogen peroxide concentration was significantly higher in barley straw ponds than in control ponds.

Discussion

P. parvum Cell Density, Toxin Level, and Ichthyotoxicity

Barley straw, at the rates and time frames used in this study, did not control *P. parvum* in terms of inhibition of population growth, toxin production, or ichthyotoxicity. Barley straw is reported to exert control on susceptible algae for 6-8 months beginning about two weeks after application (Newman 1997); however, in Experiment 1 which lasted approximately 4.5 months no such control of *P. parvum* was evident. In this study, *P. parvum* density and ichthyotoxicity dynamics appear to have been a function of water temperature. The low cell densities and lack of ichthyotoxicity from June through August probably were due to the high water temperatures (≥25oC). Cell densities and ichthyotoxicity began to increase above detectable levels when temperature dropped below 25oC. This agrees with observations that in Texas waters *P. parvum* cells have been undetectable and ichthyotoxicity has been absent when water temperatures are ≥28oC (G. Kurten, Possum Kingdom State Fish Hatchery, Graford, Texas, personal communication) and is supported by reports that the upper temperature limit for *P. parvum* growth is 30oC (e.g., Shilo and Aschner 1953).

The detection of the toxin throughout the study, even when *P. parvum* cells occurred in densities too low to be detected, suggests that the toxin molecule may be persistent for extended periods of time. Without appreciable densities of *P. parvum* there should be little or no production of new ichthyotoxin material. The toxic effect was not expressed until in September when P. parvum cell density began to increase, toxin level significantly increased, and water temperature declined (<28oC). Apparently, either the warm water during June-August or the concentration of the toxin during this period was not conducive to expression of ichthyotoxicity (Shilo and Ashner 1953). Cationic inactivation and pH probably did not contribute to the lack of ichthyotoxicity expression because the cationic content (Ca^{2+} = 296 mg/L; Mg^{2+} = 63 mg/L) and pH (pH 8.3-9.8) of the pond water were suitable for toxicity expression (Shilo 1971).

In Experiment 2, which was conducted in winter when pond water was consistently toxic and temperatures were approximately 8-14oC, ichthyotoxicity persisted while cell densities increased over time in both barley straw and control ponds. Again, barley straw did not control *P. parvum* suggesting that either this alga is unsusceptible to the algistatic chemical(s) released by decomposing barley straw or the concentration of the algistatic chemical(s) was too low to be effective. Field and laboratory studies have revealed that decomposing barley straw can provide selective control of algae, even among closely related species (Terlizzi et al. 2002; Martin and Ridge 1999). Terlizzi et al. (2002) tested the effects of barley straw extracts on 12 dinoflagellates from three taxa (Gymnodiniales, Peridiniales, and Prorocentrales) and found that

growth was stimulated in three, inhibited in four, and unaffected in five. Similarly, Martin and Ridge (1999) reported growth inhibition by decomposing barley straw in 5 of 7 species of Cyanophyta; 7 of 8 species of Chlorophyta; and 2 of 3 species of Bacillariophyta tested under laboratory conditions. These researchers also reported that the inhibition of algal population growth by barley straw was dose related. In the present study hydrogen peroxide, proposed to be likely the algistatic chemical, or a component of the algistatic chemicals complex (Newman and Barrett 1993; Pillinger et al. 1994; Newman 1997), was approximately 5.7 fold lower than the 2 mg/L reported for Microcystis aeruginosa (Newman and Barrett 1993), an alga very sensitive to decomposing barley straw (Martin and Ridge 1999). Thus, if hydrogen peroxide is the algistatic chemical, then the quantity released from the decomposing barley straw in this study was below the effective dose. However, the inhibition is said to be due not to a single chemical but rather to the synergistic interaction of a suite of inhibitory components (Newman and Barrett 1993). Therefore, until the algistatic chemicals are fully characterized and reliably measured in water, hydrogen peroxide may not offer adequate explanation for the barley straw activity. Experiments with barley straw extracts have revealed that 50 times the recommended rate for control of most alga species was required to have an effect on *P. parvum* under laboratory conditions (D. Roelke, Texas A & M University, College Station, Texas, personal communication). Therefore, we believe practical application rates of barley straw may not control *P. parvum* as suggested by results of this study.

Nutrients and Water Quality

Nutrient levels and water quality were not affected by barley straw or LLMO. Unlike Lynch et al. (2003) who found significant reductions in chlorophyll a (43.5%) and total P (65%) and an increase in water clarity (133-156%) in 4-5 months, we did not see any effect of LLMO on these variables after 4.5 months. They used a pond containing fish that received 11.3 kg of commercial feed per week and likely treated the pond with LLMO according to the supplier's instructions, conditions almost similar to ours. One obvious difference was the LLMO application rate used in the present study was lower than the rates used by Lynch et al. (2003): we used 1 L/56,718 L of pond water biweekly whereas Lynch et al. (2003) used 1 L /15,934-19,852 L of pond water biweekly. Perhaps our low application rate or water chemistry variables prevented the bacteria from reaching population levels required to noticeably reduce nutrient levels. Nutrient reduction rate by bacteria is correlated with cell density (Wetzel 1975). Barley straw also had no effect on total P, total N and ammonia-N which agree with results of an earlier study (Welch et al. 1990). Because dissolved oxygen and pH in ponds are related to phytoplankton biomass (Boyd 1990), the lack of differences in chlorophyll a partially explains the similarity in pH and dissolved oxygen levels among treatments and control groups. Welch et al. (1990) also did not see barley straw treatment effects on dissolved oxygen or pH.

