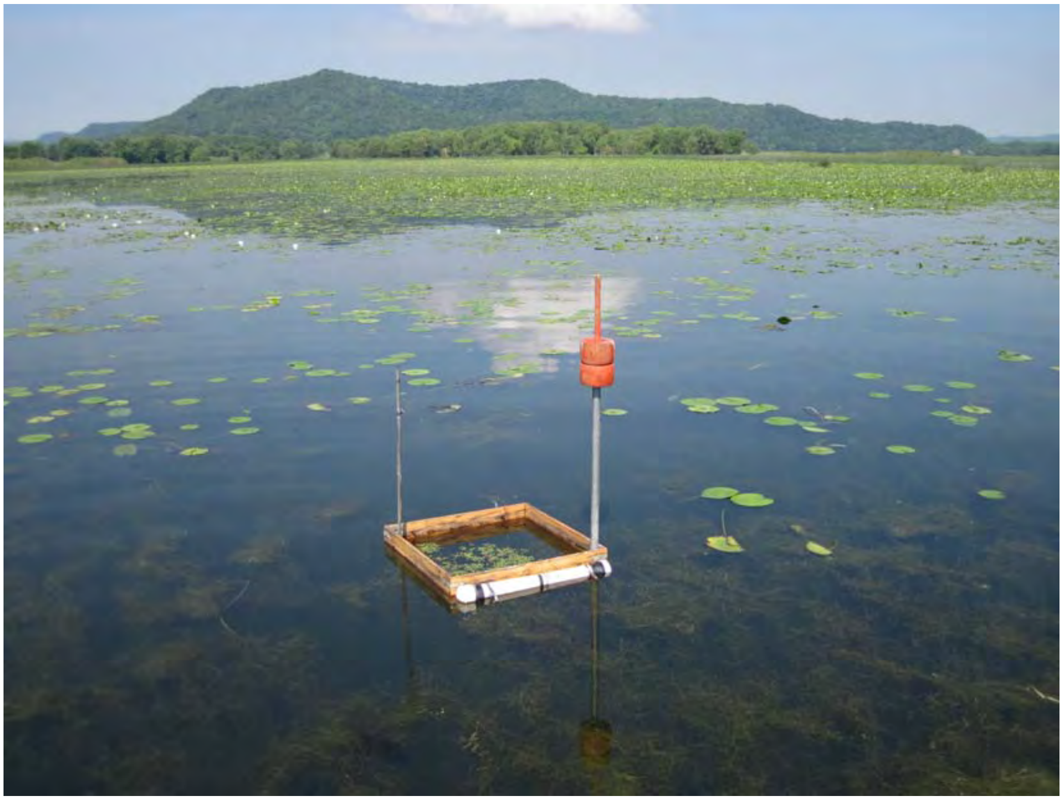
Growth, Tissue Composition and Stoichiometry of Duckweed Grown in Low Nutrient Backwaters of the Upper Mississippi River



John F. Sullivan[1] and
Shawn M. Giblin[2]
Mississippi River Water Quality Unit
Wisconsin Department of Natural Resources
La Crosse, WI
March 2012

[1] Wisconsin Dept. of Natural Resources, 3550 Mormon Coulee Road, La Crosse WI 54601 john.sullivan@wisconsin.gov, 608-785-9995

[2] Wisconsin Dept. of Natural Resources, LTRMP Field Station, 2630 Fanta Reed, La Crosse WI 54603 shawn.giblin@wisconsin.dnr, 608-781-6363

Introduction

Cultural eutrophication of aquatic systems has received considerable attention in our nations waters though much of it in the past has focused on nutrient enrichment problems in lakes. More recent attention has been made on evaluating the impacts of nutrients in riverine systems (Hilton 2006, UMRBA 2011). Recent evidence suggests that nutrient-related impacts in rivers may come in forms other than nuisance blue-green algae blooms that are typical in eutrophic lakes. The development of thick benthic or surface mats of filamentous algae or duckweed in quiescent in riverine aquatic habitats as a result of nutrient enrichment are examples of problems that may develop in these systems. These mats can result in negative impacts to aquatic life and obstruct recreational uses. Such conditions occur commonly during mid-summer periods in shallow vegetated areas of the Upper Mississippi River (UMR), especially in off-channel sloughs, backwaters and marshes (Sullivan 2008, Giblin et al. 2010).

In recent years monitoring and research has been focused on understanding the factors contributing to the development of filamentous algae or duckweed, generally referred to as metaphyton or free-floating plants (FFPs), in off-channel areas of the UMR (Sullivan 2008, Giblin et al. 2010, Sullivan and Giblin 2011 Giblin et al. Submitted, Houser et al. Submitted). The development of thick FFP mats is dependent on having adequate supply of nitrogen and phosphorus to maintain their growth and development. The sources of these nutrients are complex and variable in large floodplain rivers and includes allochtonous input from natural and cultural sources and internal loading (nutrient cycling). The fate of these nutrients in the river is strongly influenced by biological, physical and hydraulic processes. Further, since FFPs often require substrates for their initial development or quiescent surface waters to prevent their wash-out, FFPs are often associated with beds of rooted aquatic vegetation, especially submersed aquatic vegetation (SAV).

FFP research within the UMR backwaters has primarily focused on identifying conditions that limit metaphyton development, especially nutrients. Mid-summer metaphyton tissue nutrient content and stoichiometry as well as water column nutrient concentrations have often indicated nutrients were present in excess with limiting nutrient conditions being rare (Houser et al. Submitted). However more recent seasonal analysis of selected Pool 8 backwaters have identified potential water column nutrient thresholds and other physical and biological factors that may be important in influencing FFP growth (Giblin et al. Sumitted).

The objective of this work was to further define and verify nutrient conditions that limit the growth of FFP in UMR backwaters and to identify tissue nutrient thresholds and stoichiometry that are suggestive of nutrient-limited growth. We focused our study in areas where summer water column nutrient concentrations were normally low and seeded the sites with healthy duckweed placed in enclosures. This allowed us to measure duckweed growth rates, tissue nutrient composition and stoichiometry in areas where nutrient limitation was more likely and duckweed was uncommon or absent. We believed this approach would allow us to more accurately characterize nutrient conditions and tissue composition that are suggestive of nutrient limitation and help verify our evaluations made in comparative studies of FFP growth in different UMR backwaters.

Methods

Water quality surveys and duckweed growth rate measurements were made from late June to late August, 2011 in two low nutrient backwaters within the UMR floodplain. The two sites included Lizzy Pauls Pond (south of Hwy OO), a 17.5 ha isolated backwater in the eastern portion of upper Pool 5, and Target Lake, a 132.6 ha contiguous backwater in the western portion of upper Pool 8 (Figure 1). An individual site was selected in both backwaters that was characterized by low nitrogen and phosphorus concentrations during mid-summer periods based on previous surveys (Sullivan 2008, Houser et al. Submitted and Giblin et al. Submitted).

Measurements of duckweed production were made in both backwaters by seeding 0.6 m^2 wooden sampling frames (Figure 2) with a known quantity (wet weight) of duckweed obtained from a small backwater area adjacent to Broken Arrow Slough prior to each survey. Additional information on sampling frame construction and deployment procedures has been described by Sullivan and Giblin (2011). Broken Arrow Slough is a flowing side channel in upper Pool 8 southeast of Target Lake (Figure 1). Rates of duckweed production were determined by measuring the net change in wet and dry weights within the sampling frame over a period of 7 to 11 days. Relative growth rates were based on the net change in dry biomass over the sampling period: growth rate = $(\ln DW_2 - \ln DW_1)/(t_2-t_1)$ in which DW_2 and DW_1 were the dry masses at time t_2 and t_1, respectively. The dominant duckweed taxa of the seed and the resulting taxa at the end of the sampling period were noted.

A large quantity of duckweed (~150 g wet wt) was collected from the seed source with a stainless steel soil sieve (0.5 mm mesh) and placed in a clean plastic tray containing water from the seed source. Sub-samples of duckweed (~20-80 g wet wt) were collected for initial tissue analysis and to obtain duckweed for seeding the two study sites. Samples were spun in a tethered soil sieve (Sullivan and Giblin 2011) for 30 revolutions to remove excess moisture then placed in quart Ziploc bags and weighed to estimate the initial wet weights. Some site water from the seed source was re-added to the duckweed sub-samples that were to be used for growth rate measurements. Samples were placed in a cooler with a small quantity of ice to prevent samples from overheating during transit to Target Lake, Lizzy Pauls Pond or laboratory. The seed was normally transferred to the study sites within a few hours of collection. On two days (June 28th and July 15th), the duckweed seed for Lizzy Pauls Pond was held in the laboratory under fluorescent lighting for about 1 day prior to seeding the frame enclosure at the study site.

At the end of each production measurement, the entire content of duckweed within the sampling frame was removed for wet and dry weight determination. Samples were placed in small plastic trays then visually inspected to remove vegetation debris that was not duckweed. The cleaned duckweed samples were placed in plastic Ziploc bags or 2 liter containers and stored in a cooler with ice and returned to the laboratory. Additional sample cleaning was occasionally needed in the lab when broken bits of SAV were present. Excess moisture was removed from the sample as described previously then the wet weight was determined. Samples were dried at 80 C in a plant dryer for about 2 to 3 days to determine dry weight measurements. An electronic balance (Ohaus Scout Pro) was used to weigh samples. A 200 gram brass weight standard was used to verify the scale accuracy and was within (+/- 1 gram) throughout the study. Dried samples were placed into Ziploc bags and frozen prior to tissue analysis. Plant tissue analyses of duckweed samples for major and minor elements were determined by the University of Wisconsin Soil and Plant Analysis Laboratory in Verona, Wisconsin. Plant tissue element ratios (C:N, C:P and N:P) were expressed on an atomic or molar basis.

Field measurements of dissolved oxygen (DO), pH, specific conductance and water temperature were made at the surface (0.15 m), mid-depth and within 0.1 m above the sediment surface during each survey using a YSI 556 multi-parameter meter. The instrument was calibrated following manufacturer's instructions prior to each survey. In addition, a DO/temperature sonde (D-OptoLogger – Zebra-Tech LTD) was suspended horizontally about 0.2 m below the water surface in the center of the sampling frames on an iron rod. The oxygen sensor of the sondes was calibrated pre-deployment in the lab using a 2-point calibration procedure (0 and 100 % saturation) following the manufacturer's protocol. The sondes were set to log measurements at 15-minute intervals throughout the duration of the survey and were cleaned at 7 to 11 day intervals. The loggers were checked for DO calibration drift using the same 2-point calibration method at the end of the field deployment period. The maximum DO drift during post-calibration checks did not exceed +/- 0.1 mg/L at 100% saturation. The DO calibration drift at 0% saturation was within +/- 0.3 mg/L. There were no adjustments made to the recorded DO measurements.

Aquatic vegetation and qualitative estimates of free floating plants (duckweed or filamentous algae) cover were made within a 25-meter radius around the sampling site during each survey (Sullivan 2008, Giblin et al. 2010). Digital pictures were taken at each site and each frame during deployment and retrieval to document changes in vegetation cover during the study period.

