Nitrogen Fertilizer Reduction and Nutrient Budgets in Florida Largemouth Bass *Micropterus salmoides floridanus* Fingerling Rearing Ponds

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ABSTRACT

Fixed rates of organic and inorganic fertilizer are added to Florida Largemouth Bass *Micropterus salmoides floridanus* fry rearing ponds at the A. E. Wood State Fish Hatchery (AEW) to stimulate phytoplankton and zooplankton production. However, relatively high nitrogen concentrations in source water and high N:P ratios, pH and ammonia measured in ponds may indicate over-fertilization with nitrogen. We conducted a study to determine the impact of 36% reduction in inorganic N-input through reduced application frequency on Florida Largemouth Bass fingerling production and pond dynamics. Half of the ponds stocked received the standard fertilization treatment (Control) while the other half had four fewer inorganic fertilizer (Uran) applications (Low-N). The data collected was also used to construct partial N and P budgets for ponds under the two fertilization regimes. Differing N fertilizer input had no significant impact ($P > 0.05$) on any fish production parameter. The main effect of reducing N-input was to limit a secondary phytoplankton bloom, as expressed by significantly lower ($P < 0.05$) chlorophyll *a*, *b*, and *c* concentrations in Low-N ponds. There were no significant differences ($P > 0.05$) in other water quality parameters, although high pH, DO, and ammonia concentrations were more prevalent in the control ponds during the second half of the production cycle. The mean N:P ratio in control ponds in the present study (4.3:1) was considerably lower than previously reported (13.8:1), suggesting that these ponds were not over-fertilized with N during April–May. The lack of significant differences in fish production parameters suggests N-fertilization could be reduced in FLB fry rearing ponds at AEW without compromising fingerling production. However, the suppression of the secondary phytoplankton bloom indicates productivity in the second half of the production cycle was limited when N-input was reduced. There may be scope for further seasonal refinements of the fertilization regime to reduce the prevalence of high pH and requirement for pond flushing. The A. E. Wood waste treatment plant effectively removed residual nutrients from pond effluent, with hatchery effluent containing lower total N than source water. Fertilizer input represented 96.8–97.1% of N and 99.6% of P total input over the production cycle. An average of 24–25% of N and 18–27% of P-inputs were retained in harvested fingerlings. These budgets may be useful for future refinements of fertilization protocols and comparison of nutrient retention efficiencies at AEW.

INTRODUCTION

Fertilizing ponds is a well-established process that enhances phytoplankton biomass, and increases zooplankton biomass to provide forage for young fish (Geiger 1983a, b; Parmley et al. 1986; Boyd 2012a). To a point, fish production (yield) in fertilized ponds is positively correlated with phytoplankton productivity (Yusoff and McNabb 1989; Knud-Hansen et al. 1991; Boyd and Tucker 1998; Banerjee et al. 2009). Phosphorus (P) and nitrogen (N) are the key macronutrients controlling primary production in ponds (Qin 2012). Inorganic fertilizers primarily provide N and P to stimulate phytoplankton blooms but also accelerate the bacterial decomposition of organic matter (Soderberg 2012). Organic fertilizers stimulate phytoplankton blooms but also provide carbon to stimulate heterotrophic growth as an additional food source and ammonia control measure (Barkoh 1996; Coyle et al. 2012; Soderberg 2012). Combinations of organic and inorganic fertilizers in ponds are generally more effective than either treatment alone (Geiger et al. 1985; Boyd 1990; Fox et al. 1992; Soderberg 2012).

The main management approaches to fertilizing ponds include manipulating pond nutrient ratios (Qin 2012), algal bioassay fertilization (Knud-Hansen 2012), target level fertilization (Culver et al. 1993; Kurten 2001), and fixed-rate fertilizer applications (Mischke 2012). Texas Parks and Wildlife Department (TPWD) hatcheries have traditionally used fixed-rate fertilizing strategies as they are simple, routine, and don't require additional water testing (Mischke 2012). However, Kurten (1995) demonstrated the benefits of maintaining target nutrient concentrations through increased water quality monitoring at the Jasper State Fish Hatchery.

Reported pond fertilization rates vary widely (e.g., optimum N:P ratios of 2:3 to 20:1) and can be contradictory, depending on many factors including source water characteristics, climate, pond design and age, native algal and zooplankton species, and fish species cultured (Yusoff and McNabb 1989; Mischke 2012; Soderberg 2012). These levels and ratios are reviewed by Boyd (1990), Barkoh (1996), Kurten (2001), Coyle et al. (2012), Mischke (2012), Soderberg (2012), and Qin (2012). Studies in small ponds from relatively few locations with often dubious conclusions have served as guidelines for pond fertilizing regimes at sites with much different soil and water characteristics, giving mixed results (Wudtisin and Boyd 2005; Banerjee et al. 2009). In addition, most pond fertilizing regime and nutrient budget research has been conducted in earthen ponds. Since pond membranes prevent the influx and efflux of essential nutrients from sediment, fertilizer requirements differ in plastic-lined ponds (Funge-Smith and Briggs 1998; Rogge et al. 2003). Knud-Hansen et al. (1991) suggested that "optimal fertilization rates and N:P input ratios will be system specific, as internal nutrient loading is affected by such factors as pond substrate, pond history, mean depth, and water exchange/mixing characteristics." Pond fertilizing regimes thus must be tailored to site-specific conditions.

Kurten (2001) and Coyle et al. (2012) describe the development of the fertilization regime for Largemouth Bass *Micropterus salmoides* (LMB) fingerling production at the A. E.

Wood State Fish Hatchery (AEW). Glenewinkel et al. (2011) describes the current regime in use (Table 1), which was originally adopted from Hutson (1990). Both organic and inorganic N sources are used to establish and feed phytoplankton at AEW.

Soderberg (2012) suggested that two important principles of inorganic fish pond fertilization are: 1) P is nearly always the limiting nutrient to primary production (Yusoff and McNabb 1989; Boyd 1990; Coyle et al. 2012); and 2) small fertilizer doses result in large increases in fish production, whereas additional inputs increase fish production in ever-decreasing amounts (the Law of Diminishing Returns). Groeger et al. (1997) reported that the upper San Marcos River, the AEW water source, is normally relatively high in N (1.5–1.7 mg NO_3-N/L; 0.001–0.030 mg NH_4^+-N/L) and strongly P-limited (0.005–0.015 mg soluble reactive P/L; 0.015–0.030 total P/L). Measurements of source water by AEW (2006–2018; 0.7–1.6 mg NO_3-N/L; 0.004–0.012 mg NH_3-N/L; <0.002 mg P/L) and those reported by Fries and Bowles (2002) confirm this observation. This indicates that additional N-input into ponds may not be necessary (Kurten 2001).

Kurten (2001) conducted a baseline study into fertilization of Florida Largemouth Bass *Micropterus salmoides floridanus* (FLB) fingerling rearing ponds at AEW. In this study both pH as high as 10.8 and NH_4-N as high as 0.314 mg/L (not concurrently) were reported. This elevated level would not limit survival (Tomasso and Carmichael 1986) but may inhibit fry growth (Hargreaves and Kucuk 2001). The N:P ratio for this study averaged 13.8:1. The theoretical N:P ratio for balanced phytoplankton growth is 7:1 with adequate carbon availability (Welch 1980; Cromar and Fallowfield 1997). The average P concentration exceeded the minimum target of 0.03 mg/L (Culver et al. 1993; Coyle et al. 2012). Kurten (2001) concluded that the skewed N:P ratio was the result of over fertilization with N rather than P limitation. He suggested further investigation to determine if reduction or elimination of N additions after inoculation would limit stress on fry and increase growth rates. Reduced fertilizer use could also reduce cost and N waste.

Nutrient budgets are useful for "identifying and quantifying sources of nutrient gain and loss in fish ponds." (Daniels and Boyd 1989). Nutrient budgets have predominantly been reported for earthen ponds, with artificial feed or organic fertilizer supplied (Boyd 1985; Krom et al. 1985; Schroeder 1987; Knud-Hansen et al. 1991; Funge-Smith and Briggs 1998; Gross et al. 2000; Adhikari et al. 2014). Where they have been reported for lined ponds, these also had soil added (Daniels and Boyd 1989). Daniels and Boyd (1989) reported that pond liners have minimal impact on nutrient budgets since nutrient loss from earthen ponds through seepage is low. However, the sediment in earthen ponds can be a major nutrient sink both absorbing and supplying nutrients within and between production cycles (Boyd 1985, 1990, 2012a; Schroeder 1987; Hargreaves 1998). Data appears lacking for N and P budgets in lined ponds for the production of sportfish fingerlings. Such budgets are likely to be relatively site specific, being dependent on localized factors such as source water composition, climate, fertilizer type and regime, and plankton species.

Collecting water quality data can be time consuming and prone to human error. As a result, routine sampling times are typically restricted to a feasible time frame in terms of cost and labor, while assuming parameter maxima and minima are reasonably covered. Parameters that vary diurnally such as dissolved oxygen, pH and temperature are usually measured twice-daily. Other parameters such as nitrogenous compounds and alkalinity are typically measured less frequently (e.g. weekly) or only in response to deteriorating fish health. Such protocols risk missing short-term extreme values that occur outside of the routine sampling times. Data logging meters permit increased sampling frequency without concurrent increased labor and are more likely to capture the full range of parameter variance in a waterbody. This is particularly important for research where water quality is a key factor. The TPWD Inland Fisheries Analytical Services Laboratory recently acquired a Manta2 Multiprobe data logger that can measure and log eight different parameters at regular intervals. This instrument could be a valuable tool for hatchery research and operations but its effectiveness and application needs to be evaluated in hatchery pond conditions.

The aim of the present study was to 1) determine if inorganic N-input can be reduced by 36% through reduced application frequency without compromising FLB fingerling growth and survival; 2) determine the impact of 36% reduction in inorganic N-input and reduction in application frequency on pond dynamics—water quality, phytoplankton, and zooplankton; 3) construct partial N and P budgets for ponds under each fertilization regime; and 4) evaluate the effectiveness of a Manta2 Multiprobe for intensive monitoring of a variety of water quality parameters in ponds.

MATERIALS AND METHODS

Study location and design

This study was conducted at the A. E. Wood State Fish Hatchery, San Marcos, Texas in 0.4-ha (4,731,250 L) EPDM-lined ponds. Of the six ponds stocked with FLB in April 2016, half (three ponds) received the standard fertilizer treatment, while the other half (three ponds) received four fewer Uran (32-0-0 blend, Arcadian®) applications (Table 1), a difference of 5.07 kg total N per pond. All ponds received the same quantity of cottonseed meal (CSM; AO Nutrition, Ardmore, OK) and phosphoric acid (54% P_2O_5, Amberphos-54™, Nutrien, Saskatoon, Canada). Pond filling commenced on the same day FLB spawns were obtained in the hatchery and fry were stocked 5–9 days later. Test and control ponds were stocked alternately on each stocking day with approximately 200,000 fry/pond (500,000/ha; Table 2).

Ponds were prepared, FLB fry stocked, sampled, harvested and enumerated, and zooplankton were sampled twice-weekly following standard AEW protocols (Kurten 2001; Glenewinkel et al. 2011). Some of these protocols were adjusted to better standardize treatment of ponds. Liquid fertilizers were applied to ponds in individual portions and the spray rig flushed into the pond after each application to ensure equivalent nutrient input. Thresholds for flushing

ponds were widened to morning DO < 3 mg/L and afternoon pH > 10.5 to limit or eliminate pond flushing during the study. Fingerlings in all ponds were sampled on day 15 post-stocking in addition to standard sampling times. Ponds were not inoculated with zooplankton.

Water quality

Temperature, DO, and pH and were measured at 0600–0700 hours and 1530–1630 hours each day in all ponds using a YSI Model 650 MDS data logger equipped with a YSI Model 600XL probe (Yellow Springs Instruments, Yellow Springs, Ohio). A 1-L water sample was collected from inlet water on the first two days, from ponds every morning (0600–0930 hours, elbow depth next to the pond kettle), and at harvest. These samples were analyzed daily for chlorophyll a, chlorophyll b, and chlorophyll c (Method 10200 2c, Eaton et al. 2005), NH_3-N (Method 4500-NH_3 D, Eaton et al. 2005), NO_2-N, NO_3-N, PO_4-P, fluorine, chlorine, bromine, and sulfate (Method 2540 E, Eaton et al. 2005). The alkalinity of a water sample was measured (by titration using Method 2320 B, Eaton et al. 2005) every second day or daily if a large pH change (>1) was observed within the previous 12 h. Total suspended solids (TSS) was measured (Method 2540 D, Eaton et al. 2005) weekly and volatile suspended solids (VSS, Method 2540 E, Eaton et al. 2005) at harvest. Chlorophyll samples (75–400 mL) were filtered after collection and the filters frozen for later analysis. Alkalinity was measured within 6 h of collection. The remainder of the sample was frozen for later analysis.

A Manta2 Multiprobe (Model: sub 3.5, Eureka Water Probes, Austin, Texas) measured chlorophyll a, NH_4-N, NO_3-N, conductivity, DO, pH, temperature, and turbidity from mid-water column at the harvest-kettle end of a single pond every 15-min. The unit was deployed in three different ponds over the study period. We installed fresh batteries, downloaded data, and calibrated the unit every 96–120 h.

Nutrient budgets

For developing partial nutrient budgets, the total N and P-input from CSM, Uran, phosphoric acid, and initial water concentration was calculated based on the total weight and mean N and P content of each component (i.e., *nutrient concentration * total weight/volume supplied,* Adhikari et al. 2014). The N and P quantity incorporated into harvested fish biomass was calculated according to the following equation:

$$N/P \text{ content} = (B_H - B_S) * \% N/P$$

Where, B_H = Fish (fingerling) dry biomass at harvest, B_S = Fish (fry) dry biomass stocked, % N/P = Percentage N or P in fish.

To calculate N and P content of fish, five 5-g fry samples were randomly selected at stocking (assuming 275 fry/g [6,875 fry] and 20% dry weight to obtain > 5 g dry pooled sample), and 20 fingerlings per pond were randomly selected at harvest (assuming 38 mm/1.3 g fish and

20% dry weight to obtain > 5 g dry pooled sample). Sampled fingerlings were purged for 4 h in pond water filtered through a 60-μm screen to remove zooplankton. Purged fish were then weighed, and frozen at -20°C. A wet sediment sample (1 L in total) was collected from three random locations in each pond immediately following harvest. For analysis, fry, fingerling, and sediment samples from each pond were independently dried at 110°C, cooled in a desiccator, re-weighed to calculate dry matter content, ground, homogenized, and analyzed for total N and P (Davis and Boyd 1978; Mallekh et al. 1999). A ~12-g portion of each dry sediment sample was held in a furnace at 550°C for 1 h to obtain % volatile (organic) content.

