Inter-Agency Agreement to Conduct Scientific Studies Relevant to the Stormwater Treatment Areas

Agreement No. 4600003125

SUBMITTED: September 3, 2015

PREPARED FOR: South Florida Water Management District, and
Everglades Agricultural Area Environmental Protection District

PREPARED BY: DB Environmental, Inc.

Introduction

There are potential benefits to shallow water depths in PSTA systems, including increased light penetration to the benthic surface. However, because shallow water depths may be difficult to establish and maintain in full-scale STA flow paths, particularly under pulse loading conditions, the effects of water depth on PSTA performance must be better defined. Prior research with PSTA systems operated at 30 or 60 cm water depths showed no difference in phosphorus (P) removal performance, although static depth treatments outperformed variable depth treatments (CH2M-Hill 2003). The best performing experimental PSTA platforms were operated at 30 cm (STA-1W 0.2 ha test cells and mesocosms) and 9 cm (STA-1W mesocosm raceways, DeBusk et al. 2004). The STA-3/4 PSTA Cell typically has operated at a depth of 30-45 cm, with high flow events increasing average water depths to just over 60 cm.

The ability of these prior PSTA research efforts to define suitable water depth ranges has been limited in some cases by a lack of replication, and in other instances by the inability to produce ultra-low outflow TP concentrations. To overcome these limitations and provide insights into the effects of water depth on surface water TP concentrations, periphyton communities and P removal mechanisms in PSTA systems, we currently are operating a replicated outdoor mesocosm study using periphyton and macrophytes from the STA 3/4 PSTA Cell.

The current report focuses on the biological response to fluctuations in water depth and along nutrient gradients. The relative densities of macrophytes and periphyton were measured and the enzyme activity of the benthic periphyton layer was assayed as part of this evaluation. Phosphorus removal performance, TP concentrations and other water quality characteristics of these PSTA mesocosms were previously described in a recent report (DBE 2015). Nutrient contents of the macrophyte and periphyton tissues are currently being determined; those results will be presented in a future report.

Methods

Experimental Design

Operational "boundaries" of PSTA systems are being investigated in mesocosms at an experimental facility near the outflow of STA-1W. Triplicate flow ways with a local limerock substrate were established under each of four water depth treatments. The first two treatments are static in depth. Shallow treatments (23 cm) and deeper treatments (46 cm) consist of 4 tanks (each 1.8 m^2) plumbed in series. These tanks were initially established in September 2013, under constant flows that provide a hydraulic retention time (HRT) of 5 and 10 days for the shallow (23 cm) and deep (46 cm) flow ways, respectively. Delivery of a constant flow rate to both shallow and deep tanks insures equal P mass loading rate (PLR) to those treatments on an area basis.

In January 2014, additional mesocosms were established to test PSTA performance at greater water depths. Six new flow ways were constructed using larger tanks (2.8 m^2 per tank) plumbed two in series (Figure 1 and Figure 2). These systems were initially established at 46 cm depth, and flows are being delivered to provide equivalent HRT and PLR conditions to the existing mesocosms operating with 4 tanks-in-series at 46 cm depth. The first tanks in series of the new flow ways receive an equivalent PLR to the first half (first 2 tanks) of the 4-in-series systems. This approach enables a comparison of "midpoint" and "outflow" positions with equivalent HLR and P loading across static and variable-depth treatments. Key operational parameters of these systems are outlined in Table 1.

Figure 1. Mesocosms were established at a range of water depths to explore the effects of operating conditions on P removal effectiveness and biological community response. The shorter tanks (to the lower left) were plumbed 4 in-series at water depths of 23 or 46 cm. The larger mesocosms were plumbed 2 in-series and were also established at 46 cm, before transitioning to 69 cm or 92 cm water depths.