Plankton

The phytoplankton was examined at the community and major taxa (Division) levels since the Division Haptophyta consisted of only *P. parvum*, the target species of this study. The dynamics of the phytoplankton community and the algal divisions appear to have been influenced by natural conditions rather than study conditions. No significant differences in nutrient levels or zooplankton populations existed to promote differential phytoplankton growth. Similarly, zooplankton was unaffected by the treatments. The zooplankton population densities peaked in June-July and gradually declined throughout the rest of the study period. Because phytoplankton

is the main source of food for zooplankton in hatchery ponds (Geiger 1983; Ludwig et al. 1998) and other factors such as temperature and pH (Allan 1976) were similar among treatment and control groups, the similarity of the phytoplankton forage base may account for the lack of significant differences in zooplankton densities. Fish predation of zooplankton, which can significantly reduce zooplankton densities and alter zooplankton community structure in fingerling rearing ponds when fish densities are high (Brooks and Dodson 1965; Geiger et al. 1985; Morris and Mischke 1999), was probably insignificant in this study because of the low fish densities resulting from the massive toxin-related mortalities. Also, the surviving fish exhibited physical signs of stress and likely did not actively feed on zooplankton. Fish discontinue feeding when they are stressed (Randolph and Clemens 1976).

Fish Production

Fish production was poor in all ponds because of mortality resulting from *P. parvum* ichthyotoxicity. As in the ichthyotoxicity bioassay tests, fish mortality in ponds was first observed in September when water temperatures dropped to 25°C and below. For each study group 50% of the ponds lost virtually all fish, drastically increasing variability in the data and decreasing the power to detect differences in production variables. For economic or practical purposes the survival of 28.4% from LLMO ponds was better than the 6.1% from barley straw ponds, although the difference was not statistically significant. Overall, both barley straw and LLMO did not prevent ichthyotoxicity and consequently had no beneficial effects on fish production.

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Table 1.—Mean ± SE production variables for koi carp fingerlings cultured in plastic-lined ponds subjected to barley straw treatment, Liquid Live Micro-Organisms System treatment, or no treatment (control) for 140 d. No variable was significantly different among treatment and control groups ($P > 0.05$).

Treatment	Pond (ha)	Fry stocked (No./ha)	Survival (%)	Production (kg/ha)	Total length (mm)	Yield (No./ha)
Barley	0.27	175,645	6.1 ± 6.1	30.6 ± 30.5	27.3 ± 15.8	10,726 ± 10,680
LLMO	0.26	184,869	28.4 ± 19.4	47.7 ± 27.6	21.1 ± 12.2	52,712 ± 34,405
Control	0.26	181,115	22.6 ± 13.2	48.0 ± 28.3	33.6 ±11.4	42,307 ± 25,080