The measured N and P concentrations in input (inlet; first two days filling) and output (last two days' pond samples when ponds were draining) water for each pond were calculated as the sum of the mean NH_3-N, NO_2-N, NO_3-N, and chlorophyll-N, and the mean PO_4-P concentrations plus the assumed content of suspended biomass (VSS of 2 mg/L for inlet water, 60% of measured pond TSS for output water, N and P content in suspended biomass of 3 and 0.25% [Nagata 1986; Fagerbakke et al. 1996; Kütter et al. 2014; Samocha et al. 2017]).

Total N and P were measured in inlet water at pond filling and in pond water and sediment at harvest. The N and P contents in both unfiltered and filtered (64-μm) water samples were measured to estimate the content in zooplankton. Total N content in water, sediment, and fish was determined by the standard test method for total chemically bound N in water by pyrolysis and chemiluminescence detection (Method ASTM D5176-08(2015), ASTM 2015). Total P was determined by inductively coupled plasma-atomic emission spectrometry (Method 6010B, U. S. Environmental Protection Agency 1996).

Data analysis

Differences in fish production and overall zooplankton and water quality variables between treatments were assessed with a Wilcoxon test (two-sample, normal approximation) due to the small number of replicates (n=3) or a Welch's test where variances were not equal (Levene's < 0.05) using JMP 14 (SAS Institute Inc., Cary, North Carolina). All PO_4-P and chlorophyll values less than detection limits (DL: 0.03 mg/L PO_4-P and 0.002 mg/L chlorophyll a, b, c) were replaced with DL/2 (U. S. Environmental Protection Agency 2006). The NO_2-N, NO_3-N, and zooplankton data were $\log_{10} (x + 1)$-transformed before analysis due to zeros in the data (Barkoh et al. 2010). Data collected repeatedly from each pond (the first 33 days for the whole cycle, days 6–33 for the fish production cycle, and days 20–33 for the secondary phytoplankton peak) was modelled assuming a repeated measures construct with ponds as subjects, using first order autoregressive covariance structure and ML or REML estimation method (linear mixed models) using SAS Studio (SAS Institute Inc., Cary, North Carolina). The Akaike's Information Criterion and the Null Model Likelihood Ratio were used to determine the model of best fit for the data (Littell et al. 2000). We tested the effects of treatment, day post-filling, and the treatment x day interaction on each variable. Data for all analysis was considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Fish production

Differing N fertilizer input had no significant impact ($P > 0.05$) on any fish production parameter (Table 2). Fish length at 15 days post-stocking was used as a standard point of comparison given differing culture periods between ponds. Mean length at 15 days post-stock and harvest, and growth rates (mm/day and specific growth rate [%mm/day]) were all similar between fertilization regimes. Mean K values ranged from 0.71 to 1.04 and were also similar between fertilization regimes. This data suggests that reducing inorganic N-input into ponds by 36% did not compromise FLB fingerling condition or production at AEW. Further, there was no evidence that reducing inorganic N-input limited stress on fry and increased growth rates, as proposed by Kurten (2001). However, limited replication, differing culture periods, and high variation in survival between ponds in the present study limits the strength of these conclusions.

Phytoplankton and zooplankton

The main effect of reducing N-input was to limit the secondary phytoplankton bloom. Chlorophyll *a* concentrations were significantly lower ($P < 0.05$) in the Low-N ponds for both the whole and fish cycles (Table 3); while Chlorophyll *a*, *b*, and *c* were significantly lower ($P=0.021$, 0.029, and 0.032, respectively) in the Low-N ponds (means: 0.045, 0.009, and 0.005, respectively) during the period of the secondary phytoplankton bloom (days 20–33) than in the control ponds (means: 0.117, 0.031, and 0.009, respectively). Chlorophyll *b* and *c* concentrations were close to significantly lower ($P = 0.055–0.061$) in the Low-N than in the control ponds for the whole and fish cycles (Table 3). The measured chlorophyll concentrations (Figure 1) indicate phytoplankton densities. Concentrations increased for the first week at the same rate in both treatments, peaking 6–7 days after pond filling commenced. A larger secondary peak occurred around days 23–33 in the control ponds but to a much lesser degree or not at all in the Low-N ponds. Kurten (2001) reported similar bimodal trends in phytoplankton density in FLB ponds using the same fertilization regime as the control in the present study. The effect of this secondary bloom on zooplankton populations is difficult to determine from the data collected due to limited sampling times. Nitrogen input started diverging between treatments from day 8 (Table 1), after the initial chlorophyll peak. The lower N-input after this point clearly limited phytoplankton growth. The occurrence of the secondary phytoplankton peak did not significantly increase fingerling production. However, it did not create water quality conditions that were detrimental to production, i.e., significantly higher pH or lower DO. Given typically high variation in phytoplankton dynamics and fish performance between ponds, reducing nutrient input may risk stunting zooplankton populations and restricting fish growth and survival in some ponds. Supplying excess nutrients is thus advised to maximize fish productivity, provided water quality does not deteriorate and reduce fish survival and effluent does not become overly eutrophic.

Nitrogen fertilization may not only increase primary productivity but can also change the algal species composition (Sommer 1985; Soderberg 2012). Culver (1991) suggested that manipulating N levels in fry rearing ponds could create an algal species profile more beneficial to zooplankton production. Phytoplankton species were not identified in the present study; however, relative concentrations of algal pigments can indicate different species profiles. Trends and differences between treatments were similar for chlorophyll-*a*, -*b*, and -*c* (Figure 1). The data collected did not reveal any effect of N-fertilization rate on algal species. Further investigation into the phytoplankton species present in AEW ponds and the effect of fertilizer regimes on plankton species profiles may be beneficial.

Total zooplankton densities typically remained > 100/L (the recommended level at FLB fry stocking, Glenewinkel et al. 2011) in all ponds of both treatments throughout the fish cycle (Table 3). The only exception was one control pond with a density < 100/L on the day of stocking, but densities quickly increased following this point. This demonstrates that both fertilizer regimes adequately supported zooplankton development. Differing nitrogen input had no apparent effect on zooplankton densities. The ponds were rotifer-dominated (numerically), shown by the similarities between rotifer and total zooplankton densities.

Water quality

Ammonia concentrations generally remained below toxic concentrations (< 0.17 mg/L NH_3) in both treatments. The majority of higher ammonia-N concentrations were measured before fry were stocked or within the week following stocking when pH values were < 9.5 (Figure 2). This corresponded with the period of higher fertilization input at the start of the production cycle and before phytoplankton densities had peaked. However, when converted to unionized ammonia, the higher values shifted later in the cycle due to rising pH and temperature (Figure 3). Maximum unionized ammonia values that fish were exposed to were between 0.097 and 0.328 mg/L and 0.069 and 0.143 mg/L in control and Low-N ponds (Table 3). These were below the 72-h LC_{50} value of 0.67 mg/L for *M. salmoides* reported by Tomasso and Carmichael (1986) and 24-h LC_{50} value of 1.69 mg/L reported by Tidwell et al. (2000). The highest NH_3 value (0.328 mg/L) was an outlying single-day peak in a control pond, with values of 0.06–0.07 mg/L a day either side. This pond had the lowest fish production (3.61 kg/ha/d) and second-lowest survival (38.9%) of any study pond; however, factors other than ammonia may also have impacted fish performance. The peak unionized ammonia concentrations > 0.1 mg/L in other ponds also had much lower values (< 0.04–0.08 mg/L) measured a day either side, so that exposure to > 0.1 mg/L NH_3 was < 24 h. The number and range of ammonia, nitrite, nitrate, and total N peaks were similar in each treatment for the first 10 days post-filling. Higher concentrations were more frequent in the control treatment thereafter once N-input started diverging between treatments.

Centrarchids are highly resistant to nitrite and similarly resistant to nitrate compared to other warm-water fish species (Lewis and Morris 1986; Tomasso and Carmichael 1986; Tidwell

et al. 2000). Nitrite-N concentrations remained well below toxic concentrations (< 14 mg/L, Lewis and Morris 1986) throughout the culture cycle, with post-stocking NO_2-N maxima of 0.02 mg/L in both treatments (Figure 4). While the mean NO_2-N concentration and percentage of days with detectable NO_2-N were higher in the control ponds (Table 3), the concentrations were so low that this difference was not meaningful. Nitrate-N concentrations declined rapidly during the first week post-filling as ambient nitrate present in the inlet water was consumed by phytoplankton. Nitrate-N then remained < 0.22 mg/L for the remainder of the cycle (Figure 5).

Kurten (2001) reported a mean N:P ratio in FLB fry rearing ponds of 13.8:1, higher than the theoretical N:P ratio for balanced phytoplankton growth of 7:1 (Welch 1980; Cromar and Fallowfield 1997). He suggested that this skewed ratio was due to over-fertilization with N rather than P limitation as mean P was maintained above the minimum target of 0.03 mg/L. However, the mean N:P ratio measured in the present study when following the same fertilization regime (Control) was considerably lower (4.3:1) than reported by Kurten (2001) due to a lower mean NO_3-N concentration (0.193 v 0.755 mg/L, Table 3). This suggests that N was more limited than P in the present study, or that P was in excess. Phytoplankton and zooplankton productivity appeared adequate, pH rose above 10, and NH_3 above 0.05 mg/L in both treatments. Thus, increasing N-input is clearly not necessary. Several ponds had lower than the minimum P concentrations suggested by Culver (1993, 0.03 mg/L) and Sommer (1985, 0.035 mg/L) for phytoplankton growth, after day 5. Further, more phosphate will bind with the higher calcium concentrations in hard waters such as at AEW and precipitate directly from the water (Anderson 1993; Kurten 2001; Boyd 2012 a, b). This may necessitate several-fold higher P fertilization rates to achieve desired concentrations (Boyd and Tucker 1998). Thus, P concentrations also appear to have been adequate and lowering P-input could risk compromising productivity. The differing N-inputs between treatments in the present study had no significant impact ($P > 0.05$) on N:P ratios (Table 3).

Both the present study (both treatments) and Kurten (2001) reported high (> 10) initial N:P ratios that declined until about day 15 as N and P concentrations diminished. Ratios then generally remained low (< 5) for the remainder of the culture cycle in the present study (Figure 6). In contrast Kurten (2001) reported ratios gradually rising to points higher than early levels over the second half of the culture cycle due to higher sustained NO_3-N concentrations.

The lower NO_3-N concentrations observed in the present study are likely due to the difference in phytoplankton density as indicated by chlorophyll *a* concentrations. Kurten (2001) reported a mean chlorophyll *a* concentration of 14.82 μg/L in six ponds, compared to 77.21 and 42.84 μg/L in the two treatments in the present study (Table 3). Kurten (2001) reported cladoceran-dominated zooplankton communities, whereas rotifers dominated and copepod adult and nauplii densities were lower in the present study. These differences would have been driven to a large extent by season—photoperiod and temperature. Kurten (2001) followed ponds from 20 March to 4 May with mean day length of 12:48 h (range 12:08–13:25 h) and mean am-pm temperatures of 21.6–23.5°C, compared to 16 April to 7 June with 13:34 h (range 12:56–14:00 h)

and 24.5–25.9°C for the six ponds in the present study. Longer day length and higher temperature increased productivity and changed zooplankton assemblages. Higher temperatures (within tolerance limits) increase nutrient uptake by phytoplankton, ammonia volatilization, and denitrification rates (Boyd 2012b). Nutrient sources, sinks, and flows within fish ponds will vary considerably on a seasonal basis (Krom et al. 1985). In addition, Kurten (2001) only measured N and P compounds and chlorophyll *a* in ponds twice-weekly from 5–8 days after filling commenced, possibly missing many peak values. The lesson here is to be careful applying conclusions from a pond study conducted at one particular time of year to all seasons, even when the difference is only one month and 2–3°C. There is already high inherent variation between ponds within the same time period. Pond fertilizing regimes must be tailored to site-specific conditions (Knud-Hansen et al. 1991) and the optimum regime may also vary by month.

The N (Figure 7) and P (Figure 8) concentrations in all ponds of both treatments declined rapidly between days 0 and 10 post-filling, coinciding with the first peak in phytoplankton density 5–10 days post-filling. The higher concentrations in the first five days were also due to higher fertilizer input (Table 2) and lower water volumes. The concentrations remained low after day 28 post-filling, once fertilization ceased. This was also observed by Kurten (2001). The higher PO_4-P concentrations between days 13 and 28 for the control treatment (Figure 8) were all from one pond (pond 19). The three highest chlorophyll *a*, *b*, and *c* values, and two highest afternoon pH values were also recorded in this pond, although excluding these values did not change the trending differences between treatments. There were no reports of inadequate cleaning before pond 19 was filled or incorrect phosphoric acid volumes. Kurten (2001) also reported large variation in mid-cycle PO_4-P concentrations between ponds suggesting such variation is common.

Measured N and P concentrations had large spikes on the day after fertilizer applications on day 1 when water volumes were low and day 4 due to the higher dose. Measured spikes following fertilizer applications were relatively low thereafter, once established algal blooms could rapidly consume nutrients. Concentrations typically returned to pre-treatment levels within 1–3 days. This follows the pattern described by Boyd (2012a). Actual peak N and P values in ponds may have been higher as water samples were taken in the early morning following fertilizer application mid-morning the day before. Boyd et al. (2008) suggested this is a common limitation in pond fertilization experiments.

Concentrations of total N and P in pond effluents ranged up to 0.16 and 0.08 mg/L in the present study, not including content in settled pond sludge. Groeger et al. (1997) reported increased ammonium concentrations in the San Marcos River 100 m downstream of the AEW outfall. However, the construction of the AEW effluent treatment plant in 2000 (Fries and Bowles 2002) appears to have reduced this impact. Effluent water from the hatchery between 2006 and 2018 had lower total N (mean 0.802 mg N/L, range 0.091–1.869 mg N/L) than source water (mean 1.095 mg N/L, range 0.703–1.605 mg N/L). Phosphate concentrations were below detectible limits (<0.002 mg PO_4-P/L) for both source and effluent water over the same time

period. Given the effectiveness of the treatment plant, limiting nutrient releases into the San Marcos River is not a justification for reducing current pond fertilization rates.

Mean DO increased in ponds over the first week, to a greater extent in the afternoon, as phytoplankton density increased. While not significantly different ($P > 0.05$) due to high variation, mean PM DO was generally higher in the control ponds after day 17 (Figure 9). These DO trends correspond with chlorophyll trends as increasing photosynthesis produced more oxygen. The number of days with DO > 12 mg/L was also higher in the control ponds (Table 3). Some of the within-day variation in DO was caused by the different pond filling dates, a limitation of the experimental design. This resulted in differences in temperature between unpaired ponds on each day post-filling as each day does not correspond to a standard date. Temperature has the greatest immediate impact on DO, explaining the lack of similar within-day variation (independent of treatment) in the other water quality parameters measured.