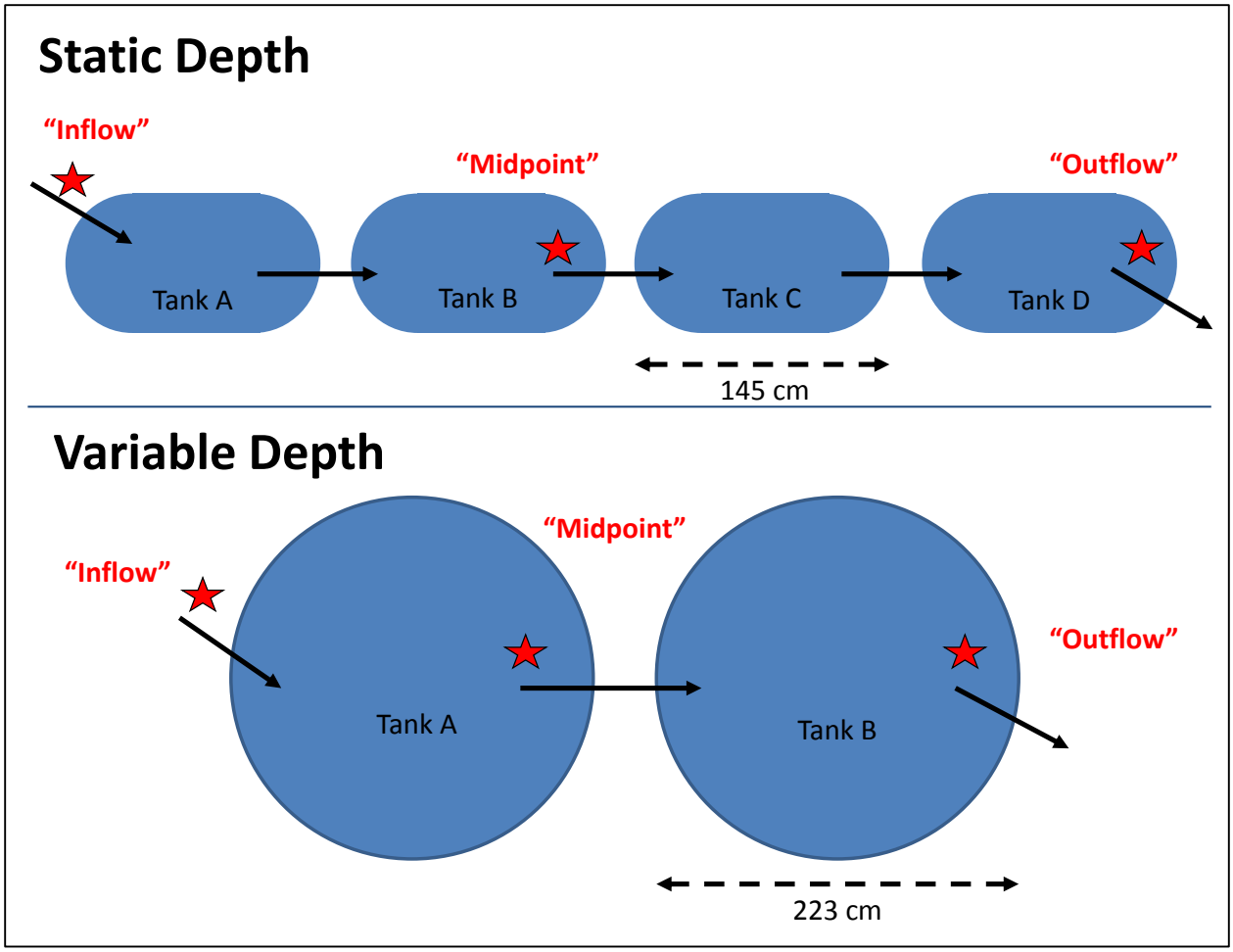

Figure 2. Process train configuration for static depth and variable depth mesocosms. Also shown are the surface water sampling locations along each flow way. Benthic samples were collected throughout the tanks to avoid resampling areas affected by earlier sampling efforts.

After an initial phase of comparable operations, the newer mesocosms were assigned to two new variable water depth treatments (46-69 cm and 46-92 cm). The transition to deeper conditions began after the water sampling on 5/29/2014 (Figure 3). On 9/15/2014, water depths were lowered back to 46 cm in the variable depth treatments, and the "shallow" conditions continued until January 15, 2015. Since January, water depths have been maintained at 69 and 92 cm in the respective variable-depth treatments to examine the P removal performance and periphyton community response to longer-duration deep water conditions.

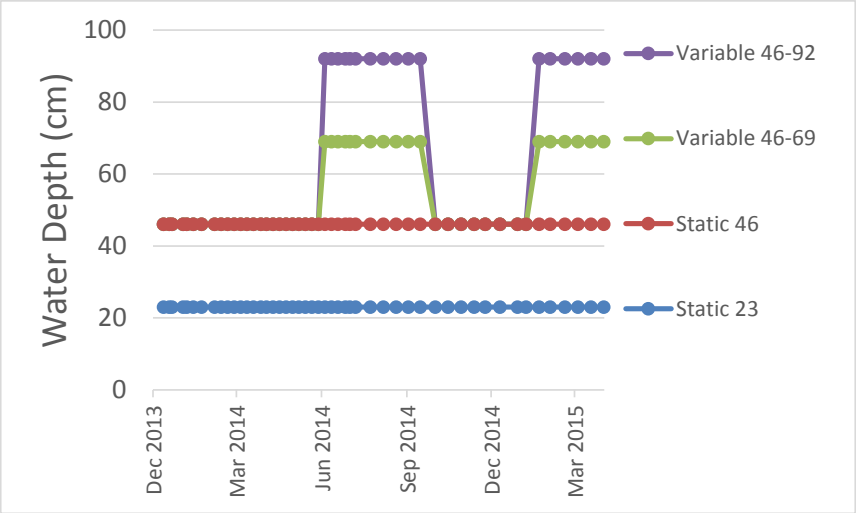

Figure 3. Water depth schedules for each of four depth treatments during the monitoring period (December 12, 2013 – April 1, 2015).

Table 1. Operational targets for experimental flow ways assigned to one of four depth treatments.