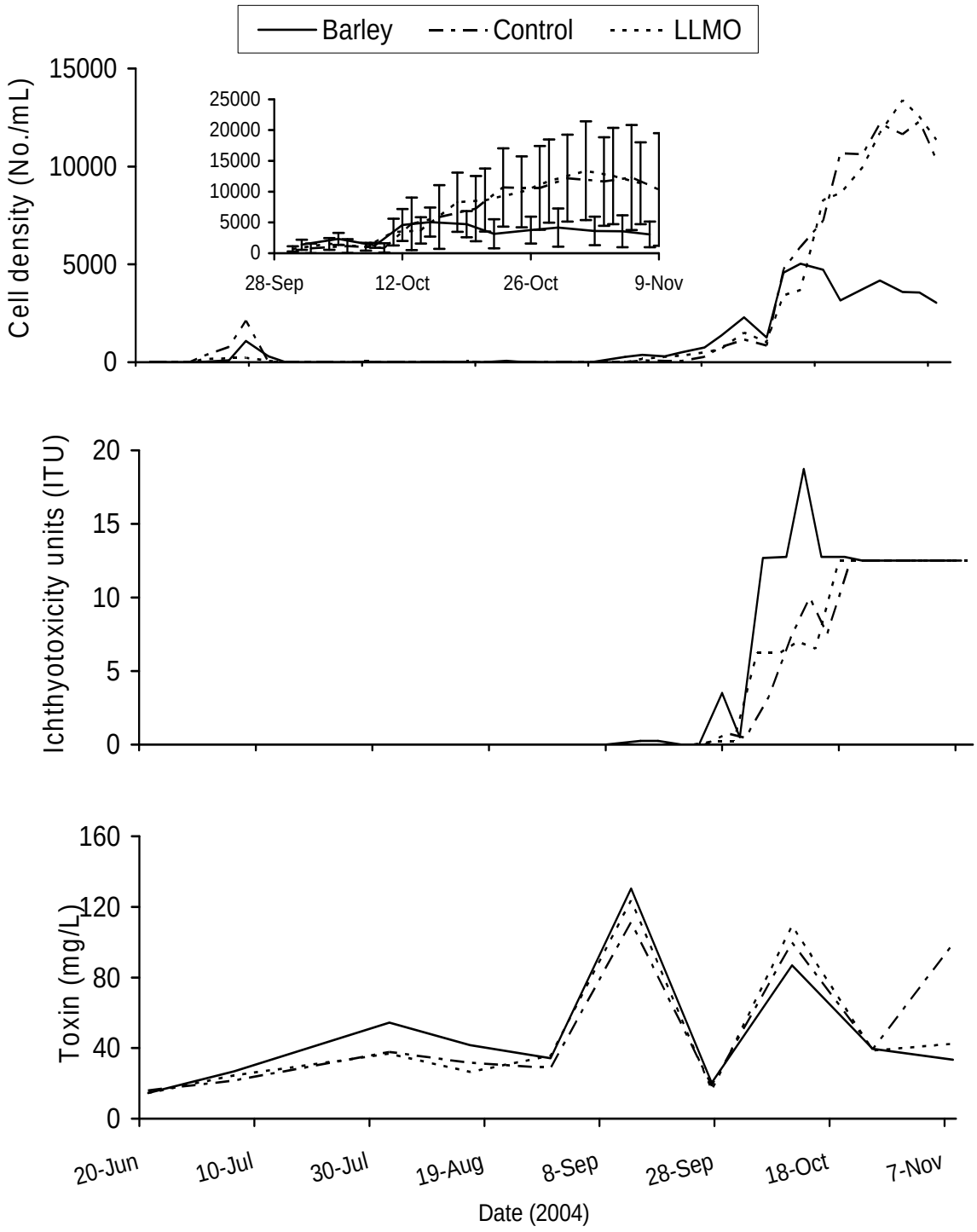

Figure 1. —Mean *Prymnesium parvum* cell densities, ichthyotoxicity, and toxin concentrations in ponds subjected to barley straw treatment, Liquid Live Micro-Organisms System treatment, or no treatment (control) in June 21-November 8, 2004 at Possum Kingdom State Fish Hatchery, Texas. Vertical bars are standard errors. There were no significant ($P > 0.05$) differences among treatments and control ponds.

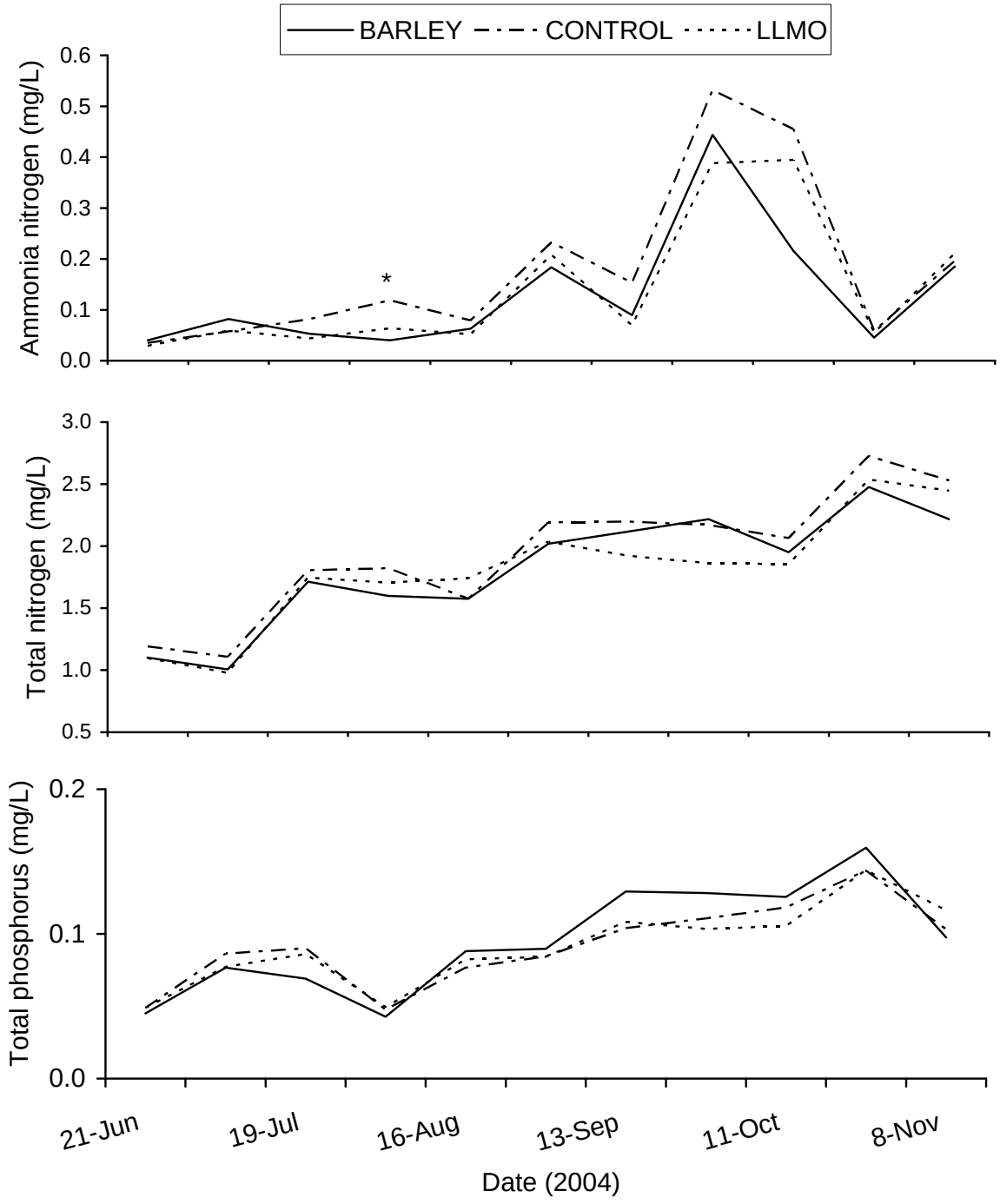

Figure 2.—Mean nutrient concentrations in ponds subjected to barley straw treatment, Liquid Live Micro-Organisms System treatment, or no treatment (control) in June 21-November 8, 2004 at Possum Kingdom State Fish Hatchery, Texas. An asterisk denotes significant ($P \leq 0.05$) differences among treatments and control ponds.