Increasing phytoplankton densities typically raise pH as CO_2 is consumed through photosynthesis, and this occurred in all ponds over time in the present study. However, the differences in chlorophyll and associated DO concentrations between treatments had only minimal impact on pH. While control ponds had more days with pH > 10, variation between ponds was high. The highest pH values were recorded after day 16 when peak phytoplankton density occurred in the control ponds. There was no significant difference in AM, PM or mean daily pH during this period (days 20–33, P=0.171, 0.061, and 0.164, respectively). Mean pH values and trends for the whole cycle were similar between treatments (Table 3, Figure 10). Pond pH generally declined 2–3 days following the final fertilizer application on day 27. High pH (> 10) was recorded in all ponds of both treatments during the study, as also reported by Kurten (2001). Inorganic fertilizer applications are often delayed or skipped if morning pH is > 9.5 in AEW fingerling rearing ponds (Hugh Glenewinkel, A. E. Wood State Fish Hatchery, San Marcos, Texas, personal communication). Morning pH rose above 9.5 between days 13 and 19 in all ponds in the present study, after two Uran applications had already been skipped in the Low-N ponds. While skipping N-inputs may have had a small impact on pH, skipping P-input instead of or in addition to N at this point could have a greater effect. Further investigation of organic and inorganic fertilizer rates and flushing protocols is recommended to moderate high pH levels. Kurten (2001) also recommended investigating options for moderating pH until the pH tolerance of FLB fry and fingerlings is better understood.

Alkalinity declined throughout the production cycle in both treatments, rapidly during the first 10 days, and at a slower rate thereafter (Figure 11). This initial decline coincided with the first peak in chlorophyll concentrations and decline of ammonia, nitrate and phosphate concentrations. The secondary chlorophyll peak in the control ponds between days 20 and 32 did not noticeably affect alkalinity compared to the low-N ponds. Phytoplankton consume alkalinity when metabolizing ammonium (3.13 g of alkalinity (as $CaCO_3$) for every gram of NH_4-N consumed) and produce alkalinity when metabolizing nitrate (4.02 g of alkalinity (as $CaCO_3$) for every gram of NO_3-N consumed; Brewer and Goldman 1976; Ebeling et al. 2006). The decline

in alkalinity in ponds indicates alkalinity production by phytoplankton metabolizing nitrate was offset by alkalinity consumption (and neutralization) through ammonium metabolism by phytoplankton, nitrification (Boyd 2012b), and heterotrophic bacterial growth stimulated by the organic fertilizer (Ebeling et al. 2006; Samocha et al. 2017). However, the difference in inorganic N-input between ponds was not large enough to generate a significant difference in alkalinity consumption (Table 3). The lowest alkalinity recorded in any pond was 89.9 mg/L as $CaCO_3$. This was well above the minimum recommended (20 mg/L as $CaCO_3$) and within the desirable range (75–200 mg/L as $CaCO_3$) of alkalinity for fish culture in ponds (Wurts and Durborow 1992; Ludwig et al. 1998; Hargreaves and Tucker 2004). The high initial alkalinity of the source water, relatively low fish biomass, and short production cycle mean that alkalinity is unlikely to decline to a point where buffering capacity or pond productivity become severely restricted. Therefore, actions to restore alkalinity such as liming are not necessary in AEW fry rearing ponds. If high pH, low DO, or high ammonia concentrations necessitate flushing ponds with fresh water, this will not only mediate the poor water quality conditions but also restore some alkalinity to improve buffering and N-metabolism.

All measured water quality variables except F, SO_4, and morning and mean daily temperature, changed significantly ($P < 0.05$) over time during the whole cycle (days 1–33). Only NO_2-N, NO_3-N, Total N, PO_4-P, N:P ratio, Br, afternoon temperature, and morning, afternoon and mean daily pH changed significantly ($P < 0.05$) over time during the fish cycle (days 6–33). However, there was no significant interaction ($P > 0.05$) between treatment and time for any variable during either cycle.

Nutrient budgets

Partial N (Table 4) and P (Table 5) nutrient budgets were constructed from the data collected during the study. These nutrient budgets were partial as, while the inputs are considered reasonably complete, there are large unknown components in the outputs. The budgets assume that ponds were entirely free of sediment at filling, staff accurately measured fertilizer applications, and the impact of N-fixation, rainfall/runoff, bird feces, invertebrate migration, and wind-blown dust was minimal. This appears to be the first attempt at producing nutrient budgets for TPWD hatchery ponds. These budgets may allow more informed pond management decisions and provide baseline data for future investigations.

The budgets show that fertilizers were the major nutrient inputs, 96.8–97.1% of N and 99.6% of P over the production cycle. The lower inlet water contribution of P (0.23–0.24%) than N (2.7–3.0%) reflects the low PO_4 concentrations in the source water. Phosphorus limitation is typical of freshwaters, including the San Marcos River (Groeger et al. 1997). Cottonseed meal supplied a large proportion of the nutrient input from fertilizers (57–67% of N and 46% of P). Organic fertilizer provides a slower release nutrient source for phytoplankton, with the high carbon content initially favoring heterotrophic bacterial production (Kurten et al. 1999; Soderberg 2012). In contrast, nutrients supplied by the inorganic fertilizers are instantly available

to phytoplankton and have a greater immediate effect on developing and sustaining the algal bloom.

While 0.2% of the P-input into ponds was from the inlet water, only ~75% of this was immediately available to phytoplankton (in the form of PO_4-P). Similarly, 0.3–0.4% of the P output was in the discharge water, with only ~21% of this immediately available. The remaining P in water was the estimated proportion in suspended biomass. The proportion of N in water bound up in suspended biomass also increased from 6–7% in inlet water to 90% in discharge water. This shift was due to increased TSS and reflects the conversion of nutrients into biomass in ponds. The higher proportion of N and P outputs in suspended biomass from the control ponds was due to higher final TSS (Table 3) generated by denser phytoplankton blooms. Much of this suspended biomass in discharge water and re-suspended sediment flushed out of the ponds at harvest is likely removed in the AEW settlement ponds and treatment plant. The total N and P content of all water samples (filtered through 64-μm screen and unfiltered) taken at pond filling and pond draining remained below detection limits, < 10 mg/L N and < 0.04 mg/L P. Therefore, the proportion of nutrients in zooplankton could not be calculated.

A substantial proportion of N and P-input into ponds was incorporated into fish biomass. Only 0.21–0.25% of N and 0.11% of P-inputs were from fry stocked. This increased to an average of 24–25% of N and 18–27% of P outputs in harvested fingerlings. These values are within the range of most published nutrient retention efficiencies: 24–43% N and 9–16% P in fertilized carp ponds (Adhikari et al. 2014), 16–26% N in fertilized Nile Tilapia *Oreochromis niloticus* ponds (Knud Hansen et al. 1991), 11–16% N in fertilized mixed carp-Tilapia ponds (Schroeder et al. 1990), 20% N and 42% P in lined and fed Striped Bass *Morone saxatilis* ponds (Daniels and Boyd 1989), 27–32% N and 30% P in fed Channel Catfish *Ictalurus punctatus* ponds (Boyd 1985; Gross et al. 2000), 34–37% N and 37–48% P in fed Turbot ponds (Mallekh et al. 1999), and 36% N and 29% P in fed marine finfish ponds (Krom et al. 1985). Many factors influence efficiencies, including temperature, fish weight, feed consumption and composition (Mallekh et al. 1999), stocking density (Adhikari et al. 2014), and fertilizer type and quantities (Knud Hansen et al. 1991). Depending on feed quality, retention efficiency is likely higher in artificial feed- than fertilizer-based systems. Efficient use of N-input may minimize problematic ammonia concentrations and increase fish yields (Knud-Hansen et al. 1991). Nutrient flow to fingerlings in the present study predominantly occurred via phytoplankton and consumed zooplankton (Parmley et al. 1986; Schroeder 1987; Schroeder et al. 1990). Some flow was also likely from fish consuming invertebrates grazing on bacterial biomass generated from the organic fertilizer (Kurten et al. 1999).

The proportion of total nutrient inputs that are incorporated into culture organisms can indicate the efficiency of nutrient utilization in ponds, both in artificial feed- (Mallekh et al. 1999) and fertilizer-based systems (Knud-Hansen et al. 1991). The proportion of N-inputs incorporated by fingerlings between treatments was similar, suggesting reducing N-input by 36% did not improve N-utilization efficiency. However, the surplus N quantity (i.e., not in fingerlings) was higher in the control ponds due to the higher input. Given the marginal

difference in discharge water N content, much of this additional N in the control ponds would have been lost through a higher rate of ammonia volatilization (Boyd 2012a) and incorporation into sediments. Final sediment volumes were not measured to verify any difference between treatments. The difference in the proportion of P-inputs incorporated by fingerlings between treatments was due to large variation in the measured P in fingerlings between ponds.

The non-protein N content of LMB (100–950 g) ranges from 7.9–10.0% of total N content (Niimi 1973). Goodyear and Boyd (1972) reported N and P content of LMB dry weight of 11.16–11.90 and 3.60–4.27%, respectively, and that composition varies with location. Davis and Boyd (1978) reported mean N and P levels of 9.77 and 3.20%, respectively, and that composition is correlated with fish size. In addition, Mallekh et al. (1999) suggested that inter-study comparisons are difficult as body composition varies with the N and P content of the diet. Therefore, the N and P content of FLB were measured in the present study rather than relying on published data to construct the nutrient budgets. The total moisture content of fry and fingerlings in the present study was 10.8–12.7% and 19.1–22.3%. Fry N and P content were 11.20 and 1.34% (dry). Fingerling N and P content from all twelve ponds were 10.1–12.3% and 1.01–4.69% (dry). These values are within the range reported in the literature for LMB, and for Striped Bass *Morone saxatilis* fingerlings reared in lined ponds, 11.51% N and 3.52% P (Daniels and Boyd 1989). There were no significant differences ($P > 0.05$) in fingerling moisture, N, or P content between treatments (Table 2).

Unaccounted outputs (74–75% of N, 72–81% of P) included N losses to denitrification ($NO_3 \rightarrow N_2$ gas) and ammonia volatilization (diffusion from water to air), N and P losses to predation and insect larvae maturing and leaving the pond within the production cycle, and N and P content in sediment. The proportion of N-input lost to denitrification and ammonia volatilization has been estimated as 14–23% in carp ponds (Adhikari et al. 2014), 30% in shrimp ponds (Funge-Smith and Briggs 1998), 30–57% in catfish ponds (Boyd 1985; Gross et al. 2000), and 50% in lined Striped Bass ponds (Daniels and Boyd 1989). The lack of anoxic sediment in the lined ponds and DO > 4 mg/L throughout the production cycles in the present study suggests a greater proportion of N would have been lost to ammonia volatilization than denitrification (Gross et al. 2000). Boyd (2012a) reported that ammonia volatilization is an important route of N loss from ponds. The volatilization rate increases with water temperature, TAN concentration, surface pH, and turbulence (Hargreaves 1998; Gross et al. 1999; Boyd 2012a).

The bulk of the remaining unaccounted N and P must have been present in the pond sediments and associated demersal invertebrates. The clay particles and sediment in earthen ponds readily absorb these nutrients, particularly P, and represent a major sink (Rogge et al. 2003; Boyd 2012a). Reports of total nutrient output in pond sediments range from 31 to 50% N and 55 to 84% P (Boyd 1985; Daniels and Boyd 1989; Funge-Smith and Briggs 1998; Adhikari et al. 2014). Lined ponds lack the sediment present in earthen ponds, so N and P mainly accumulate on the pond base through settled fish and zooplankton feces and mortalities, senescent phytoplankton, and retention in any CSM that never fully decomposed (Hargreaves

1998). Up to half of the standing phytoplankton crop in ponds may settle to the sediment each day (Schroeder et al. 1991; Hargreaves 1998). Phosphate also readily binds to calcium in hard water, such as that at AEW, forming calcium phosphate and precipitating from the water (Boyd 2012 a, b). Final sediment samples were only taken from four of the non-flushed ponds following harvest, three of which were Low-N, so no data comparison was made. Sediment samples (dry weight) from these ponds contained an average of 1.87% total N (1.45–2.62%) and 0.60% total P (0.23–1.02%). The N:P ratio averaged 3.6 (2.6–6.2). Organic content (VSS) averaged 35.8% (30.0–45.3%). This sediment likely contained a large proportion of the unaccounted N and P output, however the sediment volume at harvest in each pond could not be accurately quantified.

Manta2 multiprobe

The Manta2 multiprobe placed in several ponds during the study period provided reliable temperature, pH, DO (% and mg/L), conductivity, and chlorophyll *a* data and was particularly useful for showing short-term trends. No response was observed in any water quality variable measured in the 12 h following fertilizer applications. However, the pH peak was 0.1–0.2 units higher on the day after three of the four fertilizer applications in pond 5 when the Manta2 multiprobe was deployed. The turbidity data collected was generally reliable though was sensitive to large particles that produced extreme values. Turbidity values trended with chlorophyll *a*, as expected in phytoplankton-dominated systems with a low-turbidity water source. The nitrate and ammonia probes were not reliable, functioning intermittently and designed for higher concentrations than are typical in hatchery ponds.

Measuring water quality variables every 15 min with the Manta2 multiprobe provided information that could be missed by the normal routine of twice-daily sampling. Table 6 displays the times when minimum and maximum daily temperature, pH, and DO values were recorded in two ponds. Temperature typically peaked earlier and troughed later than pH and DO. The pond 5 data suggests that the optimum times to measure these variables in June–July to maximize the chances of recording daily extremes are 800–930 hours and 1730–1830 hours. However, hatcheries measure pond water quality based on work schedules, typically at 0600–0700 hours and 1530–1630 hours. While this will often capture close to the extreme values, biologists must be conscious that values may continue to rise in the afternoon and fall in the morning when making real-time management decisions and interpreting historical data. Some past research relying on routine hatchery water quality data, particularly where temperature, DO, or pH were the independent variables, may need to be reassessed. This also indicates that not all water quality extremes were captured in the present study. The pond 12 data, measured in April, indicates different optimum times but may be skewed by a small number of data points. The optimum time to measure water quality in ponds to capture extremes will vary throughout the year depending on photoperiod and dominant weather systems. Further data logging throughout the year will help to define these periods.

Additional relevant observations

The historical (2006–2018) NO_3-N concentrations in the San Marcos River at the hatchery intake average 1.13 ± 0.22 mg/L (range: 0.70–1.61 mg/L, n = 26). Pond inlet NO_3-N concentrations averaged 0.72 ± 0.28 mg/L (range: 0.57–1.01 mg/L, n = 19) throughout the 2016 study (Table 7). Thus some of the available N in the source water may have been depleted (predominantly incorporated by phytoplankton and bacteria) by the time it reached the ponds. Nitrogen depletion in the AEW reservoir may negate the suggestion by Kurten (2001) that additional N-input into ponds may not be necessary due to background levels in the source water.