Static Depth Treatments

	Tank			
	A	B	C	D
HLR (m/day)	0.182	0.091	0.061	0.046
PLR at 20 ppb (g P/m^2/yr)	1.33	0.67	0.44	0.33
Water Depth	HRT (days)			
23 cm	1.3	2.5	3.8	5.0
46 cm	2.5	5.0	7.5	10.0

Variable Depth Treatments

	First Tank	Last Tank
HLR (m/day)	0.091	0.046
PLR at 20 ppb (g P/m^2/yr)	0.67	0.33
Water Depth	HRT (days)	
46 cm	5	10
69 cm	7.5	15
92 cm	10	20

Measurement of Light at the Benthic Surface

Algal growth is strongly controlled by the amount of light available for photosynthesis. The intensity of photosynthetically-active radiation (PAR) at the benthic surface was determined by placing a spherical underwater quantum sensor (LiCOR, Lincoln, NE) in a recess within the outflow region of each process train, so that the sensor was even with the benthic surface (Figure 4). The PAR available at the benthic surface was determined as a percent of ambient levels, as measured above each tank. Measurements were made twice monthly from March 20, 2014 through June 18, 2014, then once monthly beginning in July 2014. The period of record in this report includes all measurements through May 27, 2015 (N = 18 events).

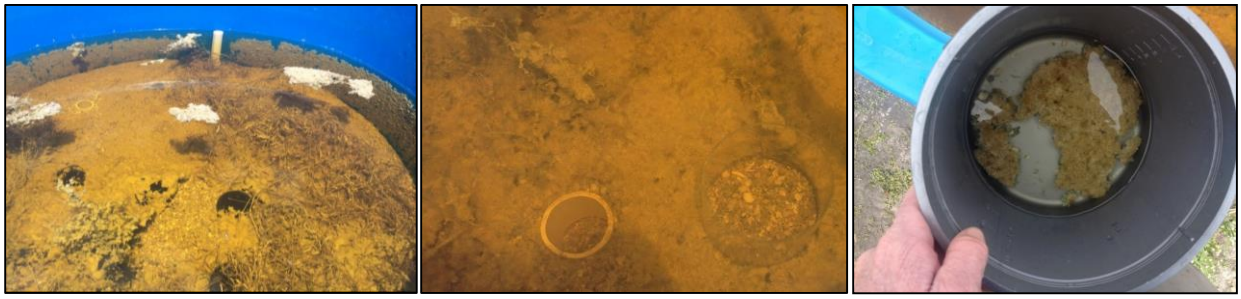

Figure 4. Periphyton in mesocosms has developed on the benthic surface, as well as tank walls, plant surfaces, and in some areas, benthic mats have separated to form floating mats (left image). The middle image shows a sampling collar used to define the area for benthic periphyton sampling, and a recess for measuring photosynthetically active radiation (PAR) at the benthic surface. The benthic periphyton mat shown in the left image was removed from the outflow region of a mesocosm on May 29, 2014.

Benthic periphyton sampling in outdoor mesocosms

Following initiation of water flows, benthic periphyton mats developed readily on the limerock mesocosm substrates. In order to compare biomass growth across depth treatments, we measured areal biomass (dry mass of periphyton per unit area of benthic surface) on three sampling dates: May 29, 2014 (prior to first deep phase); September 16, 2014 (end of 1st deep phase); and January 15, 2015 (prior to 2nd deep phase). Benthic samples were collected from each tank on each of the three sampling dates, with the following exception: static depth "B" and "C" tanks were only sampled from one replicate in May 2014.

A clean plastic bucket was submerged into the mesocosm water column next to the target sampling area. A sampling collar (14.6 cm inside diameter) was inserted into the limerock substrate. Periphyton was carefully transferred from the benthic surface by hand into the submerged bucket. Limerock gravel was also transferred, as necessary, to ensure that all periphyton was included in the sample. For each location, two grab samples were collected and

composited into one sample representing a total benthic surface area of 335 cm^2. In contrast to water sampling efforts (see Figure 2), benthic samples were collected throughout the tanks to avoid resampling areas affected by earlier sampling efforts.

Periphyton Processing

In the laboratory, excess site water from each settled sample was decanted. Gravel was rinsed free of periphyton, using DI water as rinsate in a large beaker. The sample was stirred so that periphyton fragments suspended, while the gravel settled to the bottom of the beaker. Then, the suspension was transferred into the mixer (leaving the gravel behind in the beaker). Once the gravel was isolated from the bulk of the algal material, DI was added to the beaker and swirled to dislodge any remaining algal material from the gravel. While the algae was in suspension, the liquid and algae was poured off while holding back the gravel. The gravel was inspected for any remaining periphyton fragments, then discarded. Once the gravel was removed, the algal suspension was settled, then overlying water was decanted, taking care to retain all particles.

The sample was gently stirred with a metal spoon, then "unhomogenized" samples (i.e., not blended) were transferred into 2 clean scintillation vials. The samples were then thoroughly homogenized in a food mixer. The homogenized slurry was again sub-sampled for enzyme assay (20 mL) and "homogenized" taxonomy samples (2 vials, each 20 mL). Another subsample of the remaining slurry (150 mL) was transferred into a pre-weighed plastic cup and dried to constant weight at 65°C. The remaining sample was transferred to a graduated cylinder to record the remaining volume. Periphyton samples were preserved in 4% formalin buffered with sodium borate for taxonomic identification and biovolume determination.