Water samples were collected for turbidity and nutrients (total P, total dissolved P, ammonia-N, nitrite+nitrate-N, total Kjeldahl N) and chlorophyll *a* analysis about 0.15 m below the water surface. Samples were preserved and filtered where necessary and shipped on ice to the Wisconsin State Laboratory of Hygiene in Madison, Wisconsin for chemical analysis. Dissolved ammonia nitrogen (NHx) and nitrite+nitrate-N (NOx) concentrations were often below laboratory detection limits, 0.015 and 0.019 mg/L, respectively. When this occurred, NHx and NOx values were assigned values of one-half the detection limit for plotting and statistical evaluations. Turbidity measurements were made using a Hach 2100P turbidity meter. Water depths were recorded to the nearest 0.05 ft (1.5 cm).

Daily Mississippi River discharge (cfs) was obtained from nearby gages operated by the US Corps of Engineers for Lock and Dam 5 near Minneiska, MN and Lock and Dam 8 near Genoa, WI. The study sites at Lizzy Pauls Pond and Target Lake were about 8 to 18 miles above these dams, respectively.

Basic statistics, Spearman rank correlations and Kruskal-Wallis one-way AOV, a non-parametric procedure used to evaluate differences between groupings, were derived using Statistix 8 (Analytical Software, 2003). In the Kruskal-Wallis test, a comparison of mean ranks option ($p=0.05$) was used to indentify groups with similar means.

Results and Discussion

Site conditions

Mississippi River flows were above normal during July and August 2011 based on discharge measurements reported by the US Corps of Engineers for Lock and Dams 5 and 8. River discharge approached 90,000 cfs during July then slowly decreased in mid-August (Figure 3a). Discharge averaged 64,500 and 69,200 cfs at Lock and Dams 5 and 8, respectively, during the July-August period. A comparison of these values to the Corps' flow-duration tables for these sites suggested river discharge was about two times higher than median discharge for the July-August period.

Fluctuations in river discharge were responsible for moderate changes in water depths at Target Lake ranging from about 1 to 1.5 meters during the study period. In contrast, water depths at Lizzy Pauls Pond varied little during July-August and indicated lower hydraulic connectivity with the Mississippi River main channel. Water surface elevation of Lizzy Pauls Pond was influenced by a beaver dam, which was found inside the BNSF railroad culvert at the far northern end of this backwater (north of Hwy OO). This dam raised the water surface of Lizzy Pauls Pond and negated the influence of Pool 5 stage fluctuations on this isolated backwater under discharge conditions experienced in the summer of 2011.

Water Quality Measurements

Field water quality measurements of Lizzy Pauls Pond and Target Lake revealed noticeable differences between these two backwaters (Figure 3 and Table 1). Lizzy Pauls Pond had warmer water, higher pH and lower specific conductance than Target Lake during the study period. These differences likely reflect complex differences in the sources of inflow between these backwaters, especially hydraulic exchange with the Mississippi River as well as groundwater and precipitation influences. The relatively low specific conductance of Lizzy Pauls Pond suggests this backwater was largely isolated from the influence of the main channel, which typically has a median specific conductance of approximately 400 to 550 uS/cm during summer based on the federal Long Term Resource Monitoring Program (LTRMP) stratified random sampling data for the main channel in Pools 4 and 8 (USGS 2011). Differences in macrophyte density between the two backwaters may have had some influence on water quality, especially photosynthetic and respiratory processes. In general, Lizzy Pauls Pond appeared to exhibit less SAV coverage than Target Lake but the turbidity and chlorophyll levels were generally similar (Figure 3 g,h). Maximum water depth was slightly deeper in Target Lake (Figure 3b) which may have tempered solar heating and influenced rates of cooling and contributed to temperature differences between the two sites.

Surface DO measurements revealed large variation in both backwaters (Figure 3d) ranging from near 0 to about 11 mg/L. These data reflect variable photosynthetic and respiratory processes that are influenced by daily changes in solar radiation and temperature and are typical of shallow Mississippi River backwaters containing macrophytes (Sullivan and Giblin 2011). DO levels declined significantly ($p<0.05$) with depth and indicated waters were stratified even though thermal stratification was weak (Table 2).

Plant respiration and sediment oxygen demand in combination with reduced vertical mixing due to SAV cover were likely important factors contributing to this response.

Continuous DO measurements indicated large temporal variation in concentrations ranging from near 0 to 18 mg/L (Figure 4). Median DO was 7.2 and 8.9 mg/L in Lizzy Pauls Pond and Target Lake, respectively (Table 1). Periods of hypoxic conditions (< 2 mg/L) were present in both backwaters. These conditions were experienced in mid-July to mid-August in Lizzy Pauls Pond and during August in Target Lake. In general, periods of pronounced hypoxia or anoxia were less in these two backwaters than comparable backwaters (Markle, Beiers and Round Lakes) monitored in Pool 8 in 2010 (Figure 5) by Sullivan and Giblin (2011). Although the specific reasons for the higher DO conditions in the present study were not explored, it is suspected the absence of thick mats of duckweed was an important factor. Dense duckweed mats have been associated with low DO in UMR backwaters likely in response to reduced surface re-aeration and light penetration (Sullivan 2008 and Giblin et al. 2010).

Total phosphorus (TP) and total dissolved phosphorus (TDP) exhibited greater variation and higher concentrations in Target Lake than Lizzy Pauls Pond (Figure 3i and 3j). Median TP concentrations were 0.079 mg/L in Target Lake and about one-half this concentration (0.044 mg/L) in Lizzy Pauls Pond (Table 1). These differences were attributed to differences in the dissolved fraction since the particulate fraction was generally similar in both backwaters. Median TDP concentrations were 0.044 vs 0.017 mg/L in Target Lake and Lizzy Pauls Pond, respectively. Phosphorus concentrations were quite low as typical concentrations for UMR backwaters during mid-summer based on LTRMP data are about 0.175 mg/L TP and 0.070 mg/L soluble reactive phosphorus (USGS 2011).

Total nitrogen (TN) concentrations were relatively low and normally less than 1 mg/L in both backwaters but greater variation was again noted in Target Lake, which was influenced by a NOx spike in late July (Figure 3k and 3m). TN concentrations were low as compared to median mid-summer concentrations reported for UMR backwaters for Pool 4 and 8, which ranged from about 1 to 3.5 mg/L for 1993 to 2010 (graphical browser, USGS 2011). The surge in NOx in Target Lake was likely associated with increased water exchange with the river or possibly runoff from Pine Creek, a small tributary that discharges to the northwestern boundary of Target Lake. Increased water exchange was likely during this time since there was a noticeable increase in river flow during mid- to late July (Figure 3m). Dissolved inorganic nitrogen (DIN) was very low on most sampling days (Figure 3l). NOx concentrations were often below the level of laboratory detection (0.019 mg/L). NHx concentrations were also low but exhibited moderate variation in both backwaters generally ranging from near 0 to 0.05 mg/L (Figure 3n). As with NOx, NHx was frequently reported at concentrations below the laboratory detection limit (0.015 mg/L), especially in Lizzy Pauls Pond during late July and August. Low inorganic nitrogen concentrations are common in Mississippi River backwaters with little connection to the main channel as a result of nutrient assimilation by aquatic macrophytes, algae and denitrification (Richardson et al. 2004 and James et al. 2008).

TN to TP ratios (mass basis) indicated moderate variation in Target Lake ranging from 5 to 25 in contrast to Lizzy Pauls Pond, which had relatively stable ratios of about 13 to 18 (Figure 3o and Table 1). DIN to TDP ratios were normally less than 3 in both backwaters as a result of the relatively low concentrations of NOx- and NHx-N. The exception was in late July when there was a spike in NOx concentrations in Target Lake and the DIN/TDP ratio increased to about 24 (Figure 3p).

Duckweed Production and Growth

Duckweed production exhibited moderate variation in both backwaters with the greatest range (0.0 to 3.9 g m^{-2} d^{-1}) measured in Target Lake (Figure 3q). Average production rates for Lizzy Pauls Pond and Target Lake were 2.1 and 1.7 g m^{-2} d^{-1}, respectively (Table 3). Some caution needs to be applied to measurements made in Lizzy Pauls Pond due to the inclusion of ambient *Lemna trisculca* that floated up into the frame during the production measurements at this site during July and early August. This duckweed was not found in the seed but was commonly observed growing below the surface in Lizzy Pauls Pond during this period. The inclusion of this biomass into the production measurements contributed to positive bias in the production measurements for this site during the surveys in July and early August. If these measurements are excluded, the average production was about 1 g m^{-2} d^{-1}, but the measurement was only based on two samples in August.

The average production measurements made in Lizzy Pauls Pond and Target Lake in 2011 were noticeably lower than comparable measurements (n=4) made in Pool 8 backwaters in 2010 during July and August, which averaged about 4 g m^{-2} d^{-1} (Sullivan and Giblin 2011). TN and TP concentrations measured in the Pool 8 backwaters during July-August 2010 averaged 1.9 and 0.22 mg/L, respectively (Giblin et al. Submitted). In contrast, average TN and TP concentrations measured in Lizzy Pauls Pond and Target Lake in 2011 were substantially lower (0.77 and 0.067 mg/L, respectively) and were likely an important factor contributing to lower production measurements at these sites. Production measurements in Lizzy Pauls Pond and Target Lake were also substantially lower than theoretical maximum production (20 g m^{-2} d^{-1}) as reported by Landolt and Kandeler (1987).

Relative growth rate measurements generally followed the pattern of production since these measurements are closely related (Figure 3u). Average relative growth rates were about two times higher in Lizzy Pauls Pond (0.189/d) than Target Lake (0.099/d), (Table 3). However, it is suspected the first four growth rate measurements in Lizzy Pauls Pond (July and early August) were biased high due to the movement of *L. trisulca* into the sampling frame during the production measurements as described above. Removing these values lowered the average growth rate to 0.129/d. These growth rates are substantially lower than the reported maximum (0.25 to 0.35/d) described by Landolt and Kandeler (1987) or measured by Hurlimnan-Luond (1990) and Szabo et al. (2010) in laboratory cultures.

Plotting the average growth rate versus DIN or TDP concentrations measured in Lizzy Pauls Pond and Target Lake (combined data) revealed a response consistent with measurements derived from Hulimnann-Luond (1990) and Szabo et al. (2010) based on their laboratory growth studies using *L. gibba, L. minor* and *Spirodela polyrrhiza* (Figure 6). The growth versus nutrient concentration regression plots derived from these studies provide a potential model for evaluating duckweed growth response to nutrient enrichment in UMR backwaters. The absence of surface mats of duckweeds in the vicinity of the study sites at Lizzy Pauls Pond and Target Lake in the summer of 2011 can likely be explained by relatively low levels of TN (avg. 0.7 to 0.9 mg/L) and TP (avg. 0.05 to 0.09 mg/L) which were probably insufficient for supporting growth rates necessary to yield high levels of duckweed biomass. This finding is consistent with a previous UMR study indicating that filamentous algae and duckweed development in backwaters and wetlands was low when summer average TN and TP concentrations were less than 0.95 and 0.077 mg/L, respectively (Sullivan 2008). Recent studies suggest TN and TP thresholds of 0.3 and 0.043 mg/L, respectively, below which zero metaphyton biomass was expected to occur (Giblin et al. Submitted). They found increasing biomass at higher nutrient concentrations with regression breakpoints of 1.3 mg/L TN and 0.250 mg/L TP, after which biomass reached a plateau or decreased.