Two cycles of FLB production were completed in 2016. The first (cycle 1) included the six study ponds and two other ponds stocked in April–May; the second (cycle 2) included four ponds stocked in June. The six non-study ponds were originally part of the study but their data was excluded from analysis due to lower stocking densities, higher temperatures (Mean 25.3°C in cycle 1 vs 30.4°C in cycle 2), flushing to improve water quality, and skipped fertilizer applications. The inlet NO_3-N concentrations in cycle 2 (mean ± SD: 0.428 ± 0.294 mg/L) were significantly ($P < 0.05$) lower than those in cycle 1 (mean ± SD: 0.873 ± 0.073 mg/L, Table 7), possibly due to increased phytoplankton growth in the reservoir (evidenced by higher mean pH and DO in cycle 2) at higher temperatures consuming more N. This could also be due to natural fluctuations in the San Marcos River. Kurten (2001) reported an even higher mean inlet NO_3-N concentration of 0.98 mg/L earlier in the year and at a lower temperature than in the present study. Similarly, PO_4-P traces (up to 0.07 mg/L) were measured in inlet water in cycle 1 but not in cycle 2. Further sampling could be conducted to understand seasonal changes in source and reservoir water N concentrations. Seasonal refinements to pond fertilizer regimes may be possible based on this data. The lower reservoir water N concentration during cycle 2 proved beneficial as pond productivity was already higher at the higher temperature, with associated high pH and low DO. Further N-input may have exacerbated pH and DO extremes. Other differences in inlet water quality (NO_2-N, PO_4-P, Cl, Br, and SO_4) between cycles though significant ($P < 0.05$), were not meaningful.

Further, cycle 2 ponds (filling commenced 3–6 June) and the last two cycle 1 ponds stocked (filling commenced 4-May) under both fertilization regimes were flushed due to high pH or low DO on various occasions between days 15 and 28 post-filling. The peak flushing period was between days 22 and 25 post-filling due to low DO. Only one early stocked pond (P19) required flushing due to high pH, shortly before harvest (day 33). The higher temperature in cycle 2 in conjunction with higher primary productivity were the main contributors to the low DO. However, reducing nutrient input to limit pH and DO extremes at these higher temperatures may be an option. Further investigation may be warranted.

Conclusion

The lack of significant differences in fish production parameters in this study suggests N fertilization could be reduced in FLB fry rearing ponds at AEW without compromising

fingerling production. However, the suppression of the secondary phytoplankton bloom indicates productivity in the second half of the production cycle was limited when N-input was reduced. Given typically high variation in phytoplankton dynamics and fish performance between ponds, reducing nutrient input may risk stunting zooplankton populations, restricting fish growth and survival, and increasing production time in some ponds. The differing N-input had no significant effect on water quality parameters, although limited replication may have hidden any effect. High pH, DO, and ammonia concentrations were more prevalent in the control ponds during the second half of the production cycle without appearing to impact production. A pH >10 was recorded in all ponds of both treatments. The current practice of flushing ponds when water quality parameters reach detrimental levels provides security for managing water quality under the current fertilization regime. At the same time, this study suggests that withdrawing some fertilizer input later in the production cycle in response to actual or projected poor water quality may not compromise fingerling production. There may be scope for further seasonal refinements of the fertilization regime to reduce the prevalence of high pH and requirement for pond flushing.

The mean N:P ratio in the present study (4.3:1) was considerably lower than that reported by Kurten (2001, 13.8:1) due to a lower mean NO_3-N concentration (0.193 v 0.755 mg/L). The inference by Kurten (2001) that FLB ponds therefore received N in excess was not validated by the present study. This contrast was likely driven by seasonal differences between studies, demonstrating the need to be cautious when applying conclusions from a pond study conducted at one particular time of year to another.

The nutrient budgets presented for this study quantify the N and P-inputs and outputs in FLB fingerling rearing ponds during April-May at AEW. An average of 24–25% of N and 18–27% of P-inputs were retained in harvested fingerlings. These budgets may be useful for future refinements of fertilization protocols and comparison of nutrient retention efficiencies at AEW.

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TABLE 1. Experimental fertilizing schedule for 0.44-ha (4,731,250-L) control (n=3) and reduced -N (Low-N; n=3) Florida Largemouth Bass *Micropterus salmoides floridanus* fingerling rearing ponds at the A. E. Wood State Fish Hatchery. Fertilizer included Cottonseed Meal (CSM), 1 L Phosphoric acid /0.44-ha pond (0.08 mg P/L; Amberphos-54™ = 395.71 g P/L), and 3 L Uran/0.44 ha pond (0.27 mg N/L; Uran 32-0-0 = 422.17 g N/L).

Day	Action	CSM (kg)	Phosphoric Acid (L)	Uran (L)	
				Control	Low-N
1	Filling and fertilization	22.7	1	3	3
4	Fertilization	91	2	6	6
7	Fry stocking				
8	Fertilization	22.7	1	3	
10	Fertilization	22.7	1	3	3
12	Fertilization	22.7	1	3	
15	Fertilization	22.7	1	3	3
18	Fertilization	22.7	1	3	
21	Fertilization	22.7	1	3	3
24	Fertilization	22.7	1	3	
27	Fertilization	22.7	1	3	3
35	Fingerling harvest				
Total		295.3	11	33	21

TABLE 2. Mean fish production parameters in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes. Values in brackets are ranges of raw data. Differences between treatments were assessed with a Wilcoxon test or a Welch's test where variances were not equal. SGR=Specific growth rate.

Fish production parameter	Control	Low-N	P
Stocking density (fry/ha)	502,473 (500,640–504,833)	502,335 (500,295–504,695)	1.0000
Stocked weight/fry (g)	0.004 (0.004–0.004)	0.004 (0.004–0.004)	1.0000
Stocked weight (kg)	0.73 (0.73–0.73)	0.73 (0.73–0.73)	1.0000
Stocked length (mm)	7 (7–7)	7 (7–7)	1.0000
Culture days	31.7 (24–37)	32.7 (28–40)	1.0000
Length (mm) at 15 d post-stock	21.3 (17.1–26.3)	20.4 (17.1–25.4)	1.0000
Harvest density (fingerling/ha)	251,793 (188,990–371,743)	254,736 (212,933–289,275)	0.6625
Harvest weight/ fingerling (g)	0.81 (0.69–1.00)	0.76 (0.62–0.99)	0.3827
Harvest weight (kg)	80.0 (53.4–111.3)	79.1 (53.2–114.0)	1.0000
Harvest length (mm)	45.0 (41.6–51.8)	42.9 (40.2–48.2)	0.3827
Production (kg/ha/d)	6.9 (3.6–11.6)	5.9 (4.4–7.1)	1.0000
Growth (mm/d)	1.23 (0.94–1.44)	1.11 (1.03–1.19)	0.6625
SGR (%mm/d)	6.0 (4.8–7.4)	5.6 (4.8–6.3)	1.0000
SGR (%g/d)	17.6 (14.2–22.2)	16.6 (14.0–18.6)	1.0000
K ($10^5.W/L^3$)	0.90 (0.71–1.04)	0.95 (0.88–1.02)	1.0000
Survival (%)	50.1 (37.4–74.1)	50.7 (42.2–57.6)	0.6625
Fingerling moisture content (%)	20.5 (20.0–21.1)	19.7 (18.6–20.8)	0.3827
Fingerling N content (% dry)	11.4 (10.6–12.3)	11.6 (10.8–12.3)	0.8248
Fingerling P content (% dry)	3.5 (2.3–4.7)	2.9 (1.0–4.6)	0.6625

TABLE 3. Mean daily water quality parameters and zooplankton densities in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes. Values in brackets are ranges of raw data. Differences between treatment data collected repeatedly from each pond were assessed using linear mixed models. Differences between treatments in tallied (days), minimum, and maximum data were assessed with a Wilcoxon test or a Welch's test where variances were not equal. For variables where differences were detected, different letters within each cycle indicate significance at $P < 0.05$. DO=Dissolved oxygen.

Water quality		Whole cycle (days 1–33)			Fish cycle (days 6–33)		
		Control	Low-N	P	Control	Low-N	P
Max. NH$_3$-N (mg/L)		0.60 (0.25–1.06)	0.64 (0.48–0.87)	0.6625	0.54 (0.10–1.06)	0.24 (0.09–0.45)	0.3827
Days NH$_3$-N > 0.04 mg/L	No.	12.0 (10–15)	8.7 (7–10)	0.3827	7.7 (5–11)	4.3 (3–6)	0.1904
	%	34.9 (20.0–45.5)	22.4 (15.6–28.6)	0.3827	27.8 (16.1–39.3)	13.8 (9.7–20.7)	0.1904
Days NH$_3$-N > 0.1 mg/L	No.	8.3 (3–11)	4.3 (3–6)	0.5002	4.3 (0–7)	1.3 (0–3)	0.5066
	%	24.7 (7.5–33.3)	11.2 (7.7–17.1)	0.6625	16.3 (0.0–25.0)	4.4 (0.0–10.3)	0.5066
Maximum NH$_3$ (mg/L)		0.20 (0.10–0.33)	0.11 (0.07–0.14)	0.3827	0.22 (0.10–0.33)	0.11 (0.07–0.14)	0.3827
Days NH$_3$ > 0.050 mg/L	No.	6.00 (2–8)	1.67 (1–3)	0.1775	5.7 (2–8)	1.3 (1–2)	0.1407
	%	17.8 (5.0–24.2)	4.5 (2.2–8.6)	0.1840	21.0 (6.5–28.6)	4.3 (2.8–6.9)	0.1440
Days NH$_3$ > 0.067 mg/L	No.	2.33 (0–6)	1.33 (1–2)	0.6460	3.7 (1–6)	1.0 (1–1)	0.1967
	%	6.9 (0.0–18.2)	3.5 (2.2–5.7)	0.6135	13.6 (3.2–21.4)	3.2 (2.8–3.4)	0.1937
NO$_2$-N (mg/L)		0.0035 (0.0000–0.0200)	0.0020 (0.0000–0.0300)	0.2372	0.0024 (0.0000–0.0200)	0.0004 (0.0000–0.0200)	0.3327
Max. NO$_2$-N (mg/L)		0.015 (0.006-0.020)	0.020 (0.010–0.030)	0.6248	0.010 (0.002–0.020)	0.010 (0.001–0.020)	0.8248
Days NO$_2$-N > 0 mg/L	No.	17.0 (8–26)	7.7 (6–9)	0.2683	12.0 (3–22)	2.7 (1–4)	0.2683
	%	44.0 (20.0–60.5)	19.6 (15.4–25.7)	0.1904	37.2 (9.7–57.9)	8.5 (3.2–13.8)	0.1904
NO$_3$-N (mg/L)		0.193 (0.000–1.270)	0.156 (0.000–1.590)	0.7013	0.050 (0.000–0.920)	0.017 (0.000–0.390)	0.8217
Max. NO$_3$-N (mg/L)		1.108 (0.834–1.270)	1.220 (0.960–1.590)	1.0000	0.400 (0.065–0.920)	0.200 (0.070–0.390)	1.0000
Total (measured) N (mg/L)		0.276 (0.000–1.575)	0.207 (0.000–2.025)	0.4261	0.113 (0.000–1.192)	0.034 (0.000–0.604)	0.2914
Max. Total N (mg/L)		1.349 (1.004–1.575)	1.689 (0.393–2.025)	0.3827	0.760 (0.145–1.192)	0.300 (0.117–0.64)	0.3827
PO$_4$-P (mg/L)		0.087 (0.015–0.530)	0.065 (0.015–0.810)	0.5304	0.070 (0.015–0.260)	0.034 (0.015–0.120)	0.1461
N:P ratio		4.3 (0.0–62.0)	4.7 (0.0–67.7)	0.9970	2.1 (0.0–36.3)	1.66 (0.0–40.3)	0.9195
Days N:P < 7 (N-limited)	No.	32.7 (23–39)	33.7 (31–38)	1.0000	27.0 (20–35)	31.3 (26–38)	0.5066
	%	83.5 (69.7–90.7)	85.0 (82.1–88.6)	0.6625	91.0 (80.0–100.0)	93.8 (89.7–96.8)	1.0000
Days N:P > 7 (P-limited)	No.	6.0 (4–10)	6.0 (4–7)	1.0000	2.3 (0–5)	2.0 (1–3)	1.0000
	%	16.5 (9.3–30.3)	15.0 (11.4–17.9	0.6625	9.0 (0.0–20.0)	6.2 (3.2–10.3)	1.0000
Chlorophyll a (µg/L)		77.2 (1.0–354.0)x	42.8 (1.0–162.0)y	0.0356	89.2 (15.0–354.0)x	43.4 (8.0–123.0)y	0.0325
Max. Chlorophyll a (µg/L)		220.7 (124–354)	130.7 (109–162)	0.1904	220.7 (124–354)	115.3 (102–123)	0.2652
Days Chlorophyll a ≥ 100 µg/L	No.	10.7 (5–16)	2.0 (2–2)	0.0636	10.3 (4–16)	1.0 (1–1)	0.0636
	%	32.2 (12.5–48.5)	5.1 (4.4–5.7)	0.0809	38.1 (11.4–57.1)	3.1 (2.5–3.6)	0.1255
Days Chlorophyll a ≥ 75 µg/L	No.	14.0 (10–20)	4.7 (3–6)	0.0809	13.7 (9–20)	3.0 (1–4)	0.0765
	%	41.4 (25.0–60.6)	12.0 (7.9–17.1)	0.0809	49.0 (25.7–71.4)	9.2 (3.2–14.3)	0.0809
Chlorophyll b (µg/L)		18.7 (1.0–112.0)	9.2 (1.0–46.0)	0.0612	21.6 (1.0–112.0)	9.0 (1.0–35.0)	0.0587
Max. Chlorophyll b (µg/L)		64.7 (33–112)	36.0 (29–46)	0.2683	64.7 (33–112)	31.0 (25–35)	0.2967
Days Chlorophyll b ≥ 20 µg/L	No.	12.7 (9–16)	4.0 (3–5)	0.0809	12.0 (8-16)	2.3 (1–3)	0.0765