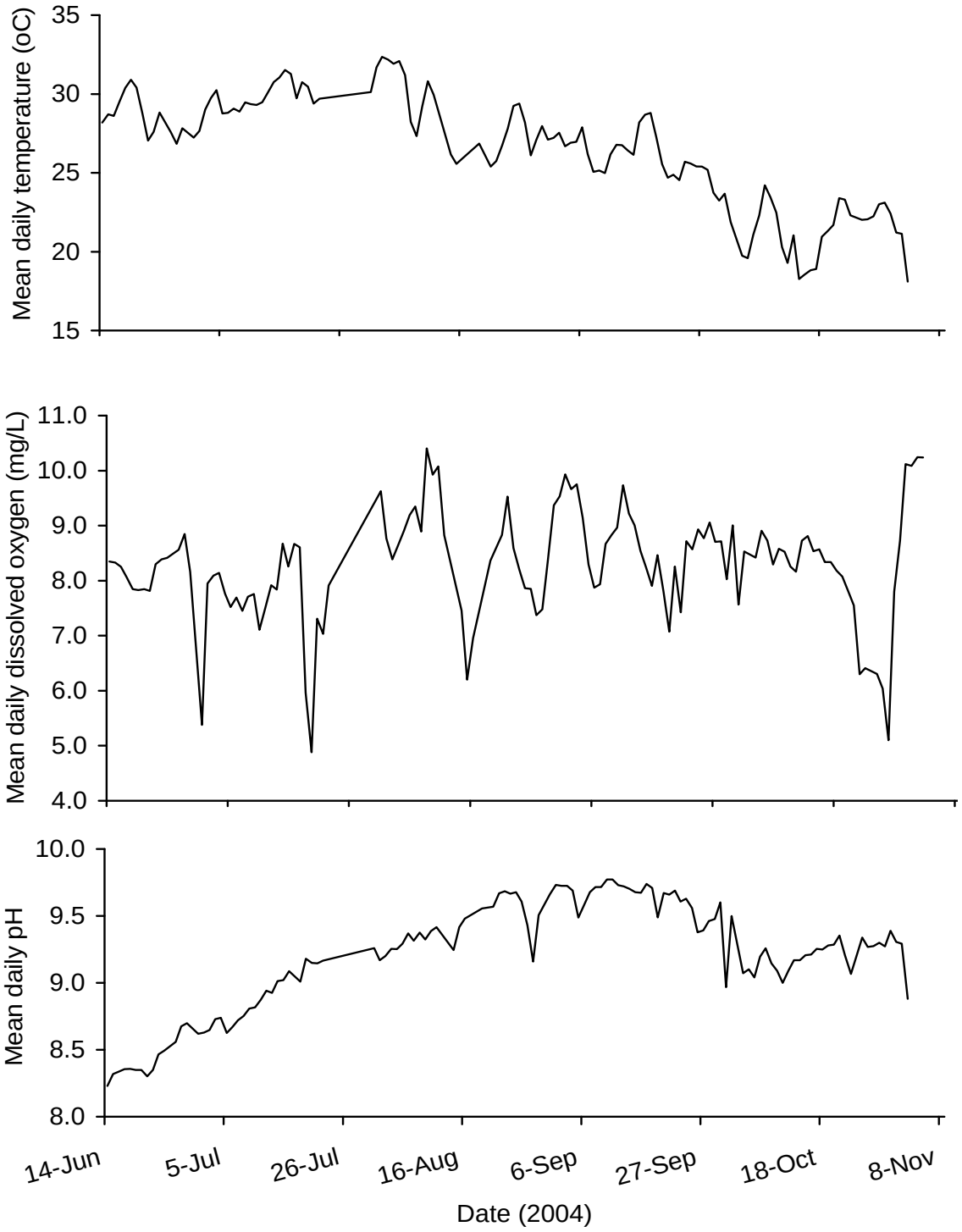

Figure 3. —Mean daily water quality variables for all ponds subjected to barley straw treatment, Liquid Live Micro-Organisms System treatment, or no treatment (control) in June 16-November 8, 2004 at Possum Kingdom State Fish Hatchery, Texas.