Duckweed Tissue Analysis

The mineral composition of duckweeds provides a means for evaluating the nutrient status of these plants and provides an indication of nutrient availability in the waters where they are collected (Szabo et al. 2010). The primary nutrients of concern are N and P since these are critical nutrients necessary to support duckweed growth (Landolt and Kandeler 1987). Carbon is also an important element in plants since it represents a major component of plant tissue and reflects the net synthesis of organic carbon associated with plant photosynthesis. It is assumed carbon is not limiting to duckweeds since most forms have free access to carbon dioxide in the atmosphere. Evaluating tissue composition and nutrient to carbon ratios (stoichiometry) of aquatic plant tissue provides means of evaluating nutrient surplus or limitation in aquatic systems (Hall and Cox 1995, Murkin et al. 1994, Verhoeven et al. 1996, USEPA 2002, Sterner and Elser 2002).

Noteworthy trends in duckweed tissue C, N and P content were found in the seed source, Lizzy Pauls Pond and Target Lake samples during the summer of 2011 (Figure 7). Carbon content was generally less than 40% and no obvious temporal trend was apparent. However, a noticeable decrease was apparent in the July 25th sample collected from Target Lake when levels fell to about 26% (Figure 7a). The C content of duckweed samples collected from Target Lake were significantly lower than samples collected from Lizzy Pauls Pond (Table 4) and may suggest duckweed grown in Target Lake were in an early phase of plant senescence due to reduced nutrient availability.

Duckweed N composition varied substantially between sites, especially during July ranging from about 1.3 to 3.8% (Figure 7b). Average tissue N in Target Lake samples was 1.85% and was significantly lower than the samples from Lizzy Pauls Pond (2.99%, Table 4). Maximum tissue N in duckweed samples collected from Upper Mississippi River backwaters influenced by municipal wastewater treatment plant discharges were reported to be about 5% (Sullivan 2008) indicating the duckweed in our study were collected in waters with less available N. Further, the marked reduction of the N content in Target Lake duckweed compared to the initial seed concentration suggested N availability was lower in Target Lake than Lizzy Pauls Pond. This was supported by the change in pigmentation of the duckweed (*Spirodela polyrrhiza*) that developed in the sampling frames of Target Lake during initial production measurements in July. Duckweed changed from green to purple (Figure 2) and likely reflected an increase in anthocyanin pigments, which has been associated with low nitrogen concentration in the growing media or ambient water (Hurlimnann-Luond 1990 and Landolt and Kandeler 1987). The lack of a similar response in tissue N or change in duckweed pigmentation in Lizzy Pauls Pond samples was surprising since water DIN levels were consistently low at this site (Figure 3l, 3m, 3n). It is suspected the tissue samples from Lizzy Pauls Pond were biased due to the movement of *L. trisulca* into the sampling frame during production measurements in July and early August as described previously. This species of duckweed typically develops below the surface and may have had access to higher levels of NHx that were released from sediments (James et al. 2008). Further, SAV coverage appeared to be lower in Lizzy Pauls Pond, which may have provided less competition for DIN and favored the development of this submerged duckweed species in this backwater.

The C:N ratio exceeded 20 in duckweed samples collected in early July and late August in Target Lake (Figure 8a). These ratios greatly exceed the estimated C:N threshold (15.5) suggesting N limitation derived from plant tissue nutrient studies of Manitoba wetlands (Murkin et al. 1994). The combination of low duckweed growth rates, change in duckweed pigmentation and high tissue C:N ratios in early July in Target Lake strongly suggest N limitation during this period at this site.

The P composition of duckweed samples was variable ranging from 0.2 to 0.5% (Figure 7c) and was substantially less than maximum levels (1-1.4%) found in water or grown in media with high P concentrations (Sullivan 2008, Szabo et al. 2004). Duckweed tissue P concentrations normally showed a marked decrease in Lizzy Pauls Pond and Target Lake when compared to the initial seed concentrations (Figure 7c). The exceptions were two samples from Lizzy Pauls Pond in early to mid-July, which showed no change or a moderate increase in comparison to the initial seed. Tissue P levels in the Target Lake samples averaged 0.25% and were significantly different than the P content of the initial seed (0.44%) and Lizzy Pauls Pond samples (0.34%), (Table 4). The general decrease in tissue P during production measurements suggests reduced availability of P at both study sites.

The C:P ratio in duckweed tissue samples from Target Lake increased substantially from the initial seed values during early to mid-July and late August with ratios approaching 500 (Figure 8b). C:P ratios exceeding 258 are suggestive of P limitation based on data derived from Murkin et al. (2004). Most of the tissue samples from Target Lake and Lizzy Pauls Pond were at or above this threshold. In contrast, the seed source C:P averaged 231 (Table 4) with all but one sample falling below 258. Tissue C:P in Target Lake duckweed averaged 373 and was significantly greater than the initial seed or from duckweed grown in Lizzy Pauls Pond. The joint occurrence of high tissue C:P and C:N ratios in July and August suggested co-limitation of both N and P in Target Lake. This is best illustrated by a plotting C:P versus N:P for the three duckweed tissue sources (Figure 9). Five of the six Target Lake samples showed a marked change in comparison to the seed source samples with a movement to a region of the graph suggesting P (C:P >258) and N (C:N >15.5) limitation. This was only noted in two of six samples from Lizzy Pauls Pond. The four samples from Lizzy Pauls Pond that did not indicate this response exhibited values similar to the seed source. These four tissue samples were likely influenced by *L. trisulca,* which had floated up from the bottom and into the sampling frame at this site during production measurements (Table 3).

The ratio of N:P in the three duckweed tissue sources were highly variable (Figure 8c). The average N:P ratio was 20.1 in samples from Lizzy Pauls Pond and was lower in the seed source and Target Lake samples, 15.3 and 16.7, respectively. However, the difference in N:P between sites was not found to be significantly different (Table 4). Duckweed N:P ratios at the latter two sites were very close to the Redfield ratio (16:1), which has been commonly found in plants and may reflect balanced levels of N and P (Redfield, 1934, Sterner and Elser 2002). N:P ratios less than 13 or greater than 23 have been associated with N or P limitation, respectively, in benthic algae (Hillebrand and Sommer 1999). Murkin et al (1994) indicated N:P ratios exceeding 22 (derived from >10 N:P mass concentration threshold) indicated P limitation. Since the N:P ratios reported here were generally within this boundary (with exception of the late August measurement in Lizzy Pauls Pond, no clear distinction of N or P limitation can be made solely on the N:P ratio alone. We believe both N and P were normally limiting in Target Lake as reflected in elevated C:N and C:P ratios. This suggests that the tissue N and P declined at similar rates and the evaluation of N:P ratios did not reflect the nutritional imbalance. This points to the need to consider tissue C concentration when performing stoichiometry evaluations of plant material.

Summary

Measurements of duckweed production, growth and tissue composition were measured in two low nutrient backwaters of the UMR during the summer of 2011. Duckweed was introduced and monitored in sampling frames deployed in Lizzy Pauls Pond (Pool 5) and Target Lake (Pool 8) at locations that normally exhibited low free floating plant development and low water column N and P concentrations.

Average water column TP concentrations were 0.045 and 0.088 mg/L in Lizzy Pauls Pond and Target Lake, respectively. The dissolved P fraction (TDP) was roughly one-half these concentrations. Average TN concentrations at these respective sites were 0.68 and 0.86 mg/L. Dissolved NHx and NOx-N were often below laboratory detection limits. The estimated median DIN concentration was about 0.02 mg/L at both sites. Target Lake experienced a spike in NOx (1.74 mg/L) in late July during a period of increased river discharge which greatly increased the average DIN for this site. It was presumed this increase was associated with inflow of elevated NOx from the river or a small tributary stream during this period. Although sub-surface growths of *L. triscula* were common at Lizzy Pauls Pond, neither Lizzy Pauls Pond nor Target Lake experienced thick surface mats of filamentous algae or duckweeds during the study period (late June to late August). These findings are consistent with previous studies in backwaters of the UMR which showed little free floating plant development in water with low N and P concentrations.

Duckweed placed in experimental frames in Lizzy Pauls Pond and Target Lake exhibited relatively low production and growth rates. Some problems were encountered in production and growth rates measured in Lizzy Pauls Pond. The movement of *L. trisulca* into the experimental frame influenced the production measurements for this site. Excluding these observations resulted in average production measurements of about 1.0 to 1.7 g/m2/day for Lizzy Pauls Pond and Target Lake, respectively. A similar approach was used to derive the average relative growth rates and yielded values of 0.129 and 0.099/d, respectively, for these two sites. These growth rates were low and substantially less than maximum values reported in the literature (0.25 to 0.35/d). The average growth rates and dissolved nutrient concentrations (TDP and DIN) measured in this study were consistent with laboratory-derived growth versus nutrient concentration relationships derived from laboratory studies reported by others.

Duckweed tissue analysis suggested a significant reduction in N and P in duckweed grown in low nutrient waters of Target Lake. Lowest tissue N and P were 1.3 and 0.19%, respectively, and represented a significant change from the seed source. These N and P contents were noticeably lower than tissue values from previous samples from UMR backwaters with higher water column nutrient concentrations. The C:N ratio (atomic basis) of duckweed grown in Target Lake exceeded 20 in early July and Lake August and exceeded the threshold (15.5) where N limitation is suggested. Anthocyanin pigment development in duckweed grown in Target Lake and relatively low growth rates were additional indicators of N limitation. C:P ratios in duckweed grown in Target Lake were high and approached 500 during similar periods of high C:N. C:P ratios exceeding 258 are suggestive of P limitation. The joint occurrence of high tissue C:P and C:N ratios in duckweed grown in Target Lake during periods in July and August suggest co-limitation of N and P at this site. The tissue composition of duckweed grown in Lizzy Pauls

Pond didn't show a similar response. It is believed the subsurface movement of ambient *L. trisculca* into the experimental frame in Lizzy Pauls Pond biased the tissue composition results. However, when observations related to this occurrence were factored out, the tissue C:P and C:N values from this site also appeared to indicate both N and P limitation.

Acknowledgements

We thank Jeff Houser, USGS, and Paul Garrison, WDNR for their comments on an early version of this report.

References

Analytical Software 2003. Statistix 8. Tallahassee, Florida.

Giblin, S.H. Langrehr, J. Sullivan and K. Hoff. 2010. Evaluation of factors influencing metaphyton abundance and distribution on navigation Pools 4, 8, and 13 of the Upper Mississippi River. Final report submitted to the U.S. Army Corps of Engineers from the U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, November 2010. LTRMP Completion Report 2009D7. 48pp.

Giblin, S.H. , J. Sullivan, J. Houser, H. Langrehr, J. Rogala and B. Campbell. *Submitted*. Temporal and spatial evaluation of factors influencing metaphyton biomass, distribution and composition within Upper Mississippi River backwaters.

Hall, J.A. and N. Cox 1995. Nutrient concentrations as predictors of nuisance *Hydrodictyon reticulatum* populations in New Zealand. J. Aquatic Plant Manage. 33:68-74.

Hillebrand, H. and U. Sommer. 1999. The stoichiometry of benthic microalgal growth: Redfield proportions are optimal. Limnol. Oceanogr. 44:440-446.

Hilton, J. M. O'Hare, M.J. Bowes. and J.I. Jones 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment. 365:66-83.

James, W.F., W.B. Richardson, D.M. Soballe. 2008. Contribution of sediment fluxes and transformations to the summer nitrogen budget of an Upper Mississippi River backwater system. Hydrobiologia 598: 95-107.