Enzyme Assay

Periphyton samples were diluted by dispensing a known volume of the enzyme subsample into a pre-weighed centrifuge tube, and adding DI as necessary, to achieve appropriate hydrolysis rates for the assays. The remainder of the diluted sample, once the enzyme assay was complete, was then dried to constant weight to calculate the enzyme assay bulk density. Results of the assay were normalized to dry weight of periphyton in the slurry as assayed.

Benthic periphyton enzyme activity was converted to µmol MUF released/cm^2/hr, using the APA per unit weight, and the dry weight biomass of periphyton per unit area ("areal biomass"). Taxonomic samples were archived and will be prioritized for identification at the conclusion of the study.

Relative Density and Species Composition of Macrophytes and Periphyton Communities in Outdoor Mesocosms

The relative density of SAV species (*Chara* and *Potamogeton*) was recorded on a 0-5 scale, with 5 indicating very dense macrophyte beds. This method was developed for use in the full-scale STA flow ways, and was adapted for mesocosm research to avoid the disturbances associated

with sampling biomass from these small systems. A similar density score was also recorded for benthic, floating, and epiphytic periphyton in each mesocosm. These scores were recorded each month to document changes in the species composition and relative density of macrophytes and periphyton during the establishment of flow-through PSTA mesocosms. Relative density values were averaged for the 8-9 locations within each tank, on each sampling date. These values were compared across replicates under each treatment to calculate a standard error for each treatment.

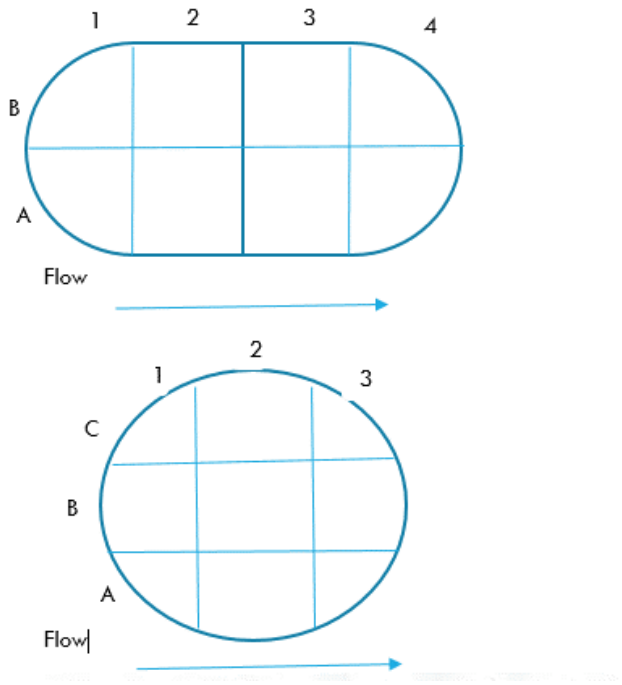

Figure 5. Sampling grid used for relative macrophyte density evaluations.

Results

Comparisons of biological response variables were made between the static depth treatments at 23 and 46 cm for each position down the four-in-series flow ways. Then, as a separate comparison, our variable depth treatments were compared for periods of shallow and deep operations. These comparisons across depth treatments were made for both the first tanks and final tanks in series.

Light conditions as a function of depth

The available PAR at the benthic surface was highest in the shallow treatments, and lowest in the depth-variable treatments (Figure 6). During shallow-phase operations, PAR levels were

similar between the two variable depth treatments operating at 46 cm, while under deep-phased operations, the increased water depth in the 46-92 cm water depth treatment reduced PAR to 33% of incident levels, as compared to 65-70% in the shallow 23 cm treatment.