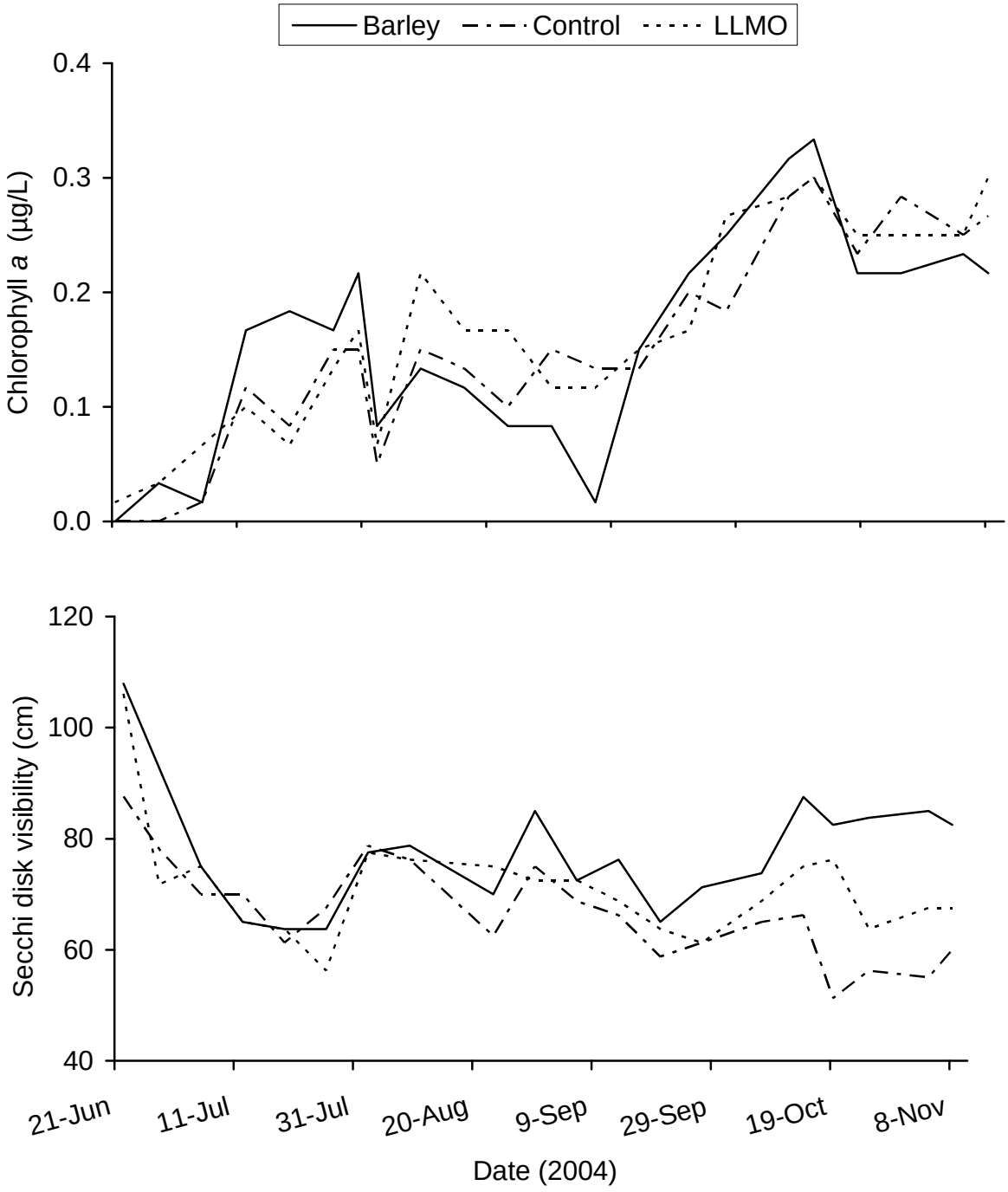

Figure 4. —Mean Secchi disk visibilities and chlorophyll-a concentrations in ponds subjected to barley straw treatment, Liquid Live Micro-Organisms System treatment, or no treatment (control) in June 21-November 8, 2004 at Possum Kingdom State Fish Hatchery, Texas. There were no significant ($P > 0.05$) differences among treatments and control ponds.

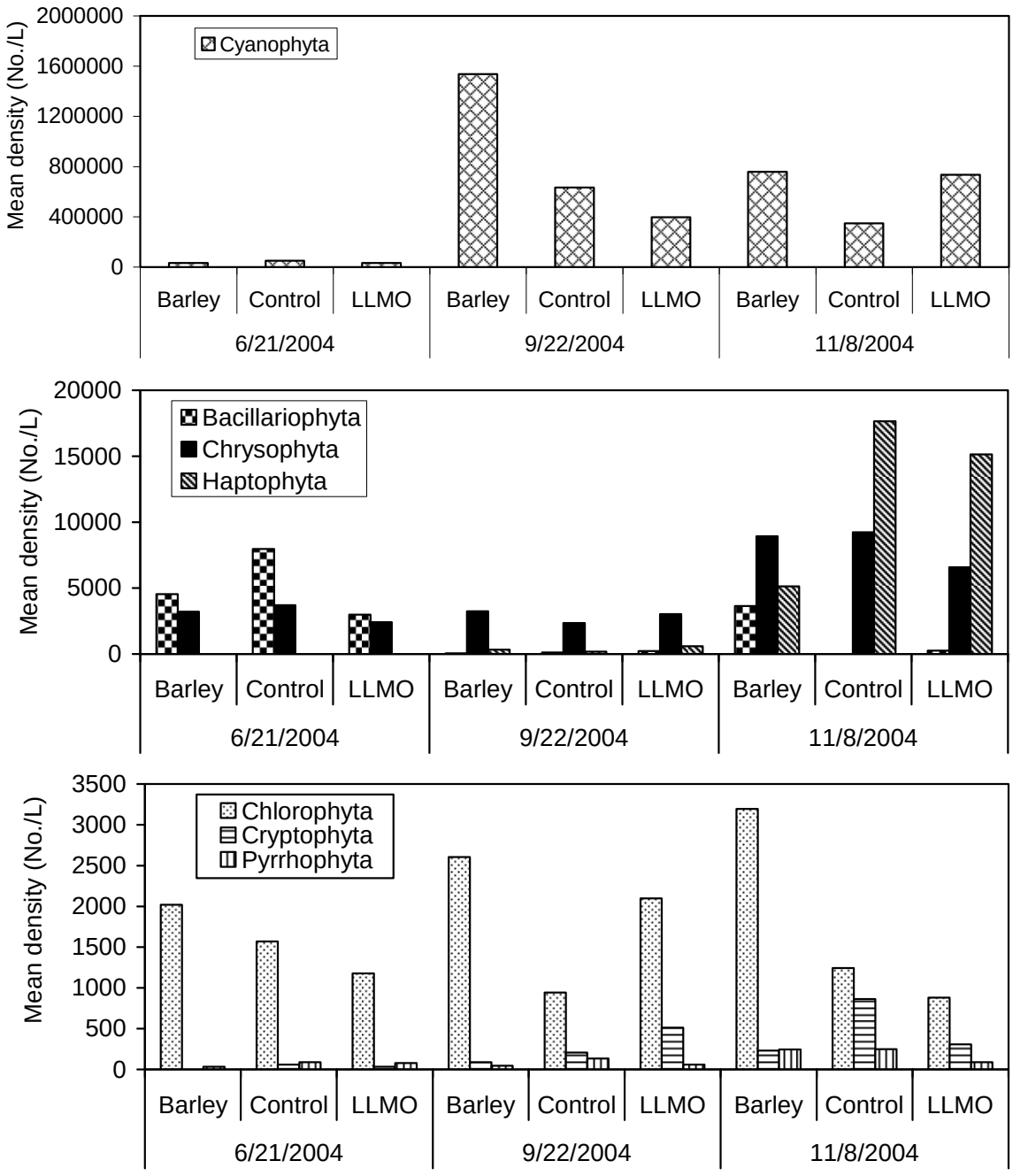

Figure 5. —Mean densities of phytoplankton taxa in ponds subjected to barley straw treatment, Liquid Live Micro-Organisms System treatment, or no treatment (control) in June 21-November 8, 2004 at Possum Kingdom State Fish Hatchery, Texas. There were no significant ($P > 0.05$) differences among treatments and control ponds.