Houser, J.N., S.M. Giblin, W.J. James, H.A. Langrehr, J.T. Rogala, J.F.Sullivan and B.R. Gray. *Submitted*. Nutrient cycling and the abundance of duckweed and filamentous algae in backwater lakes of the Upper Mississippi River.

Landolt, E., and R. Kandeler. 1987. The family Lemnaceae - a monographic study, Vol 2. Verhoff. Geobot. Inst. ETH, Stiftung Rubel, Zurich, 638 pp.

Luond, A. Hurlimann-. 1990. The development of some Lemnaceae under different nutrient conditions. Folia Geobotanica et Phytotaxonomica, 25:309-314.

Murkin, H.R., J. B. Pollard, M.P. Stainton, J.A. Boughen and R.D. Titman. 1994. Nutrient additions to wetlands in the Interlake region of Manitoba Canada: effects of periodic additions throughout the growing season. Hydrobiologia. 279/280:483-495.

James, W.F., W.B. Richardson and D.M. Soballe. 2008. Contributions of sediment fluxes and transformations to the summer nitrogen budget of an Upper Mississippi River backwater system. Hydrobiologia 598:95-107.

Redfield, A.C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. James Johnson Memorial Volume. Liverpool: Liverpool University Press. p. 176-92.

Richardson, W.B., E.A. Strauss, L.A. Bartsch, E.M. Monroe, J.C. Cavanaugh, L. Vingum and D. M. Soballe. 2004. Denitrification in the Upper Mississippi River: rates, controls, and contribution to nitrate flux. Can. J. Fish. Aquat. Sci. 61:1102-1112.

Sullivan, J.F. 2008. The use of metaphyton to evaluate nutrient impairment and proposed nutrient criteria for wetlands and backwaters in the Upper Mississippi River. Wisconsin Department of Resources, La Crosse, WI.

Sullivan, J. and S. Giblin. 2011. Continuous dissolved oxygen and water temperature monitoring in Pool 8 backwaters of the Upper Mississippi River May-September, 2010. Mississippi River Team, Wisconsin Department of Natural Resources, La Crosse, WI.

Sterner, R.W. and J.J. Elser. 2002. Ecological stoichiometery: the biology of elements from molecules to the biosphere. Princeton University Pressss, Princeton, New Jersey, USA.

Szabo, S., R. Roijackers, M. Scheffer and G. Borics. 2004. The strength of limiting factors for duckweed during algal competition. Arch. Hydrobiol. 165(1):127-140.

Szabo, S., M. Scheffer, R. Roijackers, B. Waluto, M. Braun, P. Nagy, G. Borics and L. Zambrano. 2010. Strong growth limitation of floating plant (Lemna gibba) by the submerged macrophyte (Elodea nuttallii) under laboratory conditions. Freshwater Biology 55:681-690.

U.S. Environmental Protection Agency 2002. Methods for evaluating wetland condition. #16 Vegetation-based indicators of wetland nutrient enrichment. Office of Water, Washington, DC. EPA-822-R-02-024. 22 pp.

U.S. Geological Survey. 2011. Long Term Resource Monitoring Program Graphical Water Quality Browser – Stratified Random Sampling. Upper Midwest Environmental Sciences Center, La Crosse, WI. http://www.umesc.usgs.gov/data_library/water_quality/graphical/wtr_reaches.html

Upper Mississippi River Basin Association. 2011. Upper Mississippi River Nutrient Monitoring, Occurrence and Local Impacts. A Clean Water Act Perspective. St. Paul, MN. 86 pp.

Verhoeven, J.T.A., Koerselman, W., and Meuleman, A.F.M. 1996. Nitrogen- or Phosphorus-Limited Growth in Herbaceous, Wet Vegetation: Relations With Atmospheric Inputs and Management Regimes. Trends in Ecology & Evolution 11: 494-497.

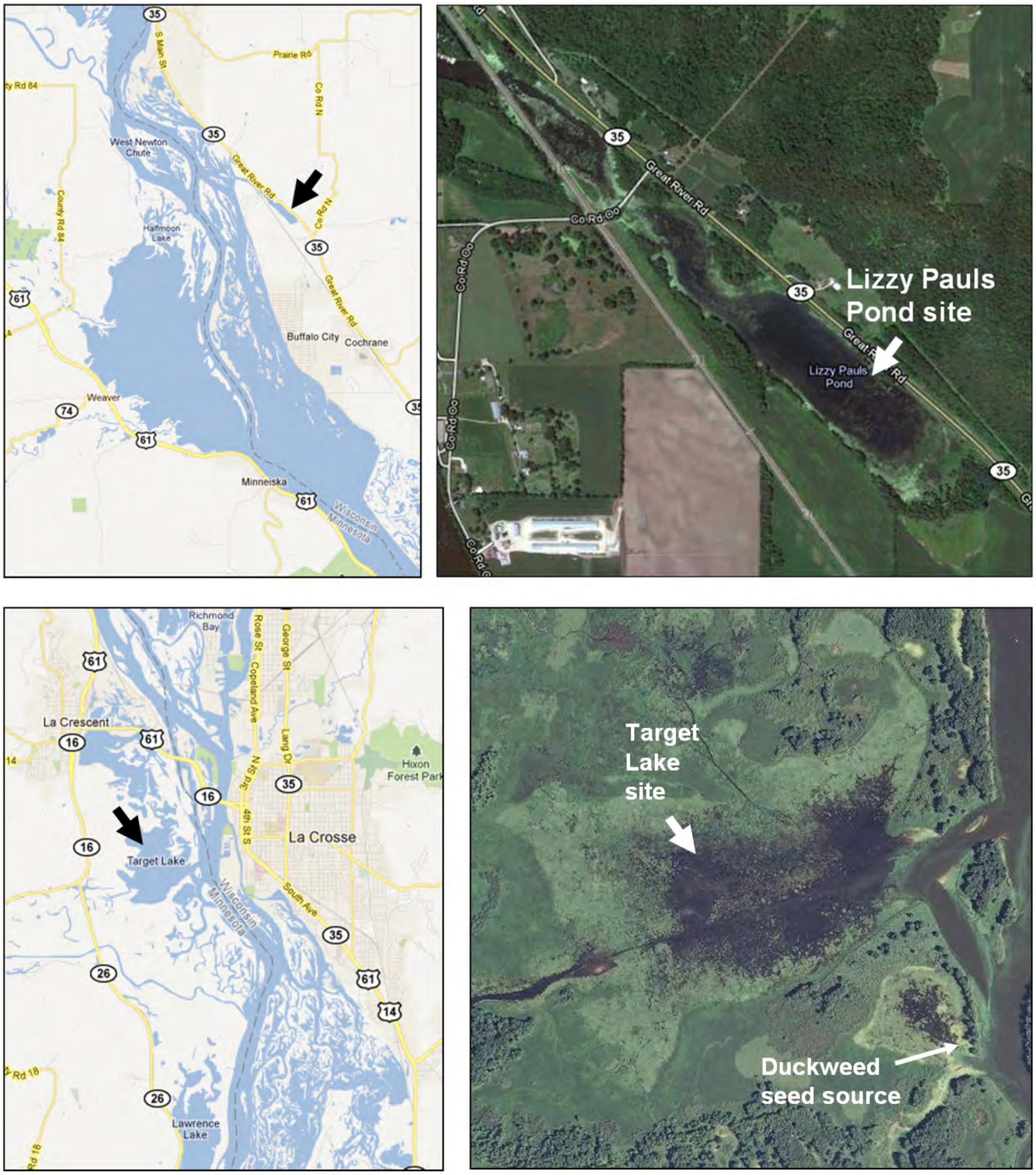


Figure 1. Site location of Lizzy Pauls Pond (top) in Pool 5 and Target Lake (bottom) in Pool 8 of the Upper Mississippi River.

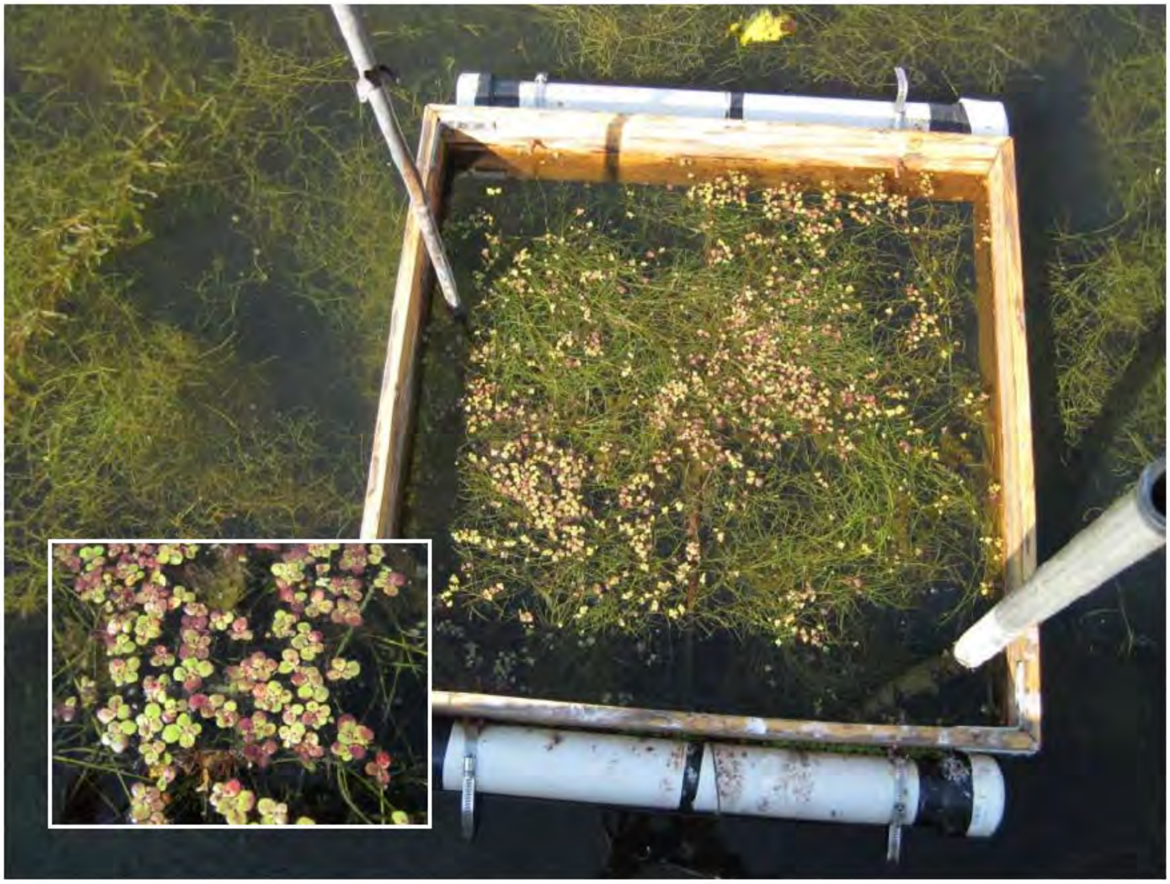


Figure 2. Experimental frame used to measure duckweed growth. This photo was taken at the Target Lake site on July 8, 2011. The insert photo is a close-up of duckweed (*Spirodela polyrrihiza*) found in the frame on this date. The submersed aquatic vegetation observed in the main photo was dominated by *Potamogeton pectinatus*.