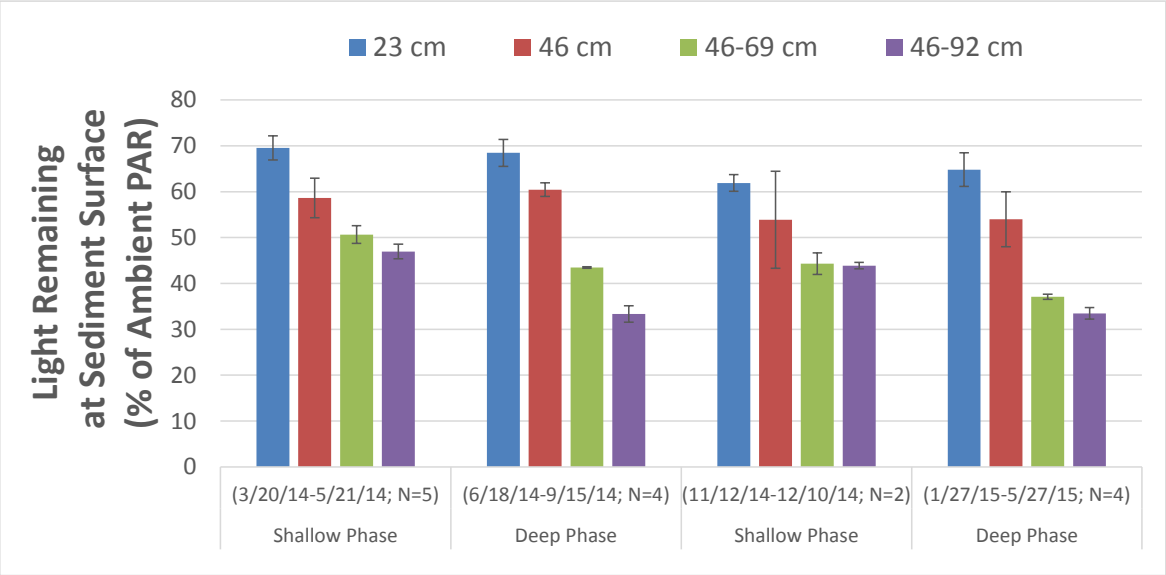

Figure 6. Light remaining at the sediment surface, as a fraction of the ambient light available above the water column, in the outflow region of mesocosms operated under four depth treatments. Error bars denote ± SE around period averages for triplicate flow ways under each treatment. Light was measured in the photosynthetically-active range (400-700 nm) during midday.

Areal Biomass of Benthic Periphyton

On May 29, 2014, the static depth mesocosms had been in flow-through operations for 9 months. Biomass of benthic periphyton remained low in the inflow region of mesocosms (1.3 ± 0.4 g/m^2) at both 23 cm and 46 cm. However, downstream tanks supported higher levels, especially at 23 cm depth (Figure 7). Repeated sampling of these communities in September 2014 and January 2015 showed increasing biomass over time for a given position along the gradient. One exception was the second tank in series for the shallow (23 cm depth) treatment, which showed no change or a slight decline in biomass over time. The trend of increasing biomass over time was also apparent in the variable depth mesocosms (Figure 8). Gains in biomass were greater in the outflow region than midpoint. It is noteworthy that benthic periphyton did increase in the outflow region between May and September 2014, when the variable depth mesocosms were operated at water depths of either 69 or 92 cm. By contrast, the midpoint sampling indicated that low biomass conditions persisted through this period in those treatments. During the subsequent shallow period (September 2014 – January 2015) areal biomass values increased in the midpoint of variable depth treatments, but to a much smaller degree than the biomass gains over the same period in the outflow region.