	%	37.6 (22.5–48.5)	10.4 (7.9–14.3)	0.0809	43.3 (22.9–57.1)	7.1 (3.2–10.7)	0.0809
Chlorophyll c (µg/L)		6.2 (1.0–22.0)	4.0 (1.0–11.0)	0.0595	7.1 (1.0–22.0)	4.1 (1.0–10.0)	0.0551
Max. Chlorophyll c (µg/L)		16.7 (11–22)	10.0 (9–11)	0.1212	16.7 (11–22)	9.7 (9–10)	0.0765
Days Chlorophyll $c \geq 5$ µg/L	No.	19.3 (18–21)	11.7 (6–15)	0.0809	18.7 (17–21)	10.0 (4–13)	0.0765
	%	56.4 (47.5–63.6)	29.9 (15.8–42.9)	0.0809	65.8 (51.4–75.0)	30.6 (12.9–46.4)	0.0809
Alkalinity (mg/L as $CaCO_3$)		133.5 (92.5–280.0)	131.7 (87.9–260.0)	0.4660	118.2 (92.5–159.2)	118.7 (87.9–162.6)	0.4959
Min. alkalinity (mg/L as $CaCO_3$)		101.4 (92.5–105.9)	99.9 (87.9–122.6)	0.6625	101.4 (92.5–105.9)	99.9 (87.9–122.6)	0.6625
F (mg/L)		0.14 (0.09–0.29)	0.13 (0.05–0.18)	0.1134	0.14 (0.11–0.16)	0.13 (0.05–0.16)	0.1474
Cl (mg/L)		17.8 (13.6–20.7)	17.7 (10.8–20.7)	0.9253	18.0 (15.9–20.7)	18.0 (15.6–20.7)	0.7737
Br (mg/L)		0.62 (0.09–1.61)	0.58 (0.09–1.65)	0.4800	0.52 (0.09–1.61)	0.50 (0.09–1.24)	0.8621
SO_4 (mg/L)		22.9 (18.0–27.3)	22.7 (14.6–27.4)	0.9163	23.0 (19.6–27.3)	22.9 (19.8–27.4)	0.7672
TSS (mg/L)		22.1 (2.5–97.5)	16.9 (2.7–103.7)	0.3827	20.0 (9.0–51.5)	12.8 (7.0–24.0)	0.0809
Max. TSS (mg/L)		60.0 (31.0–97.5)	49.2 (20.0–103.7)	0.6625	36.7 (27.5–51.5)	18.2 (10.5–24.0)	0.0809
DO (mg/L)*	AM	9.1 (4.1–15.8)	8.9 (2.7–16.4)	0.7169	9.5 (4.1–15.8)	9.0 (2.7–16.4)	0.7644
	PM	12.1 (6.3–20.7)	11.3 (5.6–20.7)	0.2319	12.7 (8.4–20.7)	11.6 (8.0–20.7)	0.2142
	Daily	10.7 (5.9–18.3)	10.2 (5.4–17.9)	0.3914	11.2 (6.7–18.3)	10.4 (7.8–17.9)	0.4285
	Min.	5.0 (4.1–6.4)	5.1 (2.7–7.1)	1.0000	5.0 (4.1–6.6)	5.3 (2.7–7.1)	1.0000
	Max.	20.2 (19.5–20.7)	18.8 (16.6–20.7)	0.3827	20.2 (19.5–20.7)	18.3 (15.1–20.7)	0.3827
Days DO > 12 mg/L	No.	15.7 (13–17)	10.7 (9–13)	0.1157	14.3 (13–15)	9.0 (7–12)	0.0765
	%	48.6 (41.9–56.7)	29.9 (21.4–40.6)	0.0809	55.5 (48.4–68.2)	31.2 (18.9–46.2)	0.0809
Temperature (°C)	AM	24.6 (20.1–28.2)	24.5 (20.7–28.1)	0.8738	24.8 (20.1–28.2)	24.7 (20.7–28.1)	0.9253
	PM	25.9 (21.7–31.3)	25.9 (21.6–31.5)	0.8128	26.0 (21.7–31.3)	25.8 (21.6–31.5)	0.7878
	Daily	25.3 (21.4–28.6)	25.2 (21.7–28.7)	0.8791	25.4 (21.4–28.6)	25.4 (21.7–28.7)	0.8936
pH	AM	9.3 (8.0–10.5)	9.3 (7.9–10.1)	0.8060	9.5 (8.1–10.5)	9.5 (8.1–10.1)	0.8856
	PM	9.5 (7.8–10.5)	9.5 (7.8–10.3)	0.6230	9.7 (8.7–10.5)	9.7 (8.7–10.3)	0.5645
	Daily	9.4 (7.8–10.5)	9.4 (7.8–10.2)	0.9134	9.6 (8.5–10.5)	9.6 (8.5–10.2)	0.8400
	Max.	10.4 (10.3–10.5)	10.2 (10.1–10.3)	0.1212	10.4 (10.3–10.5)	10.2 (10.1–10.3)	0.1212
Days pH > 9.8	No.	14.3 (12–18)	15.0 (11–20)	1.0000	14.3 (12–18)	15.0 (11–20)	1.0000
	%	44.0 (40.0–50.0)	41.1 (33.3–55.6)	0.6625	54.2 (50.0–58.1)	50.5 (37.8–71.4)	0.7631
Days pH > 10.0	No.	11.3 (8–16)	6.7 (2–15)	0.3827	11.3 (8–16)	6.7 (2–15)	0.3827
	%	34.5 (26.7–44.4)	18.6 (4.8–41.7)	0.3827	42.1 (36.4–51.6)	23.5 (5.4–53.6)	0.6625
Zooplankton (no./L)							
Cladocera		49 (0–409)	29 (0–208)	1.0000	64 (0–409)	32 (0–208)	0.6625
Copepod adult		1.2 (0.0–6.5)	3.1 (0.0–36.8)	0.6625	1.4 (0.0–6.5)	3.6 (0.0–36.8)	0.6625
Copepod nauplii		7.1 (0.0–38.9)	5.1 (0.0–26.0)	0.6625	8.7 (0.0–38.9)	5.9 (0.0–26.0)	0.6625
Rotifer		683 (4–2,163)	663 (37–3,028)	1.0000	750 (4–2,163)	781 (113–3,028)	1.0000
Total zooplankton		739 (13–2,367)	700 (45–3,046)	1.0000	824 (24–2,367)	823 (119–3,046)	1.0000
Min. total zooplankton		57 (13–134)	99 (45–193)	0.3827	89 (24–134)	257 (119–461)	0.1904
Max. total zooplankton		1,874 (1,092–2,367)	1,913 (876–3,046)	1.0000	1,874 (1,092–2,367)	1,913 (876–3,046)	1.0000

*These minimum DO values were recorded on the morning of harvest when pond water levels were low. If all final morning DO values are excluded, the mean and min morning DO values become: 9.20 (5.43) and 8.98 (5.91) mg/L for control and low-N over the whole cycle and 9.56 (5.85) and 9.16 (6.57) mg/L for control and low-N over the fish cycle.

TABLE 4. Nitrogen inputs, outputs and balance in EPDM-lined 0.4-ha (4,731,250-L) ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.

Components (Mean±SD)	Control		Low-N	
	kg/0.4 ha	%	kg/0.4 ha	%
Inputs				
Water				
Measured [a]	0.862 ± 0.164	2.51	0.819 ± 0.118	2.80
Estimated in suspended biomass [b]	0.057 ± 0.000	0.17	0.057 ± 0.000	0.19
Cottonseed meal	19.490 ± 0.000	56.63	19.490 ± 0.000	66.51
URAN	13.932 ± 0.000	40.48	8.866 ± 0.000	30.25
Fry	0.072 ± 0.000	0.21	0.072 ± 0.000	0.25
TOTAL INPUT	34.413 ± 0.164	100.00	29.304 ± 0.118	100.00
Outputs				
Water				
Measured [a]	0.034 ± 0.042	0.10	0.023 ± 0.017	0.08
Estimated in suspended biomass [b]	0.315 ± 0.082	0.92	0.235 ± 0.049	0.80
Fingerlings	8.305 ± 2.085	24.13	7.267 ± 3.273	24.80
Unaccounted [c]	25.759 ± 1.960	74.85	21.779 ± 3.403	74.32
TOTAL OUTPUT	34.413 ± 2.125	100.00	29.304 ± 3.303	100.00

[a] Measured nitrogen content in water is the sum of NH_3-N, NO_2-N, NO_3-N and chlorophyll-N.

[b] Estimated nitrogen within suspended biomass calculated from VSS (organic suspended solids content) and organic N content of 3%.

[c] Unaccounted includes losses to denitrification and ammonia volatilization, content in sediment, and larger invertebrates not captured in water samples.

TABLE 5. Phosphorus inputs, outputs and balance in EPDM-lined 0.4-ha (4,731,250-L) ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.

Components (Mean±SD)	Control		Low-N	
	kg/0.4 ha	%	kg/0.4 ha	%
Inputs				
Water				
Measured [a]	0.014 ± 0.014	0.17	0.015 ± 0.016	0.19
Estimated in suspended biomass [b]	0.005 ± 0.000	0.06	0.005 ± 0.000	0.06
Cottonseed meal	3.676 ± 0.000	45.62	3.676 ± 0.000	45.62
URAN	0.001 ± 0.000	0.01	0.001 ± 0.000	0.01
Phosphoric acid	4.353 ± 0.000	54.02	4.353 ± 0.000	54.02
Fry	0.009 ± 0.000	0.11	0.009 ± 0.000	0.11
TOTAL INPUT	8.058 ± 0.014	100.00	8.059 ± 0.016	100.00
Outputs				
Water				
Measured [a]	0.007 ± 0.010	0.09	0.005 ± 0.008	0.06
Estimated in suspended biomass [b]	0.026 ± 0.007	0.32	0.019 ± 0.004	0.24
Fingerlings	1.470 ± 0.785	18.24	2.196 ± 1.709	27.25
Unaccounted [a]	6.555 ± 0.803	81.35	5.839 ± 1.717	72.45
TOTAL OUTPUT	8.058 ± 0.789	100.00	8.059 ± 1.712	100.00

[a] Measured phosphorus content in water is the PO_4-P concentration.

[b] Estimated phosphorus within suspended biomass calculated from VSS (organic suspended solids content) and organic P content of 0.25%.

[c] Unaccounted includes content in sediment and larger invertebrates not captured in water samples.

TABLE 6. Times when maximum and minimum daily values of temperature, pH and dissolved oxygen were recorded by a Eureka Water Probes Manta 2 water quality sonde in Florida Largemouth Bass *Micropterus salmoides floridanus* fry rearing ponds at the A. E. Wood State Fish Hatchery from 18–22 Apr (Pond 12, n=4) and 17 June–4 July 2016 (Pond 5, n=15–18). The measurements were taken every 15 min at half-pond depth next to the kettle.

	Temperature		pH		Dissolved oxygen	
	Time maximum	Time minimum	Time maximum	Time minimum	Time maximum	Time minimum
Pond 12 (18–22 Apr)						
Mean	18:45	10:11	19:56	7:52	19:07	9:22
Median	18:37	9:37	20:30	7:52	19:07	9:00
Range	17:45–20:00	9:00–12:30	17:15–21:30	7:30–8:15	18:15–20:00	7:30–12:00
Pond 5 (17 June–4 July)						
Mean	17:25	9:07	18:09	8:07	18:12	8:22
Median	17:45	9:15	18:37	8:00	18:22	8:15
Range	13:30*–18:45	8:30–9:45	13:30*–19:45	7:00–10:45	13:45*–20:15	7:15–9:00

*The early maxima outliers were recorded on a cloudy, wet day on 25-June. Removing those figures shifts the mean maximum time for each parameter approximately 15 min later.

TABLE 7. Mean water quality variables of samples collected from the inlet of ponds filling at the A. E. Wood State Fish Hatchery from 16 Apr–5 May (Cycle 1 [six study ponds plus two others], n=14) and 30 May–6 June 2016 (Cycle 2 [four ponds], n=7). Values in brackets are ranges. Differences between cycles were compared with a Students t-test or Wilcoxon test. Different letters within each row indicate a significant difference at $P < 0.05$.

Water quality	Combined cycles	Cycle 1	Cycle 2	P
NH_3-N (mg/L)	– (<0.04–0.171)	– (<0.04–0.066)	– (<0.04–0.171)	–
NO_2-N (mg/L)	0.002 (0.000–0.007)	0.0004 (0.000–0.002)x	0.0046 (0.004–0.007)y	<0.0001
NO_3-N (mg/L)	0.724 (0.000–1.010)	0.873 (0.760–1.010)x	0.428 (0.000–0.677)y	<0.0075
PO_4-P (mg/L)	0.010 (0.000–0.070)	0.015 (0.000–0.070)x	0.000 (0.000–0.000)y	<0.0089
Alkalinity (mg/L as $CaCO_3$)	241.7 (203.5–300.0)	243.1 (203.5–300.0)	239.4 (231.1–249.4)	0.3643
Chlorophyll a (μg/L)	– (<0.002–0.011)	– (<0.002–0.002)	– (<0.002–0.011)	–
Chlorophyll b (μg/L)	– (<0.002–0.002)	– (<0.002–<0.002)	– (<0.002–0.002)	–
Chlorophyll c (μg/L)	– (<0.002–<0.002)	– (<0.002–<0.002)	– (<0.002–<0.002)	–
F (mg/L)	0.15 (0.09–0.29)	0.14 (0.09–0.29)	0.15 (0.13–0.17)	0.7553
Cl (mg/L)	18.0 (16.6–20.7)	18.4 (16.9–20.7)x	17.2 (16.6–19.4)y	0.0138
Br (mg/L)	0.86 (0.41–1.61)	1.03 (0.46–1.61)x	0.52 (0.41–0.66)y	<0.0001
SO_4 (mg/L)	23.8 (21.5–26.5)	24.6 (22.8–26.5)x	22.2 (21.5–22.8)y	<0.0001
TSS	21.0 (1.5–103.7)	27.6 (1.5–103.7)	3.5 (2.0–5.5)	0.6824
Reservoir temperature (°C) [a]	24.0 (22.2–26.7)	23.01 (22.2–24.2)x	26.0 (25.2–26.7)y	<0.0001
Reservoir DO (mg/L) [a]	8.9 (7.2–9.9)	8.7 (7.2–9.5)x	9.3 (9.1–9.9)y	0.0137
Reservoir pH [a]	7.95 (7.81–8.11)	7.92 (7.81–8.04)x	8.00 (7.89–8.11)y	0.0240