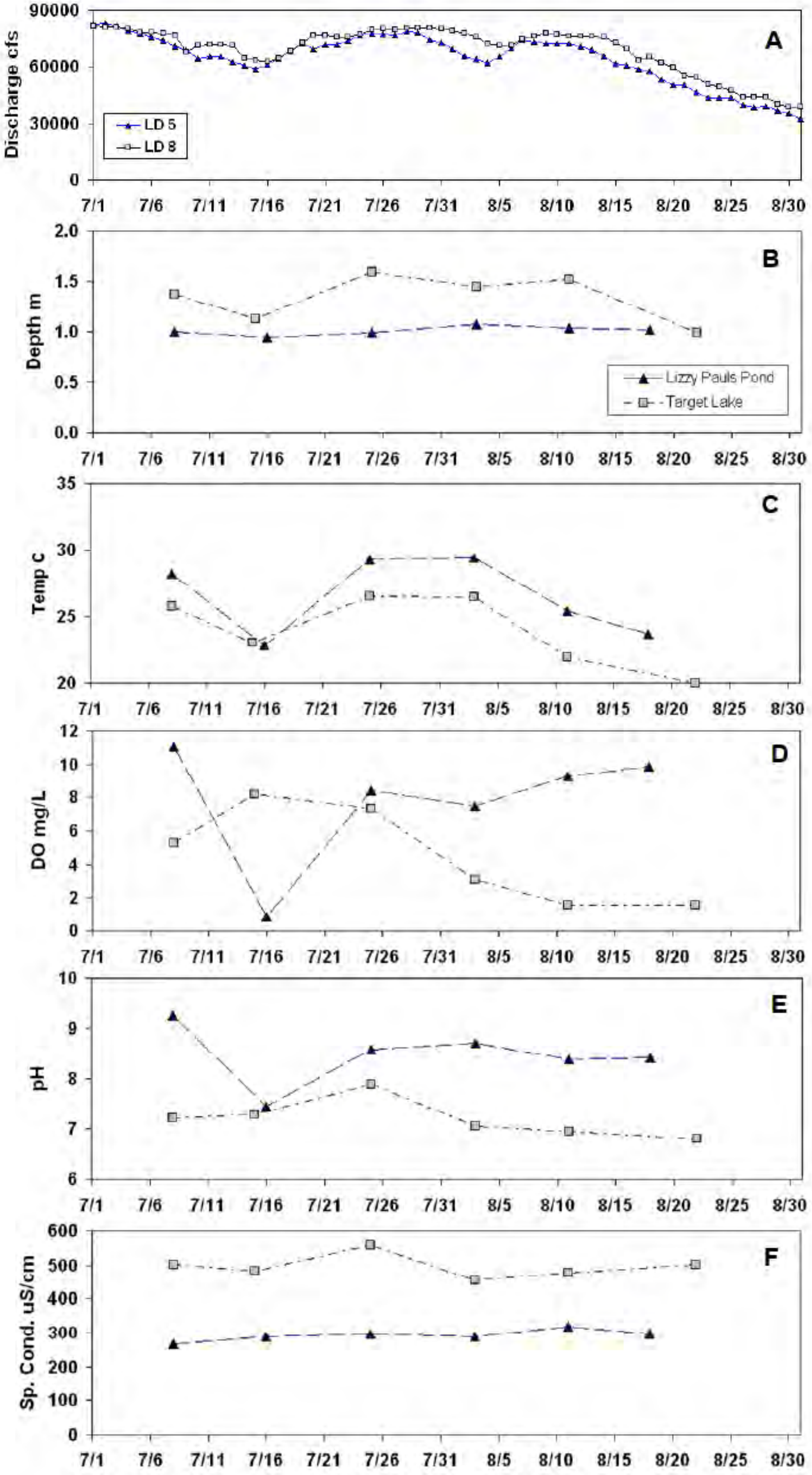


Figure 3. Field and laboratory measurements from Lizzy Pauls Pond (Pool 5) and Target Lake (Pool 8) in the Upper Mississippi River in the summer of 2011.

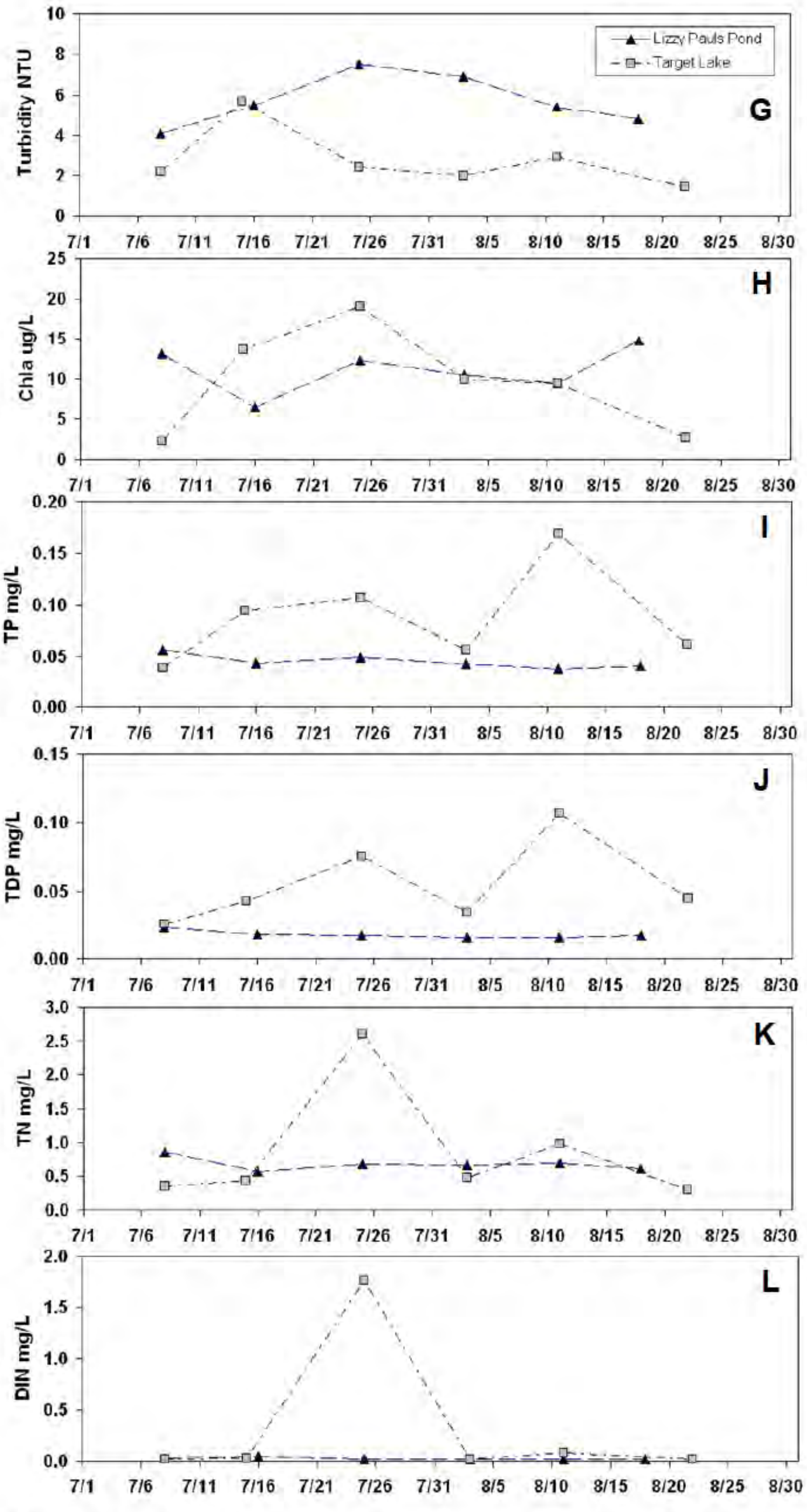


Figure 3 (continued).

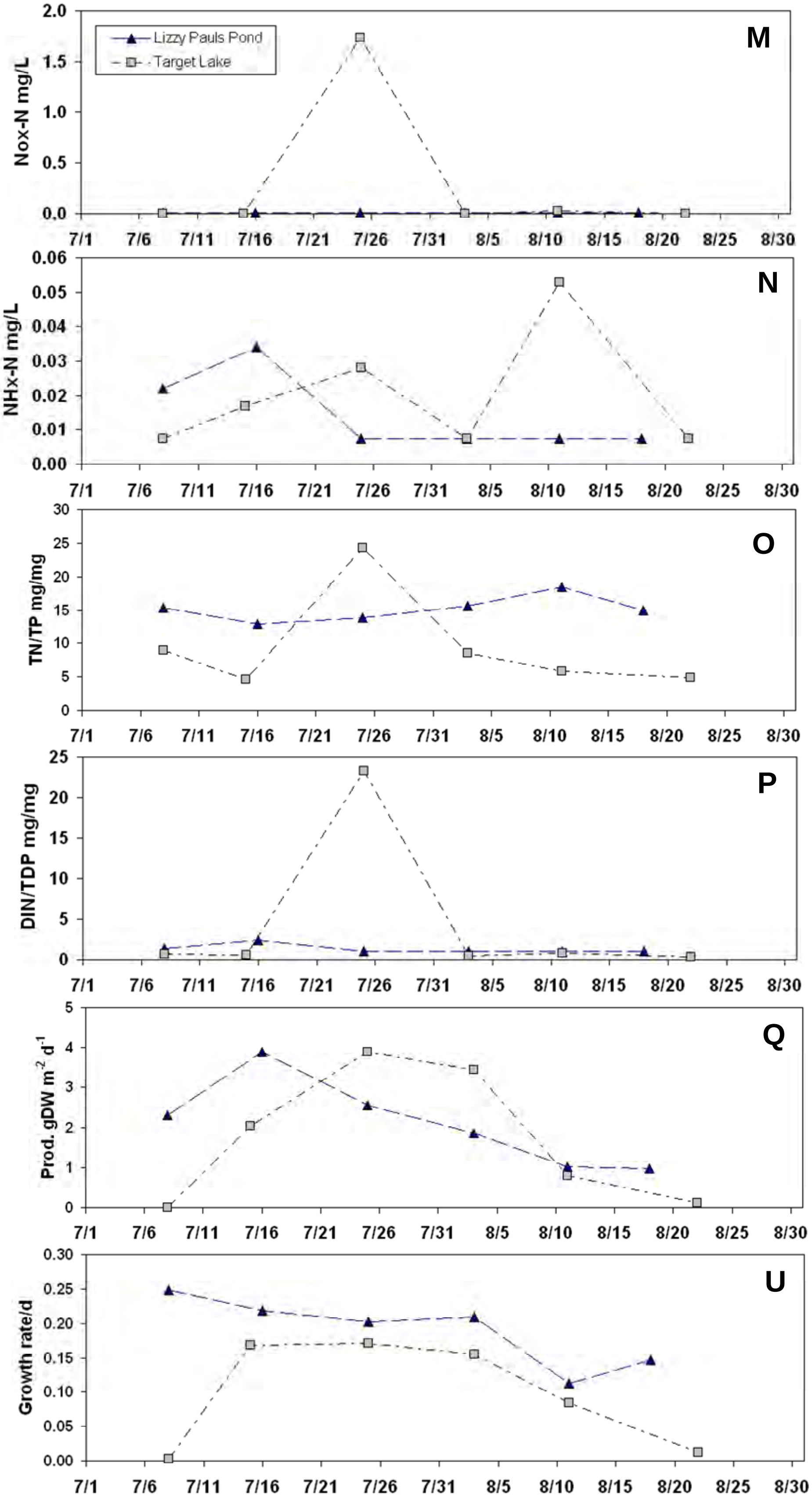


Figure 3 (continued).