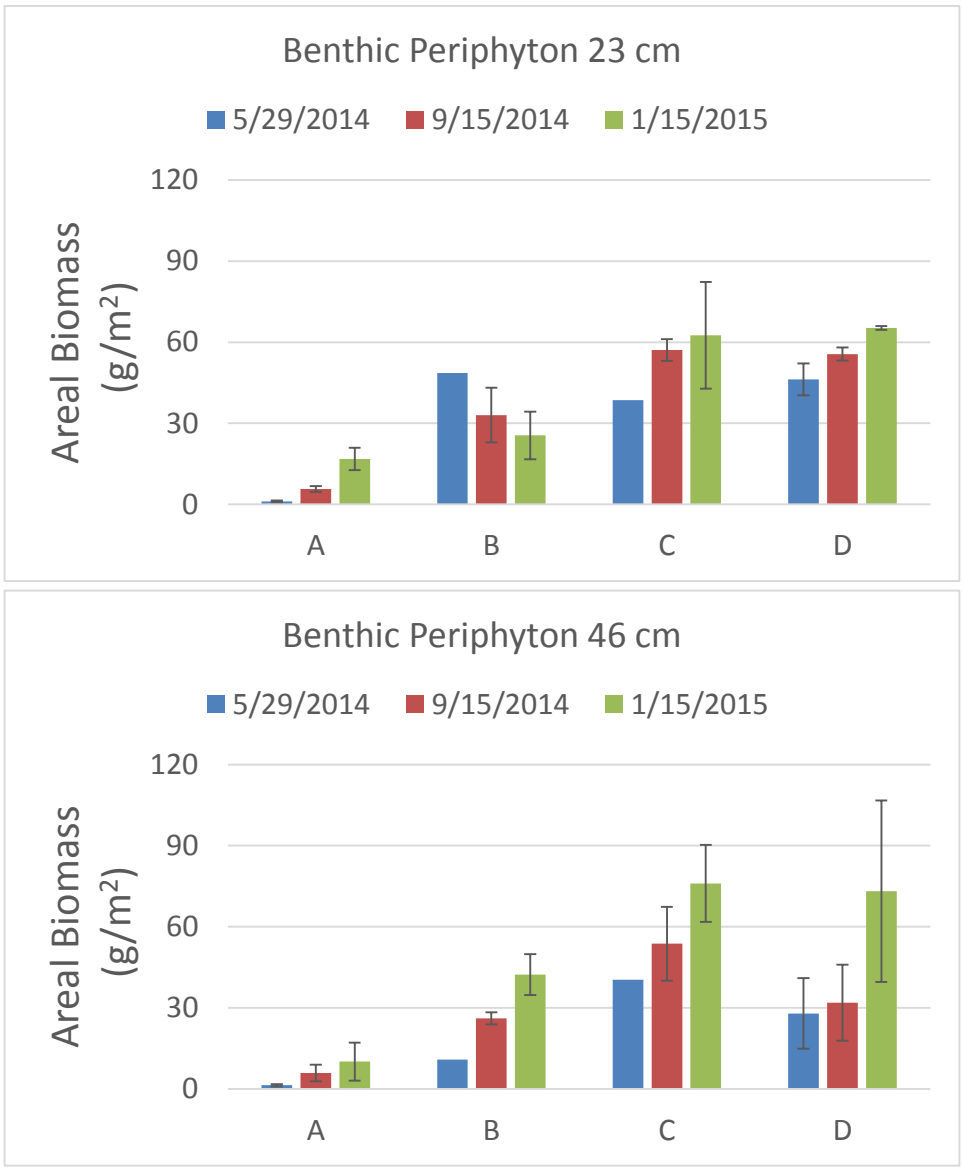

Figure 7. Areal biomass development on the benthic surface of mesocosms operated at either 23 cm or 46 cm water depths. The process trains consisted of four tanks in series, with inflow into "A" tanks and outflow from "D" tanks. The error bars denote the standard error around the mean values from triplicate process trains under each depth treatment.

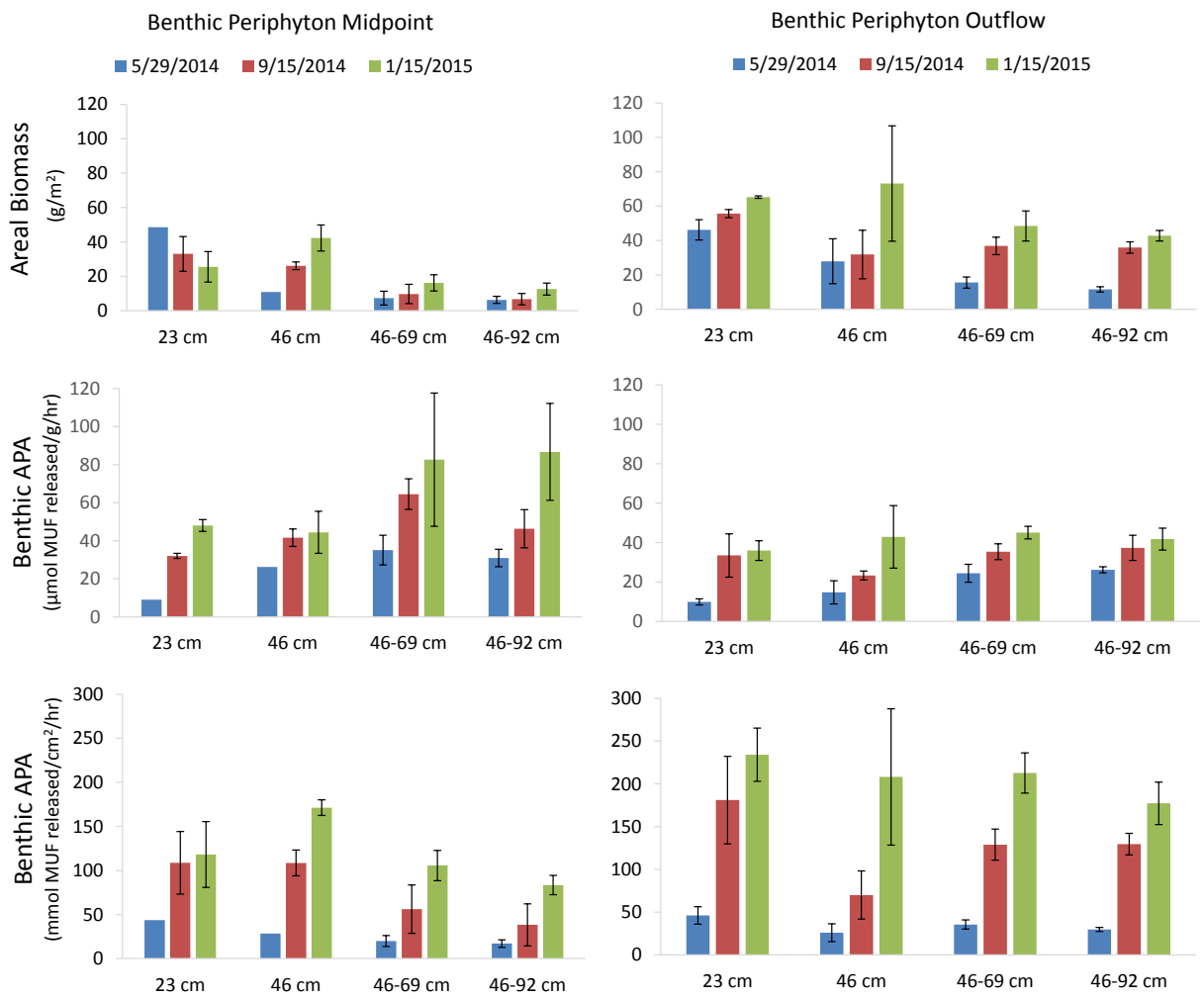

Figure 8. Areal biomass of benthic periphyton grown in mesocosms operated at either static water depth (23 or 46 cm) or variable water depth, on three sampling dates (Top panels). The midpoint and outflow of each flow way was sampled. Benthic periphyton was assayed for alkaline phosphatase activity (APA) and normalized to the dry weight of the periphyton (middle panels) and the benthic surface area supporting periphyton growth (bottom panels). The error bars denote standard error around the mean of values from three replicates under each treatment.