[a] Mean daily measurements (AM + PM / 2) in the AEW reservoir on the day of inlet sample.
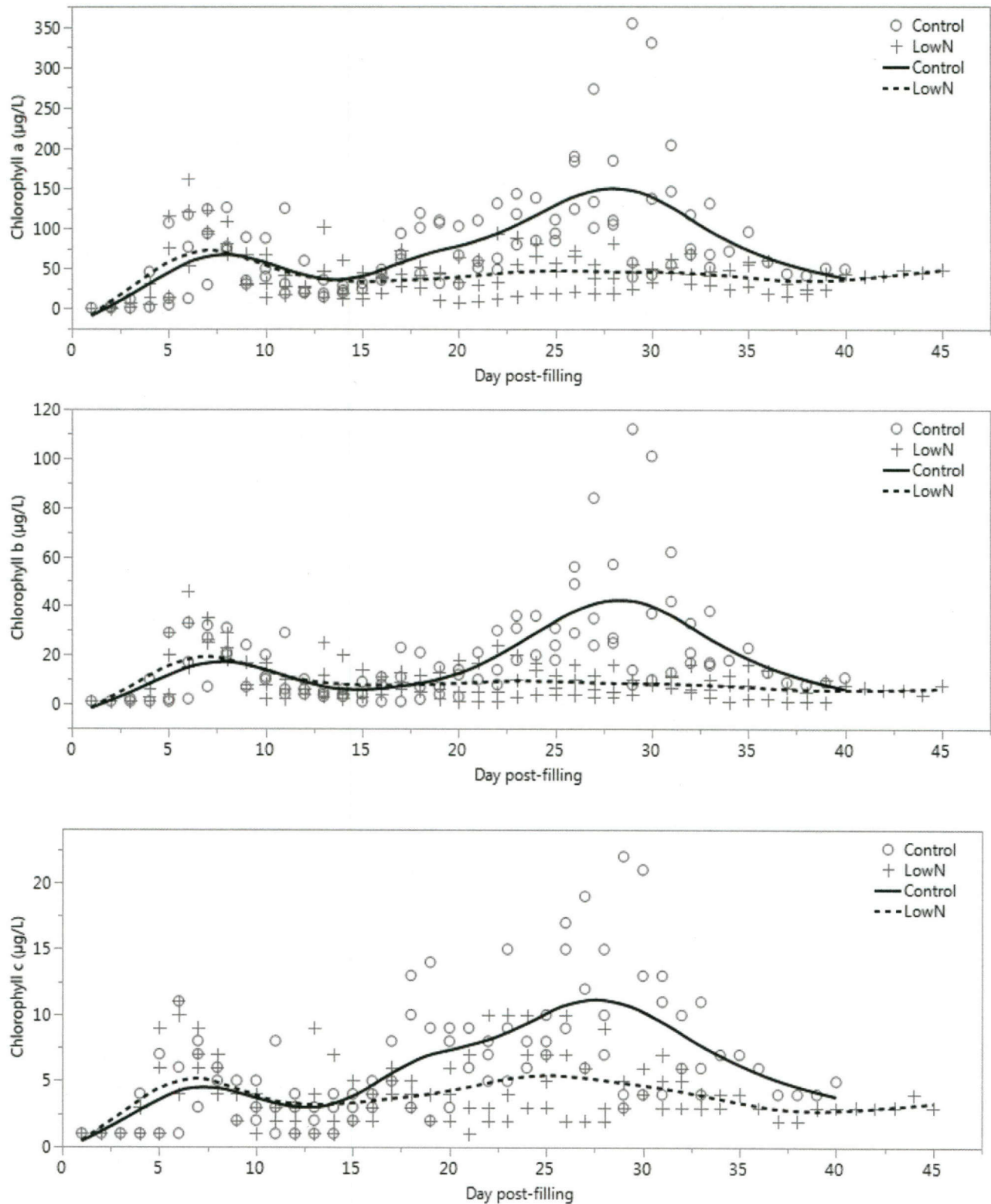
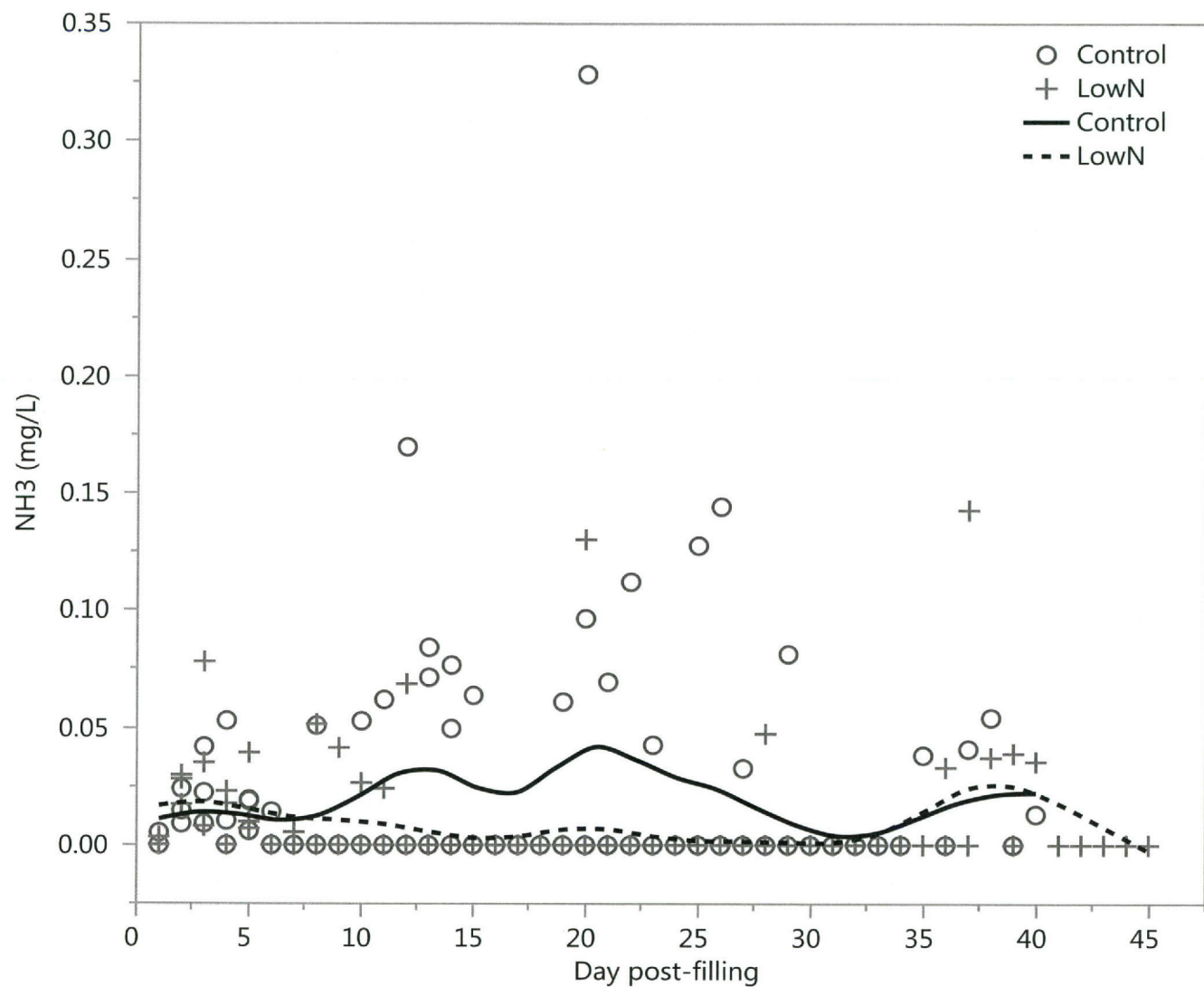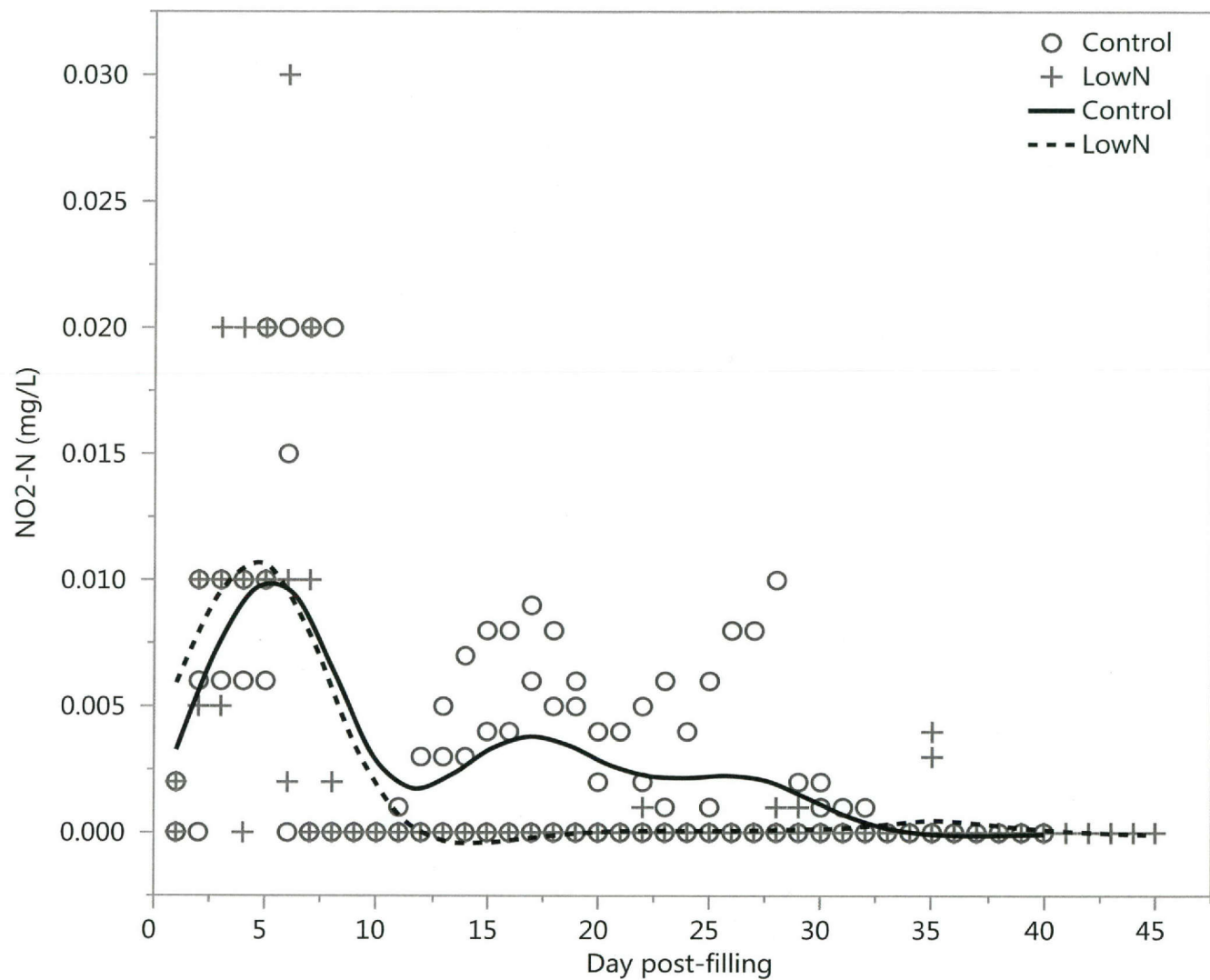FIGURE 1. Chlorophyll *a*, *b*, and *c* concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes
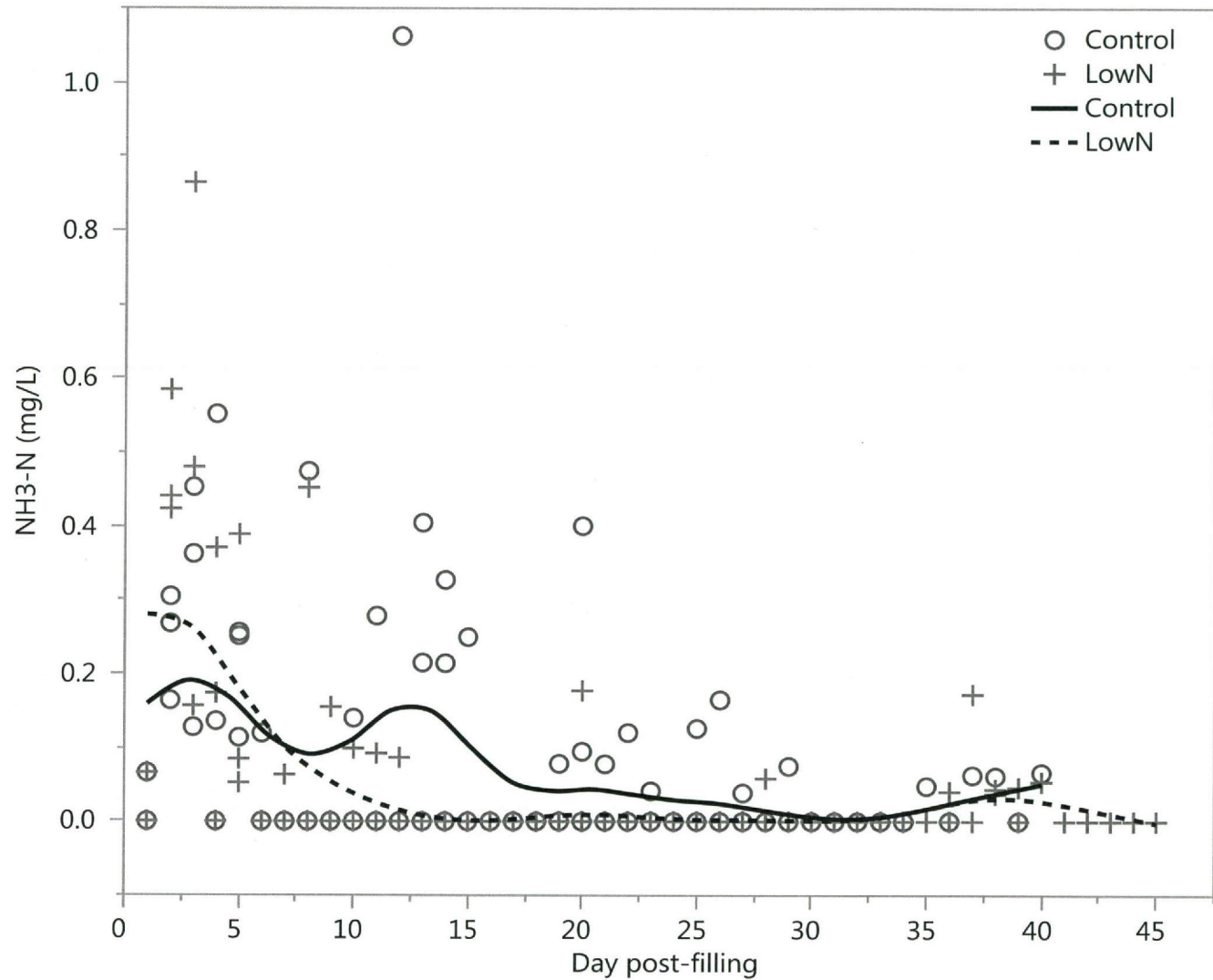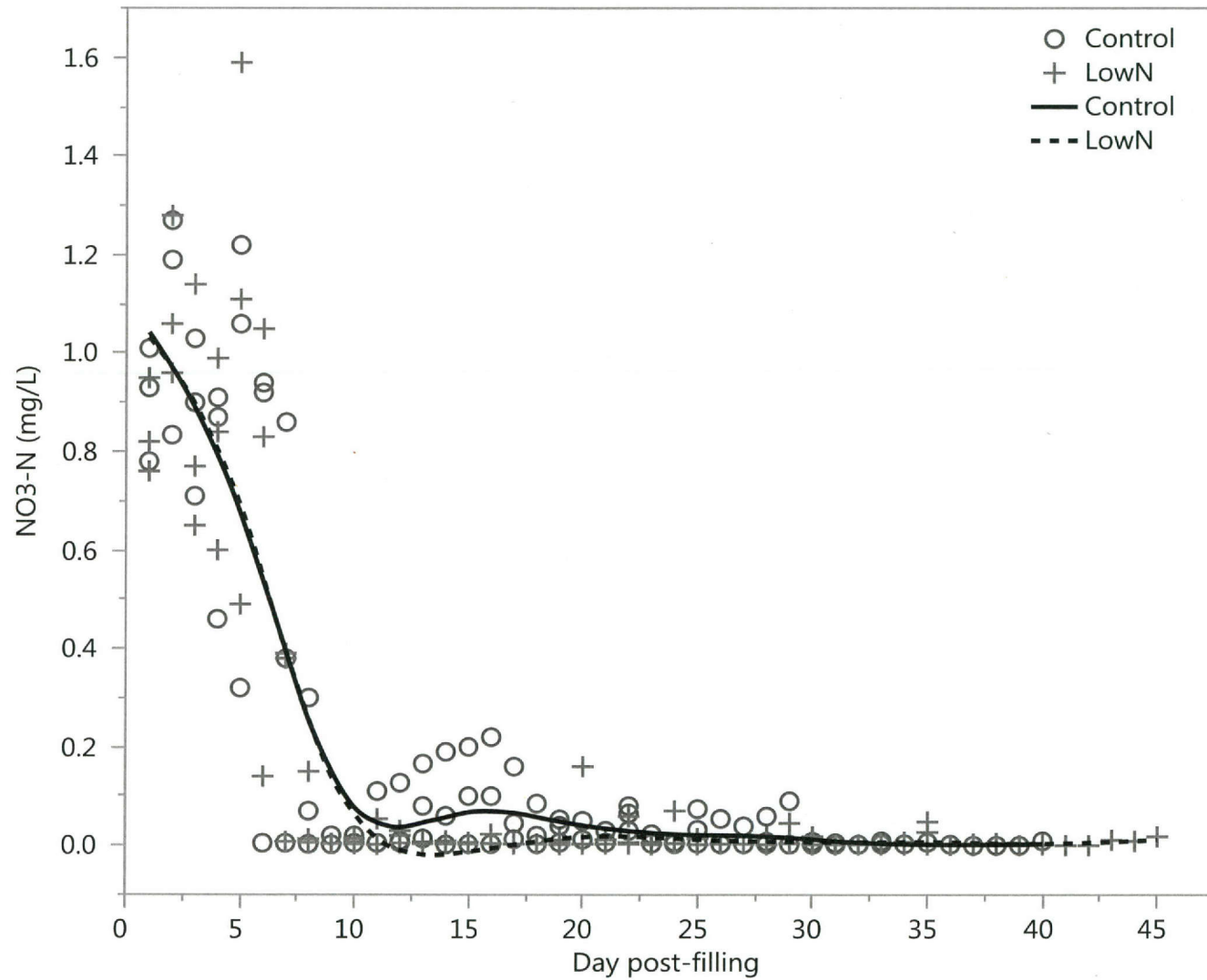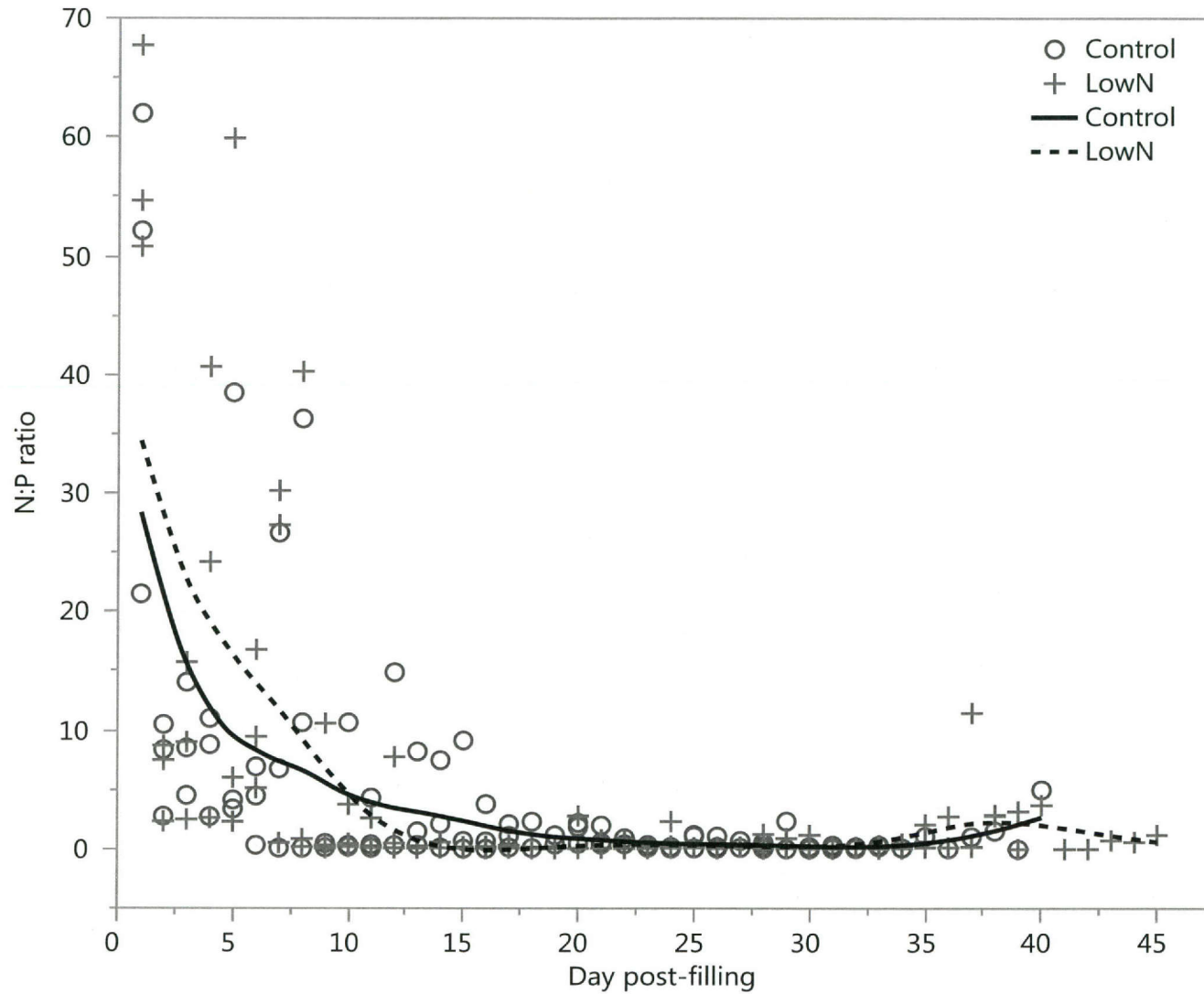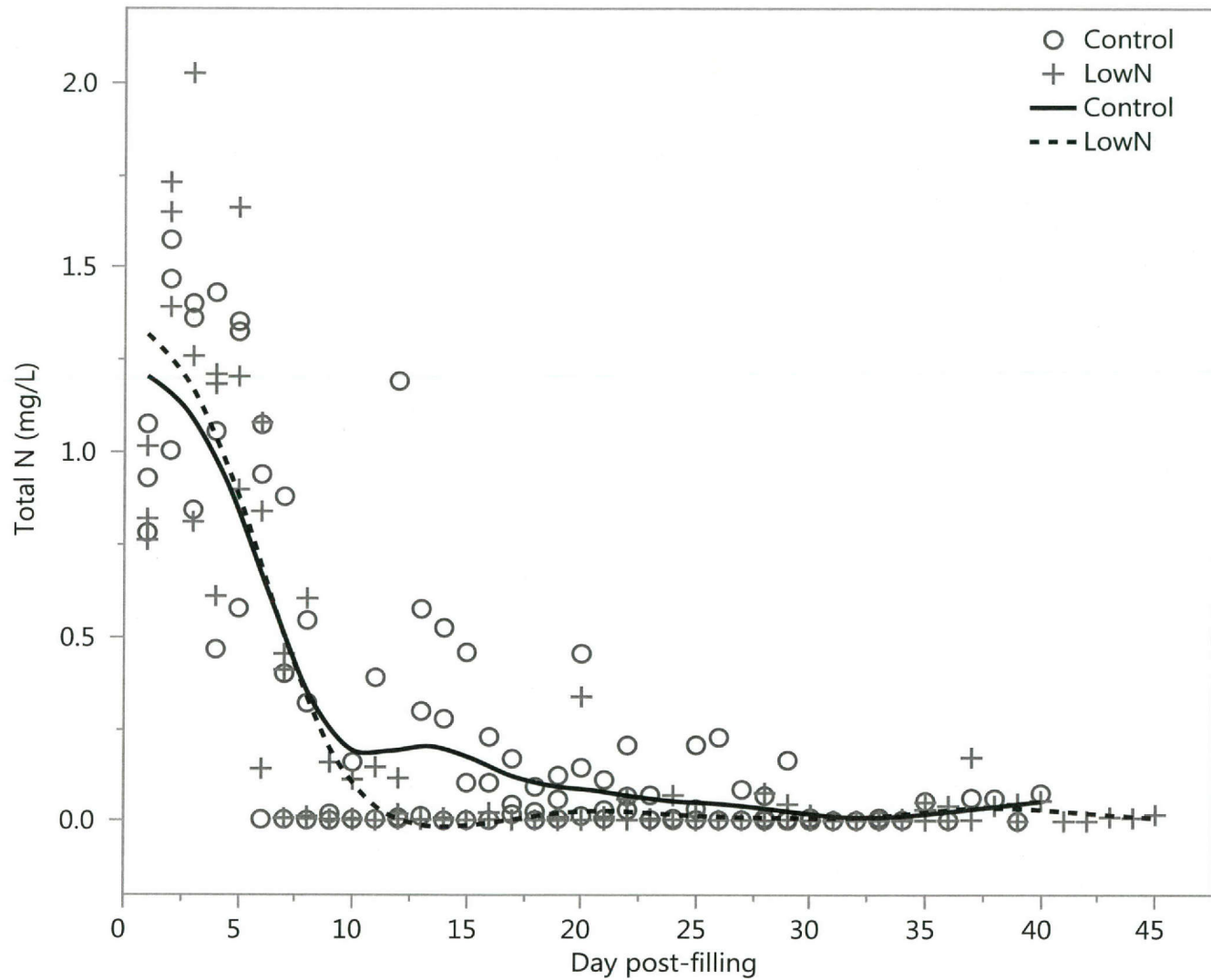
FIGURE 2.   Total ammonia-nitrogen concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes. All values < detection limits (0.04 mg/L) converted to 0 mg/L.