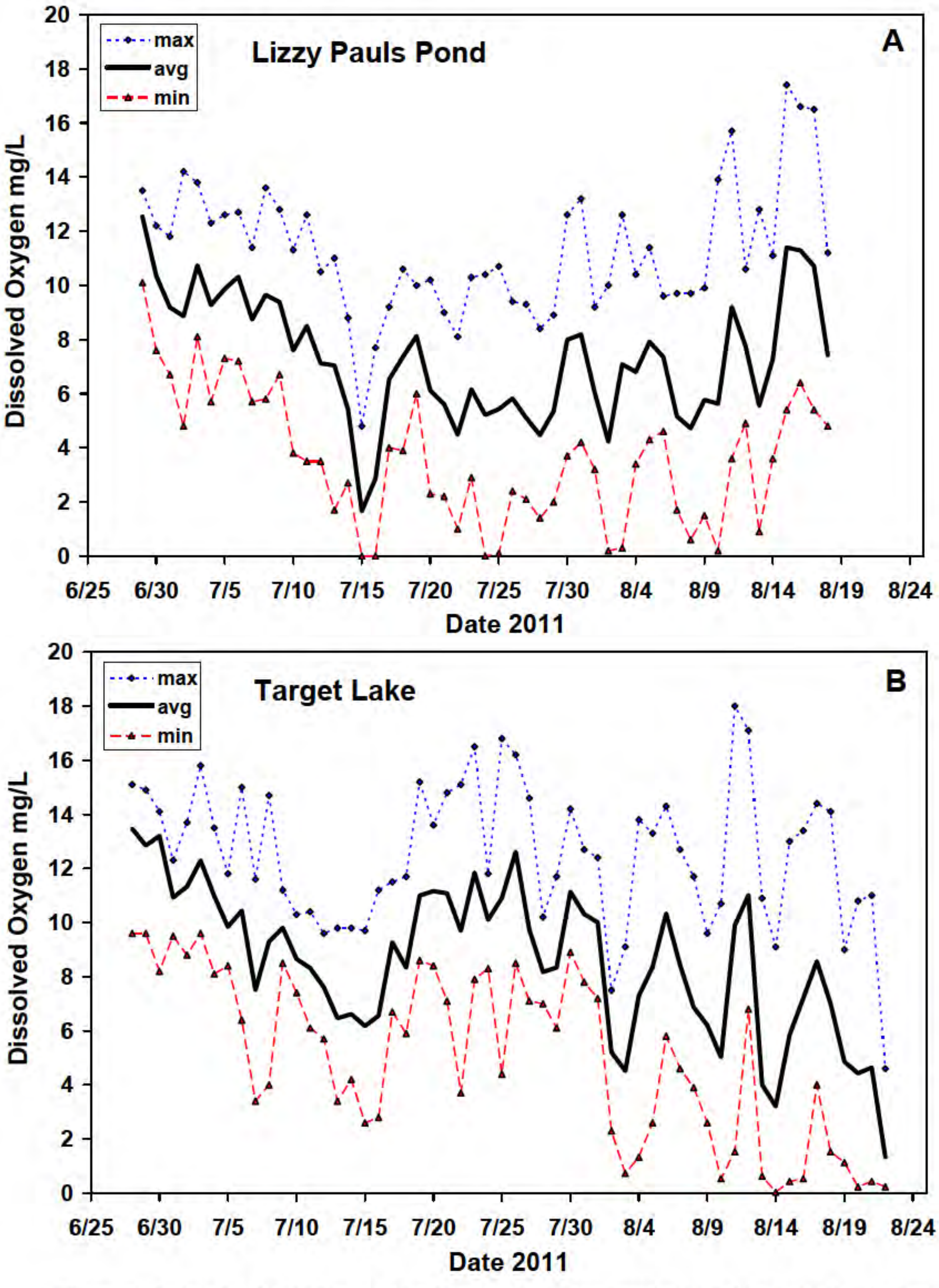


Figure 4. Summary of continuous dissolved oxygen measurements collected in Lizzy Pauls Pond (**A**) and Target Lake (**B**) of the Upper Mississippi River. Data are daily maximum, average and minimum values based on a 15-minute logging interval.

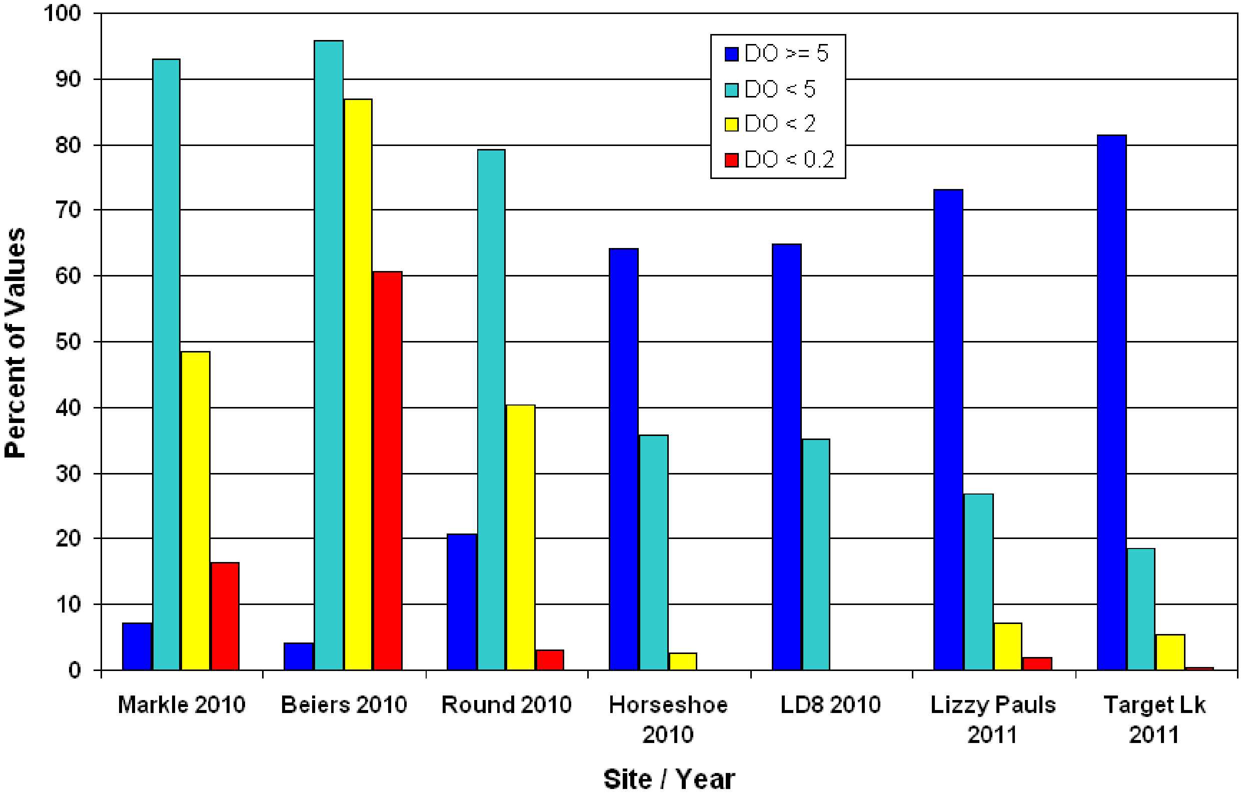


Figure 5. Frequency of occurrence of various dissolved oxygen (DO) concentrations (>=5, <5, < 2 and <0.2 mg/L) based on continuous dissolved oxygen measurements in Upper Mississippi River backwaters and the main channel (Lock and Dam 8) in July-August 2010 and 2011 (this study).

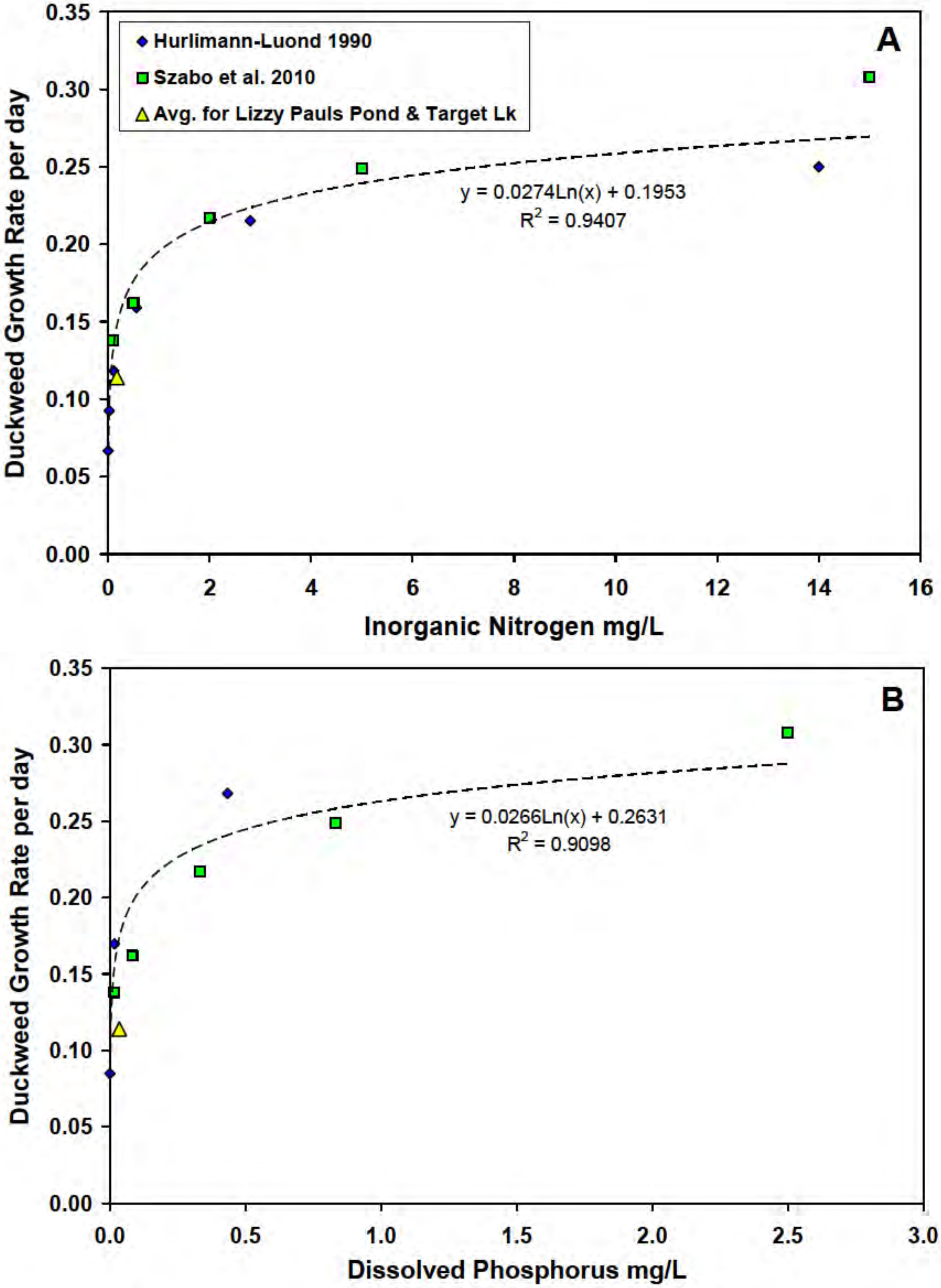


Figure 6. Duckweed growth rate (natural log) versus dissolved inorganic nitrogen (**A**) and dissolved phosphorus (**B**) derived from laboratory measurements and from average measurements made in Lizzy Pauls Pond and Target Lake.