Biological community development over time

The benthic periphyton mat became established within a few months in the outflow region of the 23 cm deep flow ways (Figure 9). The benthic periphyton in upstream tanks were slower to colonize, and the first tank in series (A) never developed dense mats. *Chara* and *Potamogeton* also did not develop into dense stands, with relative density scores typically less than 3 (Figure 10 and Figure 11).

Macrophyte and periphyton responses to water depths

Effects of water depth on benthic periphyton and macrophytes are shown in Figures **12 – 14**. *Chara* density in the B tanks was higher at 46 cm than 23 cm late in the period of record, but little effect of depth was evident for *Chara* at other positions in the flow way (tanks A, C, or D). Relative density of the benthic periphyton generally was not negatively affected by deeper (46 cm) water depths, with the exception of the first few months for the B, C, and D tanks. The last tank in series in the shallowest treatment maintained a very dense benthic mat, while the average density of the last tank in series for the 46 cm treatment was somewhat lower. *Potamogeton* density was consistently higher at 46 cm than 23 cm in the first tank in series. This relationship, however, was not observed consistently at other positions further down the gradient.

Figure 9. Relative density of benthic periphyton in each of four tanks in series of process trains operated at static water depths of either 23 cm or 46 cm. Values represent the average (± SE) of triplicate mesocosms under each depth treatment, and 8 measurements within each tank for each date.