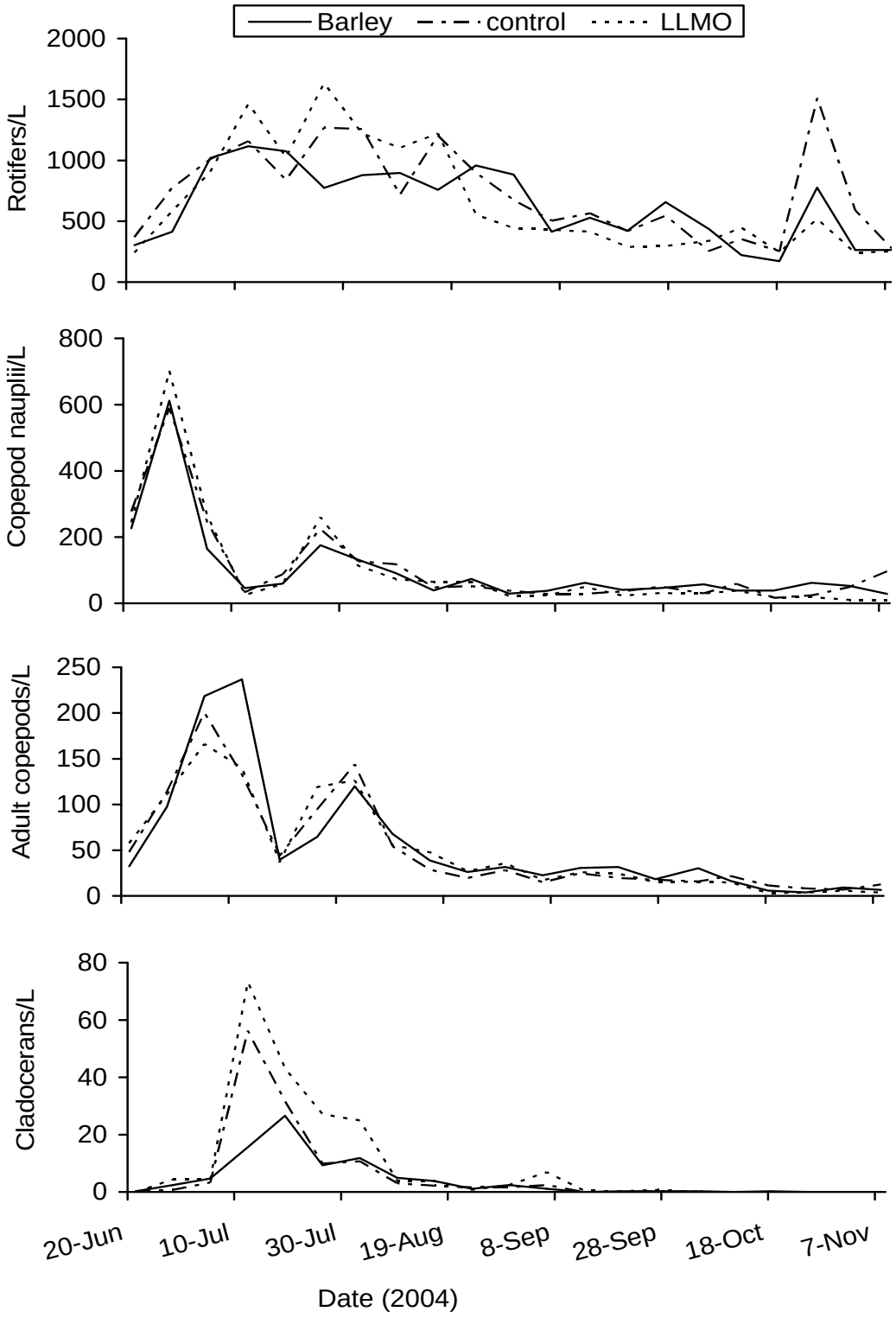

Figure 6. —Mean zooplankton densities in ponds subjected to barley straw treatment, Liquid Live Micro-Organisms System treatment, or no treatment (control) in June 21-November 8, 2004 at Possum Kingdom State Fish Hatchery, Texas. There were no significant ($P > 0.05$) differences among treatments and control ponds.