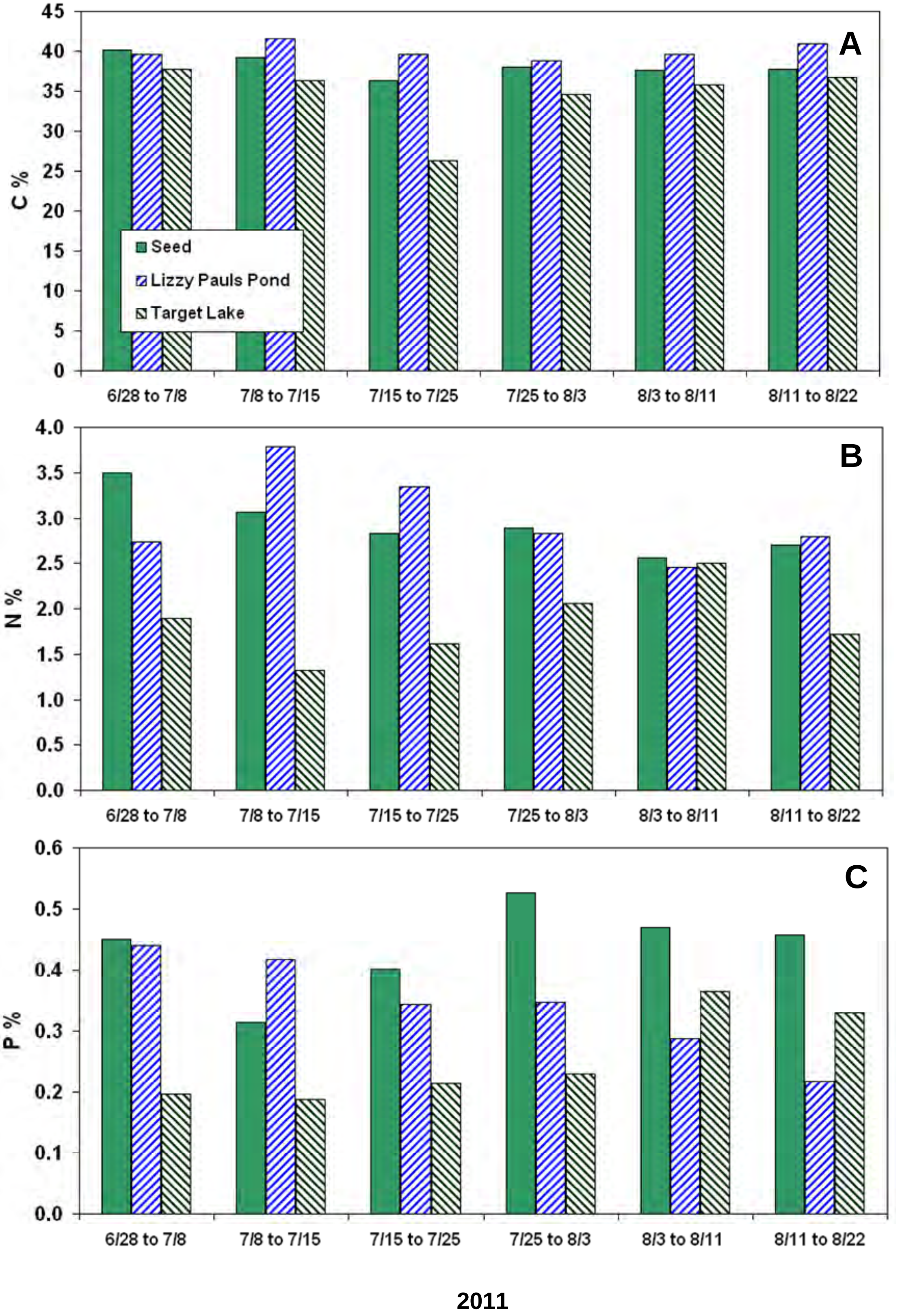


Figure 7. Percent carbon (**A**), nitrogen (**B**) and phosphorus (**C**) content in duckweed tissue samples collected from the seed source, Lizzy Pauls Pond and Target Lake during the summer of 2011.

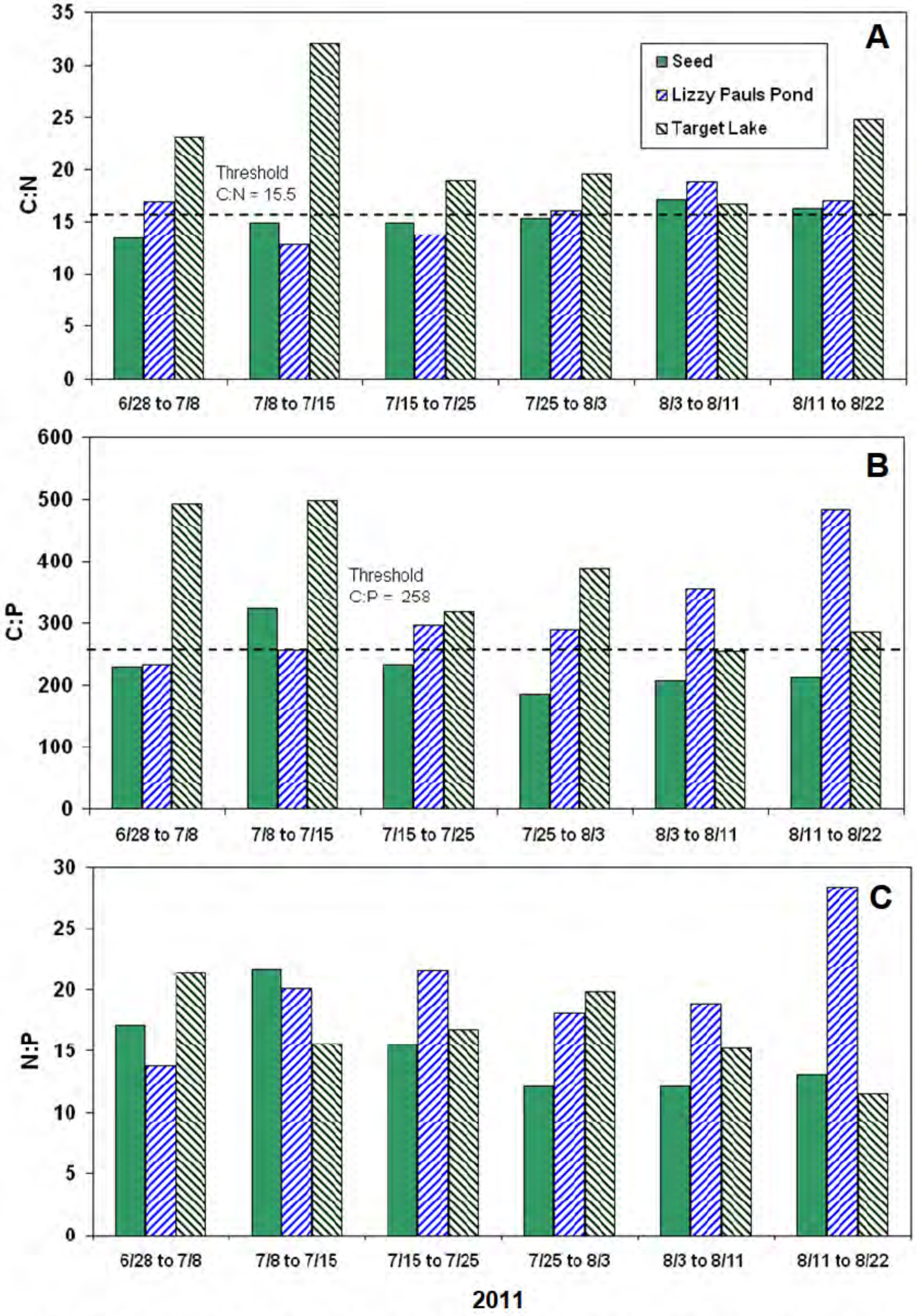


Figure 8. Carbon to nitrogen (**A**), carbon to phosphorus (**B**) and nitrogen to phosphorus ratios (**C**) measured in duckweed tissue samples collected from the seed source, Lizzy Pauls Pond and Target Lake during the summer of 2011. Ratios are expressed on an atomic basis.

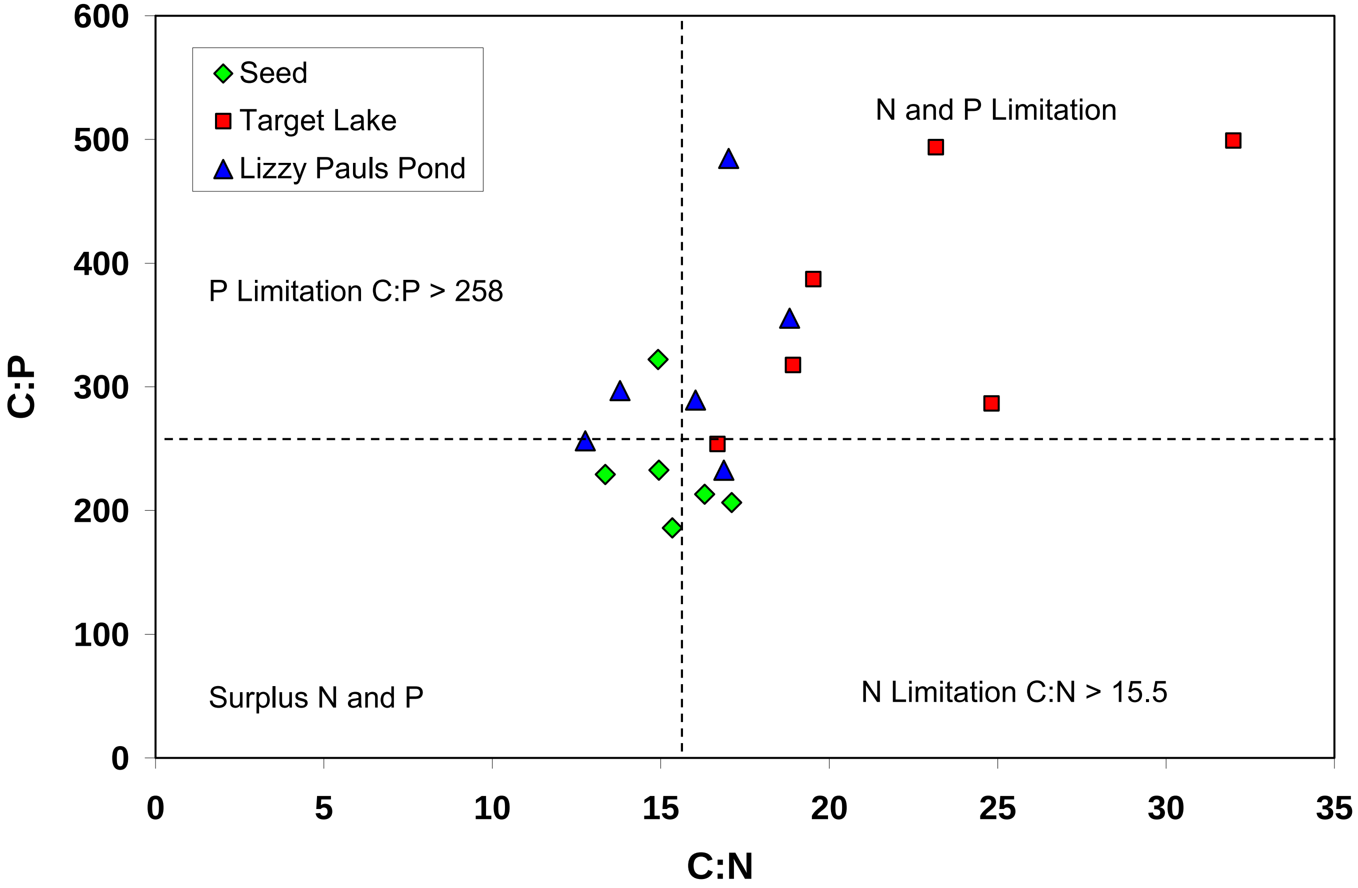


Figure 9. Carbon to phosphorus versus carbon to nitrogen ratios (atomic basis) of duckweed tissue samples collected from the seed source, Target Lake and Lizzy Pauls Pond during the summer of 2011. Thresholds suggesting phosphorus and nitrogen limitation were derived from Murkin et al.1994.