FIGURE 3.   Unionized ammonia concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes. All values < detection limits (0.04 mg/L) converted to 0 mg/L.

FIGURE 4. Nitrite-nitrogen concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.
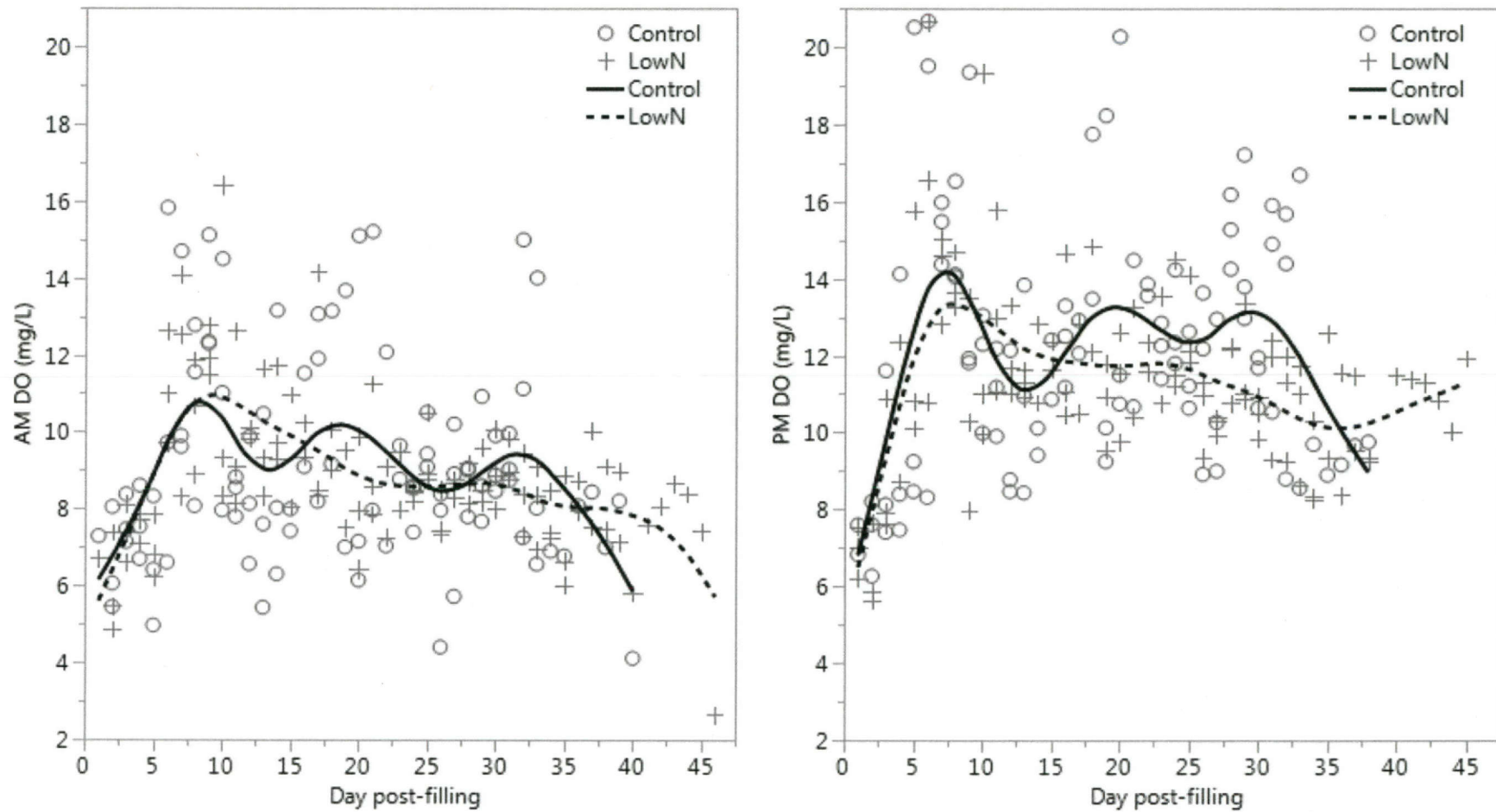
FIGURE 5. Nitrate-nitrogen concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.