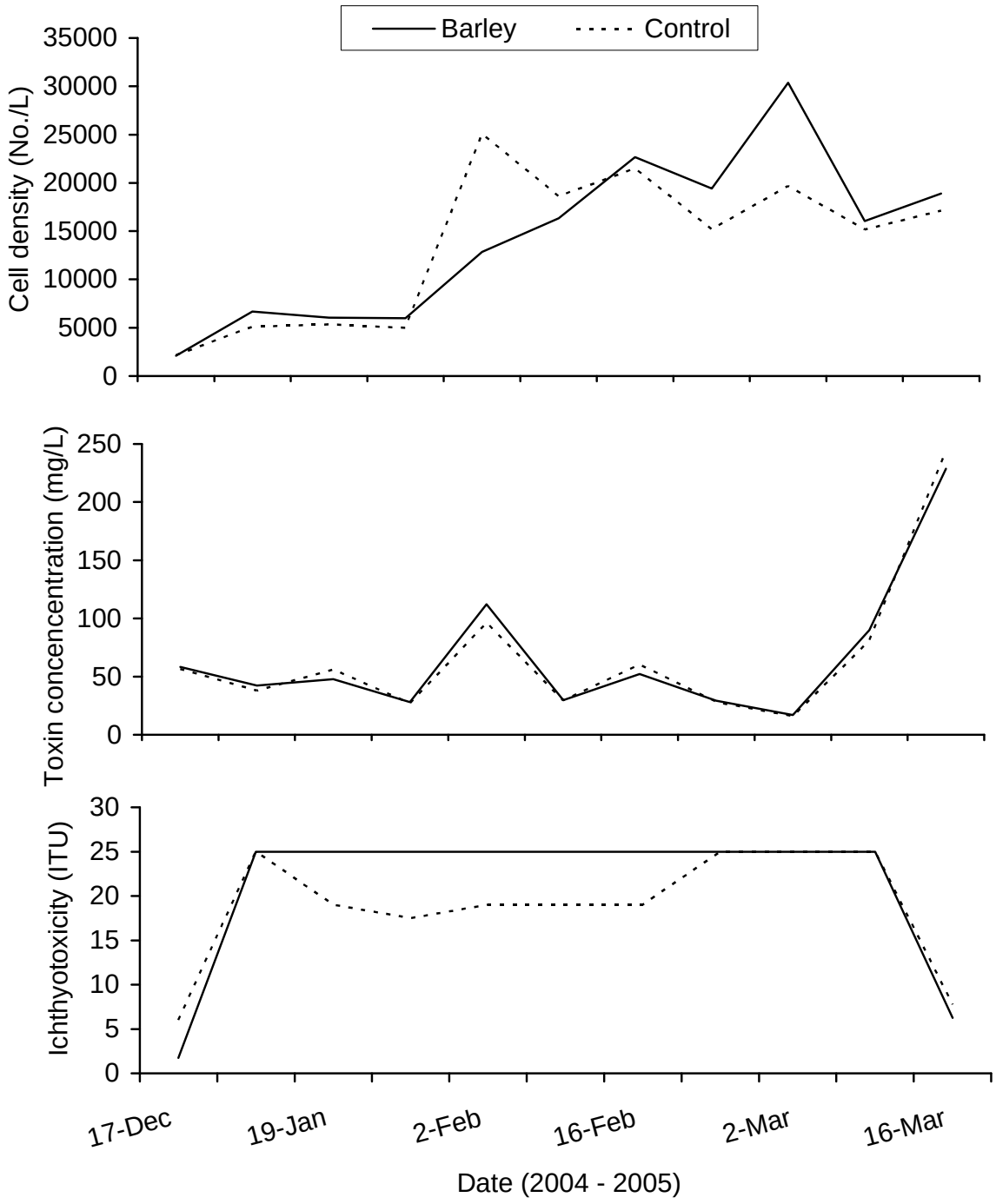

Figure 7. —Mean *Prymnesium parvum* cell densities, ichthyotoxicity, and toxin concentrations in ponds subjected to barley straw treatment or no treatment (control) in December 2004-March 2005 at Dundee State Fish Hatchery, Texas. There were no significant ($P > 0.05$) differences between treatment and control ponds.