Table 1. Summary of field and laboratory water quality measurements collected during duckweed growth measurements made in Lizzy Pauls Pond and Target Lake during the summer of 2011. Underline values represent estimated values derived by assigning concentrations one-half the laboratory detection limit.

	Lizzy Pauls Pond					**Target Lake**				
Measurement	**N**	**Median**	**Avg.**	**Min.**	**Max.**	**N**	**Median**	**Avg.**	**Min.**	**Max.**
Depth m	6	1.01	1.01	0.95	1.07	6	1.41	1.34	0.99	1.59
Dissolved Oxygen mg/L	6	8.9	7.9	0.9	11.1	6	4.2	4.5	1.6	8.2
Dissolved Oxygen mg/L[1]	4666	7.2	7.1	0.0	17.4	5027	8.9	8.4	0.3	18.0
Temperature C	6	26.8	26.5	26.5	29.4	6	24.4	23.9	20.0	26.5
Temperature C[1]	4666	27.8	28.0	21.9	35.8	5027	26.8	26.6	19.9	33.4
Sp. Conductivity uS/cm	6	294	294	294	320	6	491	496	456	559
pH	6	8.50	8.47	7.46	9.25	6	7.14	7.20	6.80	7.89
Turbidity NTU	6	5.5	5.7	4.1	7.5	6	2.3	2.8	1.4	5.7
Chlorophyll *a* ug/L	6	11.4	11.1	6.5	14.8	6	9.6	9.5	2.3	19.1
Ammonia-Nitrogen mg/L	6	0.008	0.014	0.008	0.034	6	0.012	0.020	0.008	0.053
Nitrite+Nitrate-Nitrogen mg/L	6	0.009	0.009	0.009	0.009	6	0.009	0.300	0.009	1.74
Inorganic Nitrogen mg/L	6	0.024	0.017	0.017	0.044	6	0.022	0.321	0.017	1.77
Total Kjeldahl-Nitrogen mg/L	6	0.67	0.67	0.56	0.85	6	0.45	0.56	0.29	0.96
Total Organic Nitrogen mg/L	6	0.66	0.66	0.53	0.83	6	0.44	0.54	0.28	0.91
Total Nitrogen mg/L	6	0.67	0.68	0.57	0.86	6	0.46	0.86	0.30	2.60
Total Diss. Phosphorus mg/L	6	0.017	0.018	0.016	0.023	6	0.044	0.055	0.026	0.11
Particulate Phosphorus mg/L	6	0.027	0.027	0.022	0.033	6	0.026	0.033	0.013	0.062
Total Phosphorus mg/L	6	0.044	0.045	0.038	0.056	6	0.079	0.088	0.039	0.17
Inorg. N / Dissolved P Ratio	6	1.1	1.3	1.0	2.4	6	0.7	4.4	0.4	23.3
Total N / Total P Ratio	6	15.1	15.2	12.9	18.4	6	7.2	9.5	4.6	24.3
Duckweed production g/m^2 dw	6	2.08	2.10	0.98	3.90	6	1.42	1.72	0.01	3.89
Duckweed growth rate/day	6	0.206	0.189	0.112	0.248	6	0.120	0.099	0.003	0.171

[1] Continuous monitoring results for July and August using a 15-minute logging interval.

Table 2. Field water quality measurements collected at Lizzy Pauls Pond (Pool 5) and Target Lake (Pool 8) of the Upper Mississippi River in the summer of 2010. Underline values in bold represent concentrations that differed significantly ($p<0.05$) with depth (top versus bottom) based on a two-sample t-test ($p<0.05$).

Site	**Sample Location**	**Sample Depth m**	**Temp. C**	**Dissolved Oxygen mg/L**	**pH**	**Specific Conductance us/cm**
Lizzy Pauls Pond	Surface	0.15	26.4	<u>**7.2**</u>	8.31	298
n= 7	Middle	0.46	25.3	5.8	7.97	311
	Bottom	0.91	24.3	<u>**2.9**</u>	7.56	329
	Diff: Bot-Surf	0.76	-2.1	-4.3	-0.75	31
	%change		-8.0	-59.4	-9.0	10.4
Target Lake n= 7	Surface	0.15	24.7	<u>**5.6**</u>	7.40	498
	Middle	0.63	24.2	4.3	7.25	506
	Bottom	1.24	23.6	<u>**1.4**</u>	6.99	525
	Diff: Bot-Surf	1.09	-1.1	-4.2	-0.41	27
	%change		-4.5	-74.6	-5.5	5.4

Table 3. Duckweed biomass, production and growth rate (natural log) measurements made in experimental frames deployed in Lizzy Pauls Pond (Pool 5) and Target Lake (Pool) of the Upper Mississippi River in the summer of 2011.

Start Date	End Date	Duration Days	Backwater Area	Start Biomass g wet wt.	% dry wt.	g dry wt	Dom. Taxa	End Biomass g wet wt.	% dry wt.	g dry wt	Dom. Taxa	Production g dw/m2/d	Growth rate/d
06/29/11	07/08/11	9	Lizzy Pauls P.	19	7.8	1.5	S. polyrhiza	238	5.9	13.9	S. poly, L. tri	2.30	0.248
07/08/11	07/16/11	8	Lizzy Pauls P.	57	6.9	4.0	S. polyrhiza	312	7.3	22.7	L. trisulca	3.90	0.218
07/16/11	07/25/11	9	Lizzy Pauls P.	33	8.1	2.7	S. polyrhiza	208	7.9	16.4	S. poly, L. tri	2.55	0.202
07/25/11	08/03/11	9	Lizzy Pauls P.	23	7.8	1.8	S. polyrhiza	149	7.9	11.8	L. trisulca	1.85	0.209
08/03/11	08/11/11	8	Lizzy Pauls P.	31	10.9	3.4	L. minor	110	7.6	8.3	S. polyrhiza	1.02	0.112
08/11/11	08/18/11	7	Lizzy Pauls P.	29	8.0	2.3	mix	82	7.8	6.4	S. polyrhiza	0.98	0.146
			Avg:	32	8.3	2.6		183	7.4	13.3		2.10	0.189
06/28/11	07/08/11	10	Target Lake	33	7.8	2.5	S. polyrhiza	27	9.6	2.6	S. polyrhiza	0.01	0.003
07/08/11	07/15/11	7	Target Lake	54	6.9	3.8	S. polyrhiza	108	11.4	12.3	S. polyrhiza	2.03	0.169
07/15/11	07/25/11	10	Target Lake	64	8.1	5.2	S. polyrhiza	246	11.6	28.5	S. poly, L. min	3.89	0.171
07/25/11	08/03/11	9	Target Lake	79	7.8	6.2	S. polyrhiza	277	8.9	24.8	S. polyrhiza	3.45	0.155
08/03/11	08/11/11	8	Target Lake	37	10.9	4.0	L. minor	100	7.9	7.9	L. min, S. poly	0.80	0.084
08/11/11	08/22/11	11	Target Lake	73	8.0	5.8	mix	80	8.2	6.6	S. polyrhiza	0.12	0.012
			Avg:	57	8.3	4.6		140	9.6	13.8		1.72	0.099

Table 4. Plant tissue analysis of duckweed samples collected during duckweed growth and production measurements in Upper Mississippi River backwaters during the summer of 2011. A Kruskal-Wallis AOV applied to the ranks was used to test for significant differences ($p<0.05$) between sites. Groups that differed significantly from each other are indicated in bold. Letters designate groups with similar means.

Plant Tissue	Seed Source				Lizzy Pauls Pond				Target Lake				Kruskal-Wallis AOV
Measurement	N	Avg.	Min.	Max.	N	Avg.	Min.	Max.	N	Avg.	Min.	Max.	P
N %	6	**2.92 ab**	2.57	3.50	6	**2.99 a**	2.45	3.79	6	**1.85 b**	1.32	2.50	**0.0005**
P %	6	**0.44 a**	0.31	0.53	6	**0.34 ab**	0.22	0.44	6	**0.25 b**	0.19	0.36	**0.0040**
C %	6	**38.16 ab**	36.30	40.10	6	**40.02 b**	38.85	41.52	6	**34.56 a**	26.28	37.73	**0.0001**
Ca %	6	**3.72 ab**	2.27	4.90	6	**3.22 b**	2.42	4.22	6	**6.79 a**	4.76	13.18	**0.0005**
K %	6	**3.13 a**	2.70	3.49	6	**2.00 b**	1.84	2.19	6	**2.01 b**	1.00	2.74	**0.0005**
Na %	6	**0.76 ab**	0.74	0.97	6	**0.92 a**	0.99	1.02	6	**0.55 b**	0.58	0.66	**0.0002**
Mg %	6	0.73	0.67	0.83	6	0.79	0.72	0.87	6	0.73	0.61	0.94	0.2105
S %	6	0.41	0.36	0.46	6	0.33	0.21	0.50	6	0.36	0.27	0.43	0.1162
C:N atomic	6	**15.3 b**	13.4	17.1	6	**15.9 ab**	12.8	18.8	6	**22.5 a**	16.7	32.0	**0.0030**
C:P atomic	6	**231 b**	186	322	6	**319 ab**	232	484	6	**373 a**	254	499	**0.0132**
N:P atomic	6	15.3	12.1	21.6	6	20.1	13.8	28.5	6	16.7	11.5	21.3	0.1917
Al ug/g	6	**634 a**	179	1617	6	**102 b**	40	163	6	**238 ab**	90	503	**0.0015**
B ug/g	6	535	383	648	6	654	559	732	6	455	297	677	0.0589
Cu ug/g	-	-	-	-		15.0	8.9	20.6	-	-	-	-	-
Fe ug/g	6	**1525 b**	886	2460	6	**3591 a**	1744	5386	6	**1425 b**	667	2005	0.0020
Mn ug/g	6	**3630 ab**	2550	4962	6	**6107 a**	2553	10487	6	**1870 b**	1156	2715	**0.0004**
Zn ug/g	-	-	-	-	6	65.1	44.5	87.9	-	-	-	-	-