FIGURE 6. The ratio of nitrogen/phosphorus concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.
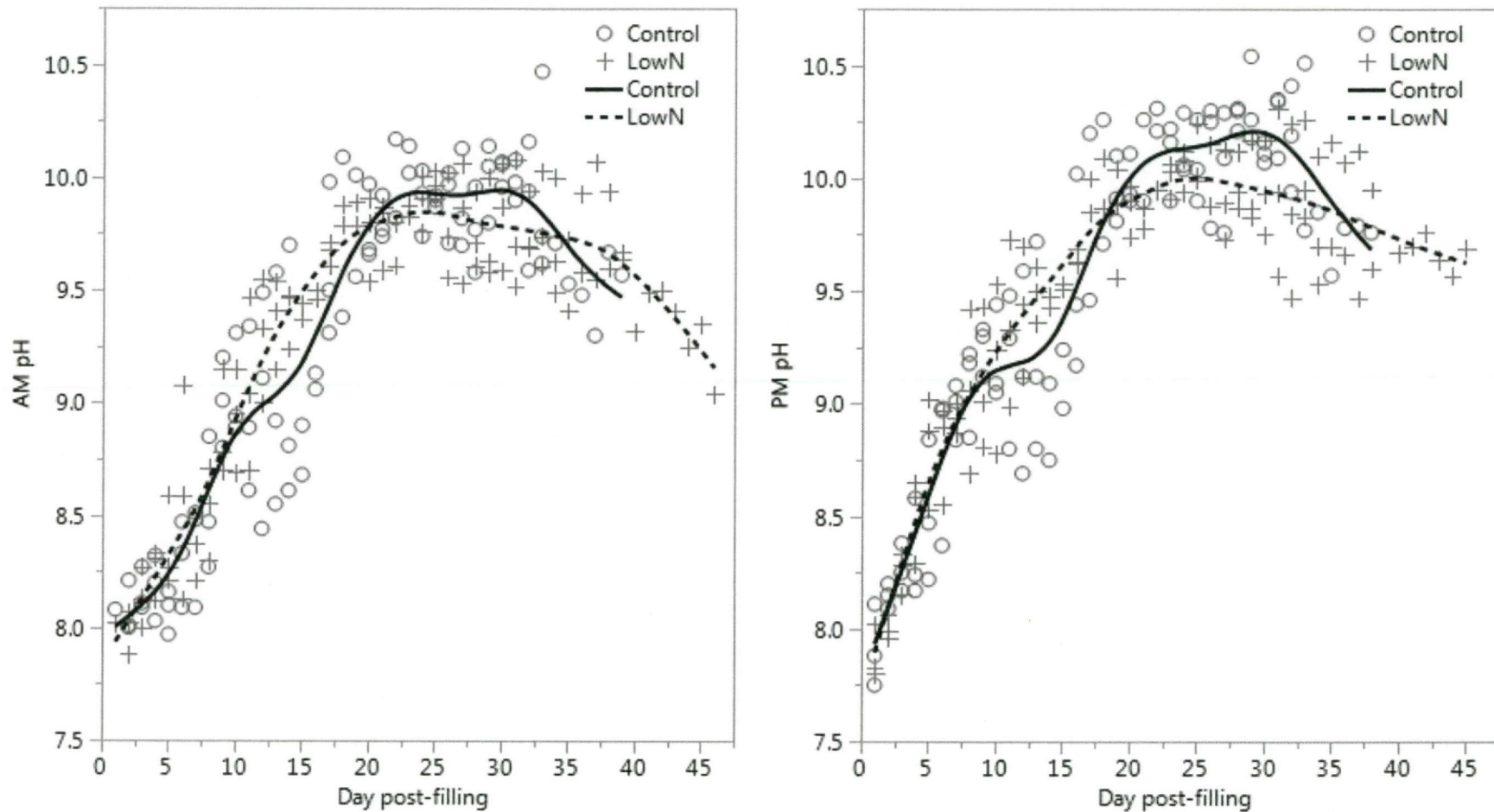
FIGURE 7. Total nitrogen concentrations (NH_3-N + NO_2-N + NO_3-N) in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.
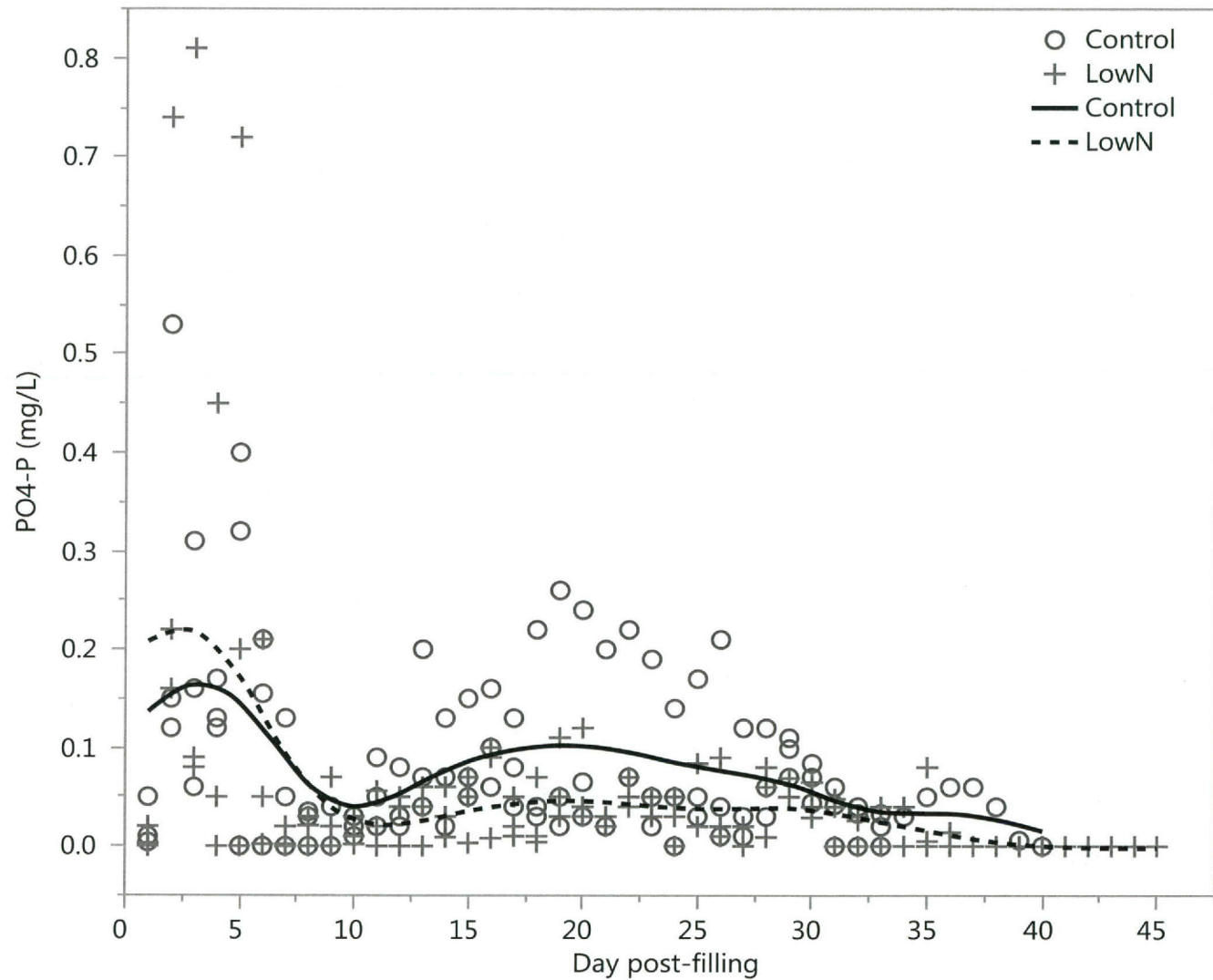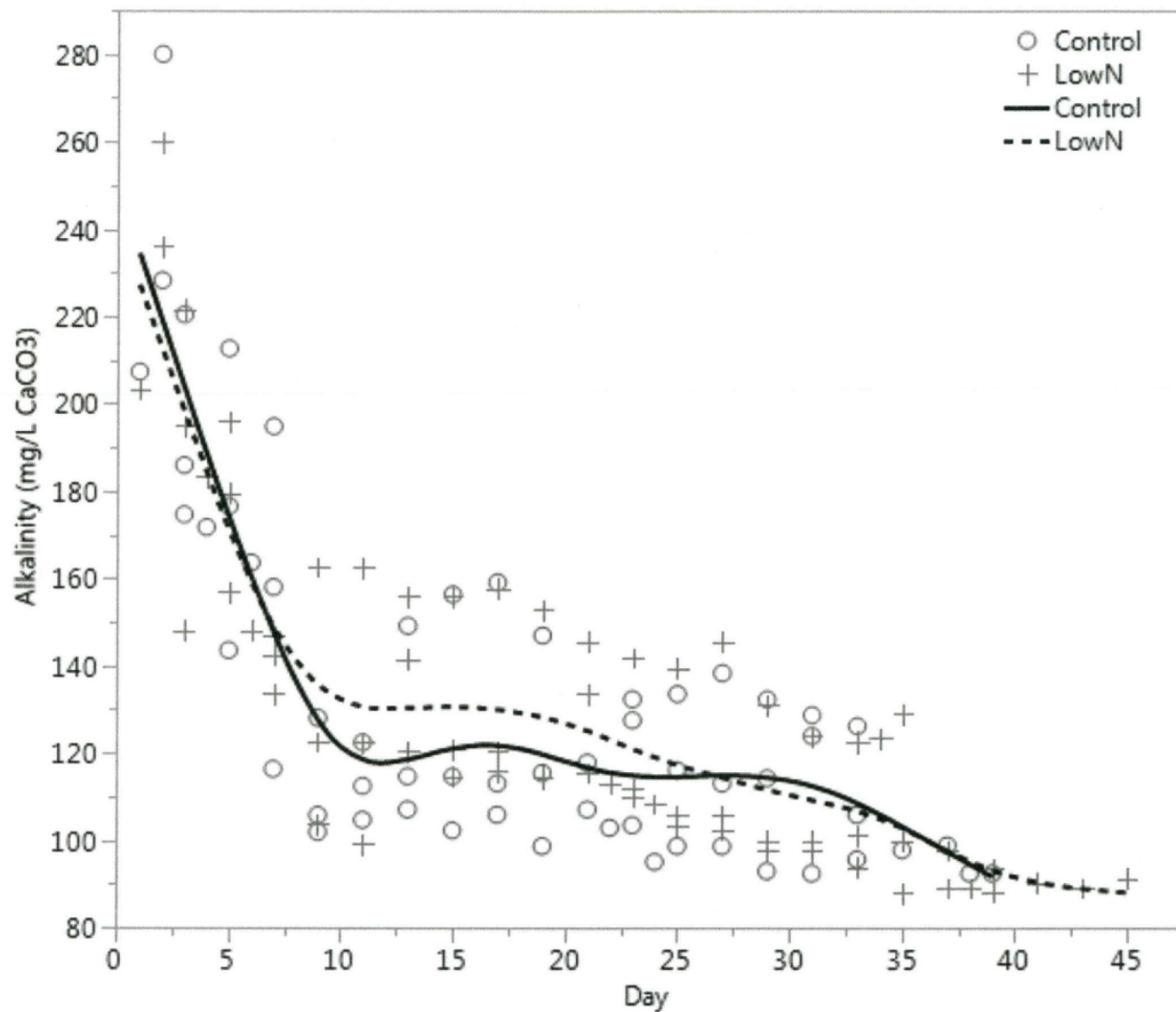
FIGURE 8.   Phosphorus concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.

FIGURE 9. Morning and afternoon dissolved oxygen (DO) concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.

FIGURE 10. Morning and afternoon pH in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.

FIGURE 11.   Alkalinity concentrations in six EPDM-lined 0.4-ha ponds stocked with Florida Largemouth Bass *Micropterus salmoides floridanus* fry from 16–23 April 2016 at the A. E. Wood State Fish Hatchery and fertilized following standard (Control; n=3) and reduced-N (Low-N; n=3) fertilization regimes.
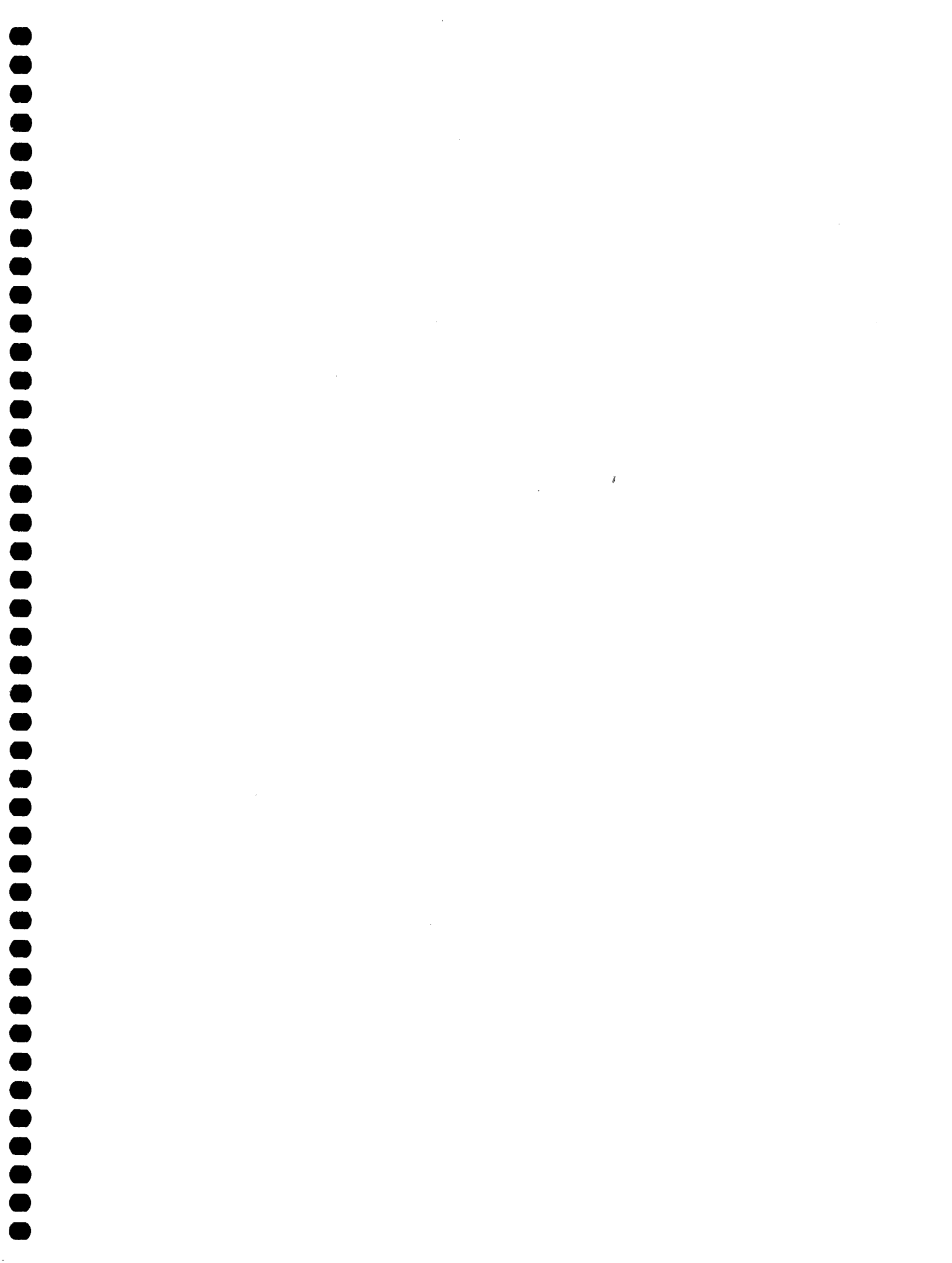
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