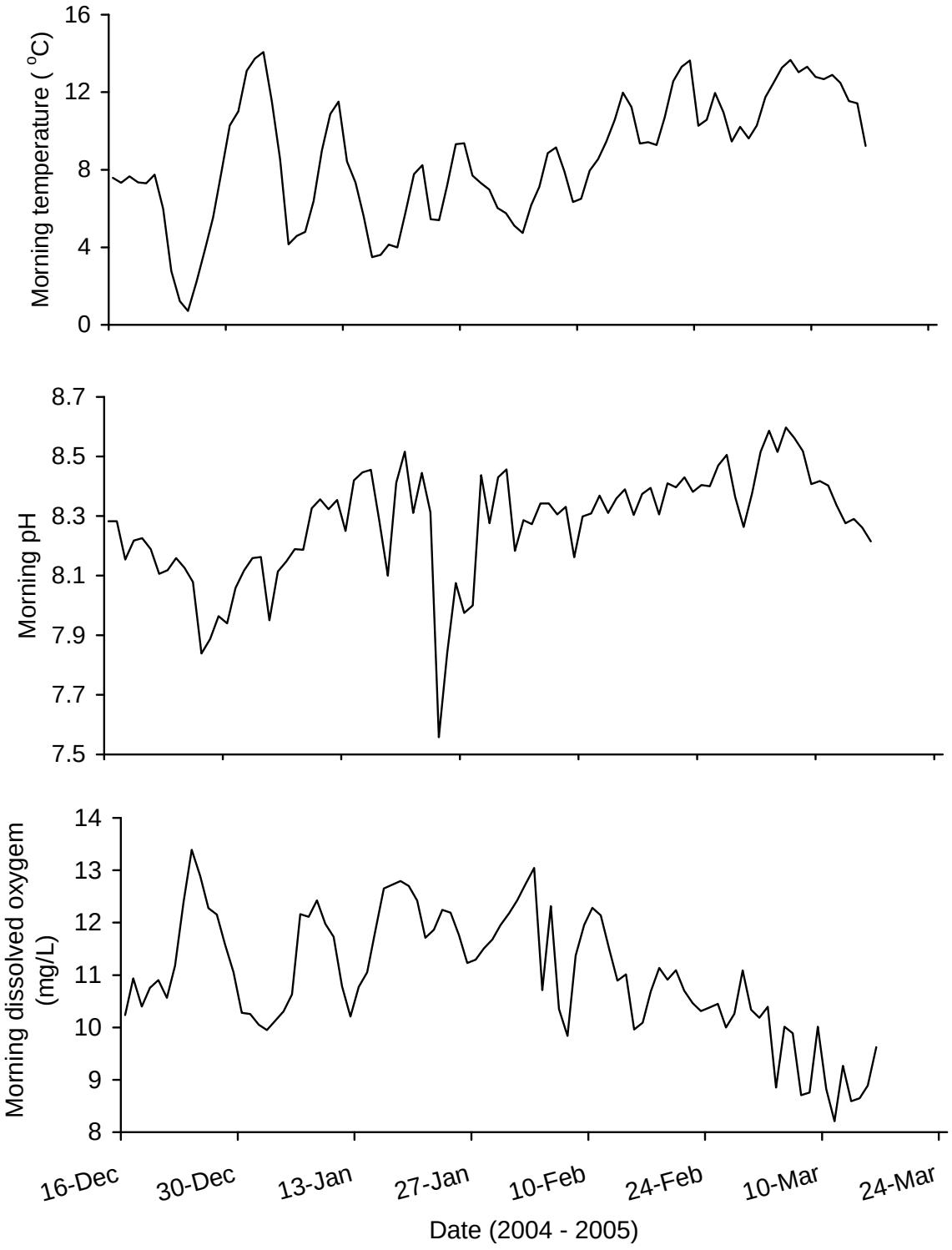

Figure 8. —Mean water quality variables in ponds subjected to barley straw treatment or no treatment (control) in December 2004-March 2005 at Dundee State Fish Hatchery, Texas. There were no significant ($P > 0.05$) differences between treatment and control ponds.

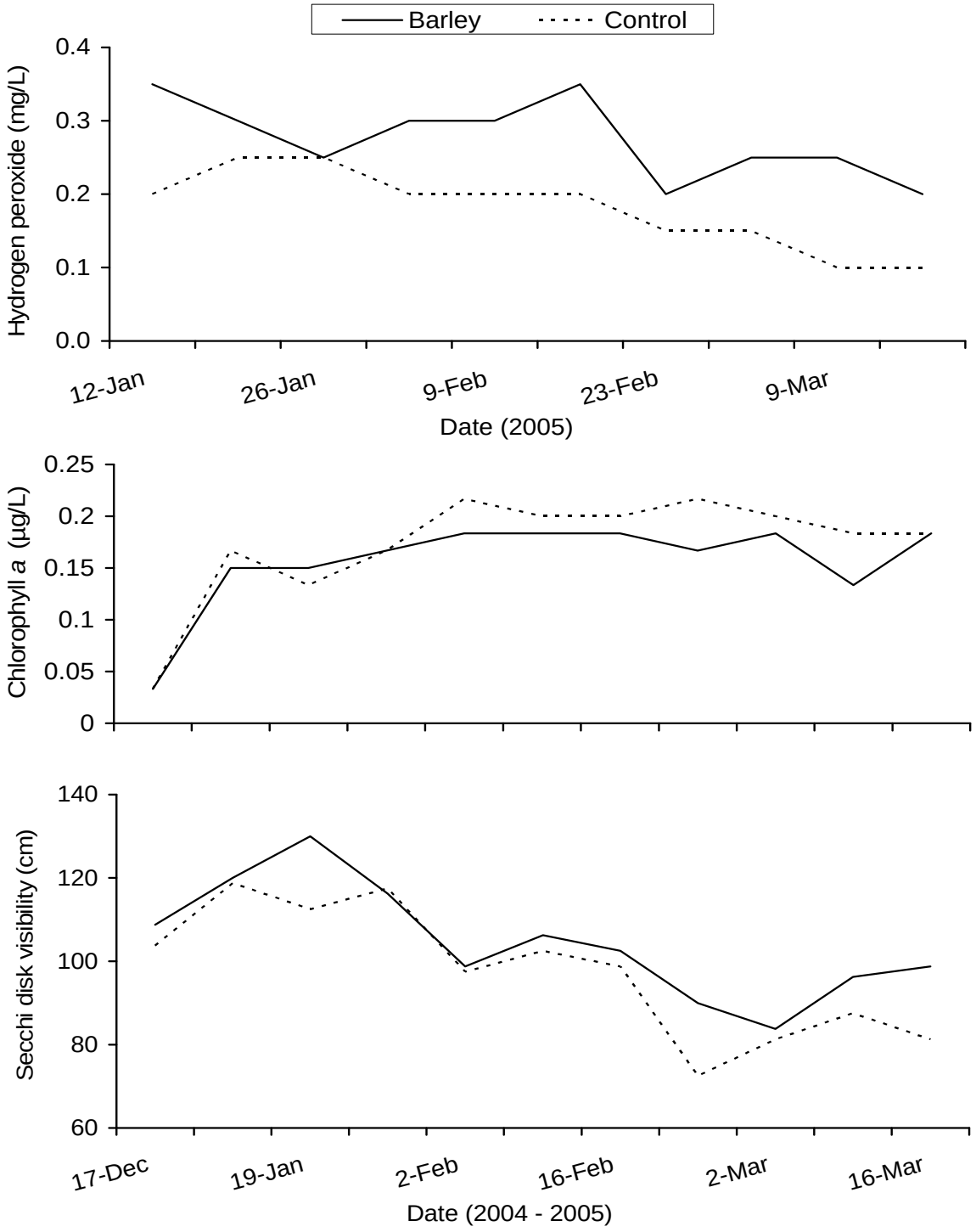

Figure 9. —Mean hydrogen peroxide, chlorophyll a and Secchi disk visibility in ponds subjected to barley straw treatment or no treatment (control) in December 2004-March 2005 at Dundee State Fish Hatchery, Texas. Between treatment and control, the difference in chlorophyll a or Secchi disk visibility was not significant ($P > 0.05$) but the difference in hydrogen peroxide was significant ($P \leq 0.05$).