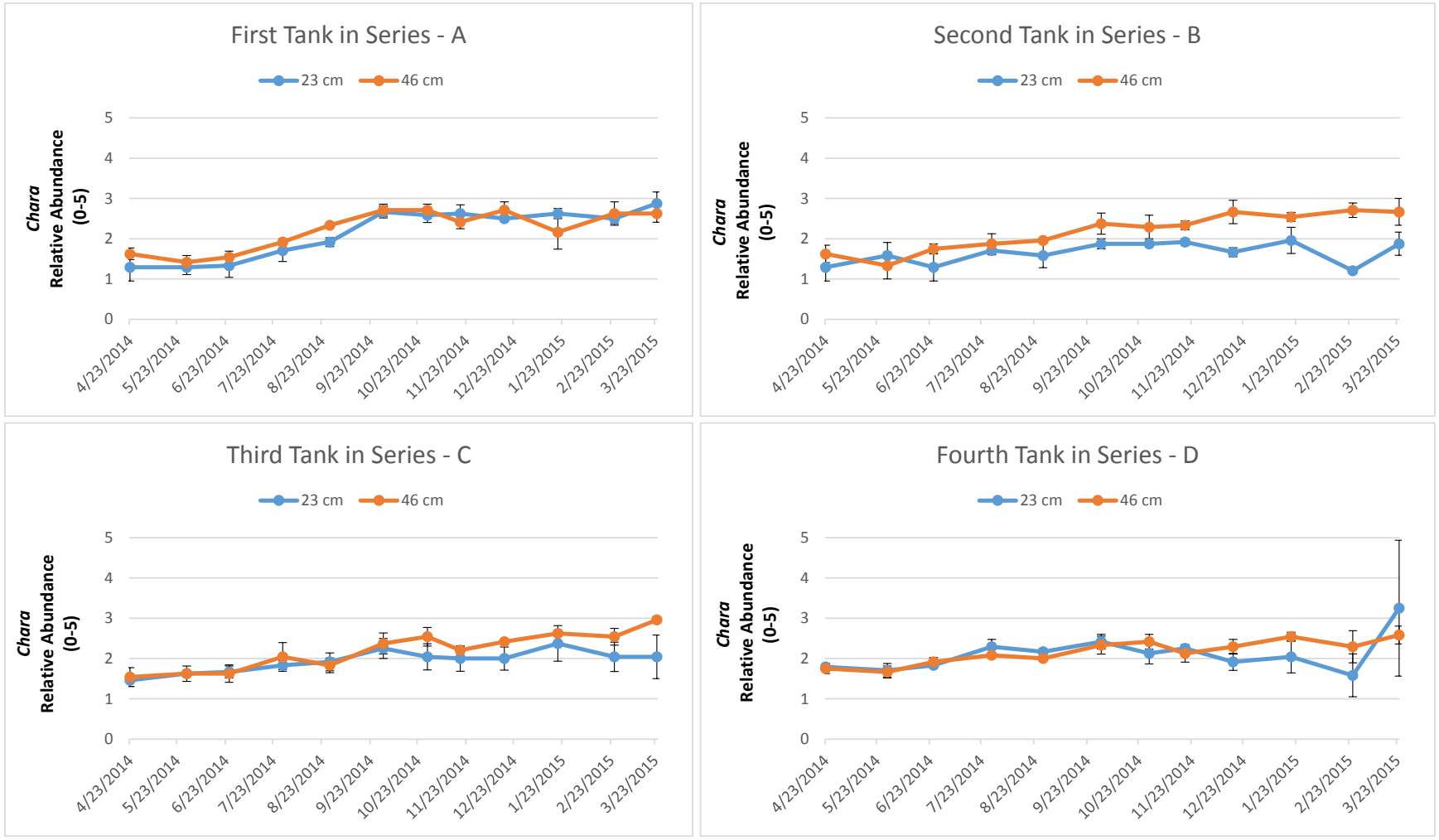

Figure 10. Relative density of *Chara* in each of four tanks in series of process trains operated at static water depths of either 23 cm or 46 cm. Values represent the average (± SE) of triplicate mesocosms under each depth treatment, and 8 measurements within each tank for each date.

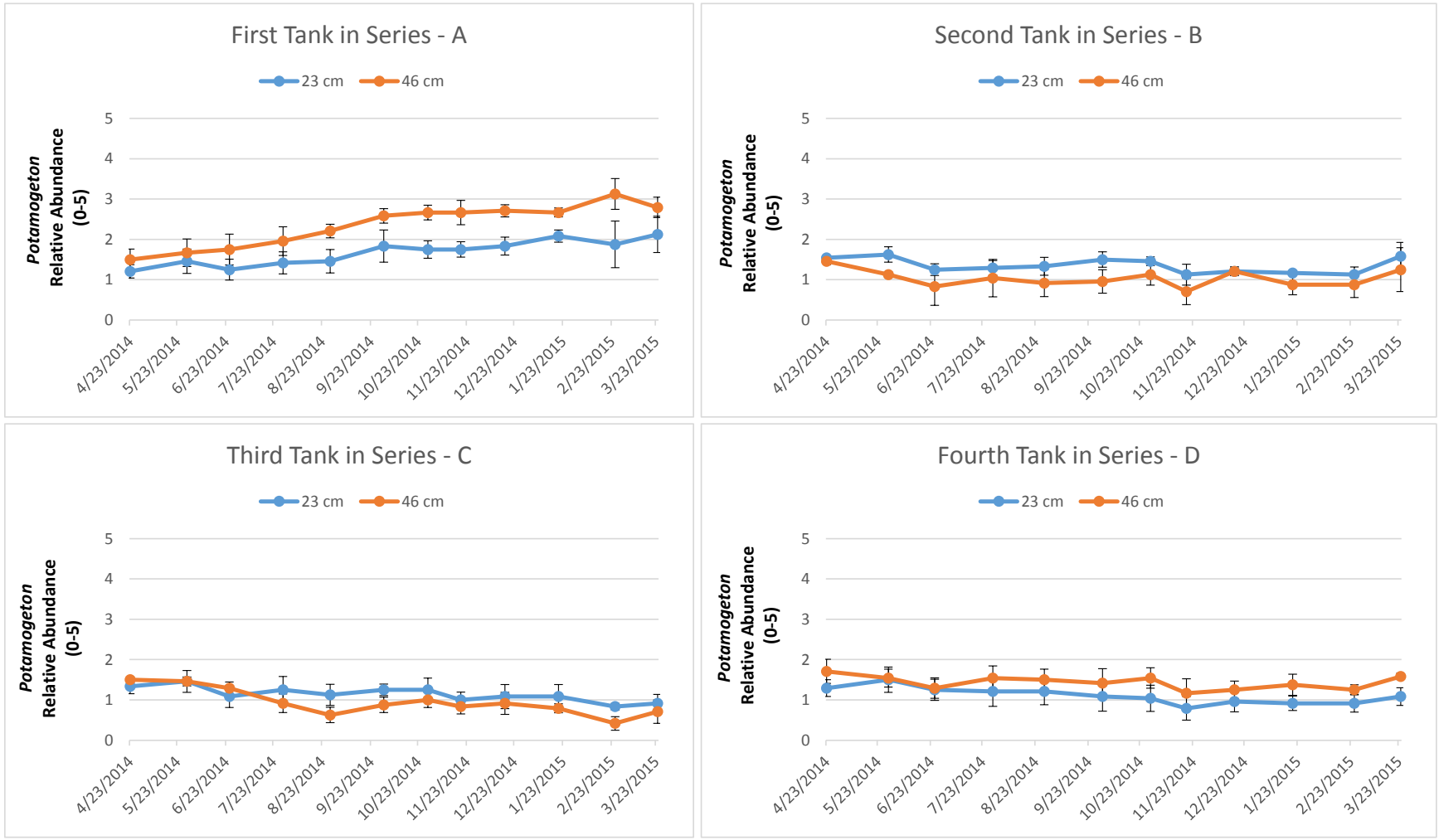

Figure 11. Relative density of *Potamogeton* in each of four tanks in series of process trains operated at static water depths of either 23 cm or 46 cm. Values represent the average (± SE) of triplicate mesocosms under each depth treatment, and 8 measurements within each tank for each date.

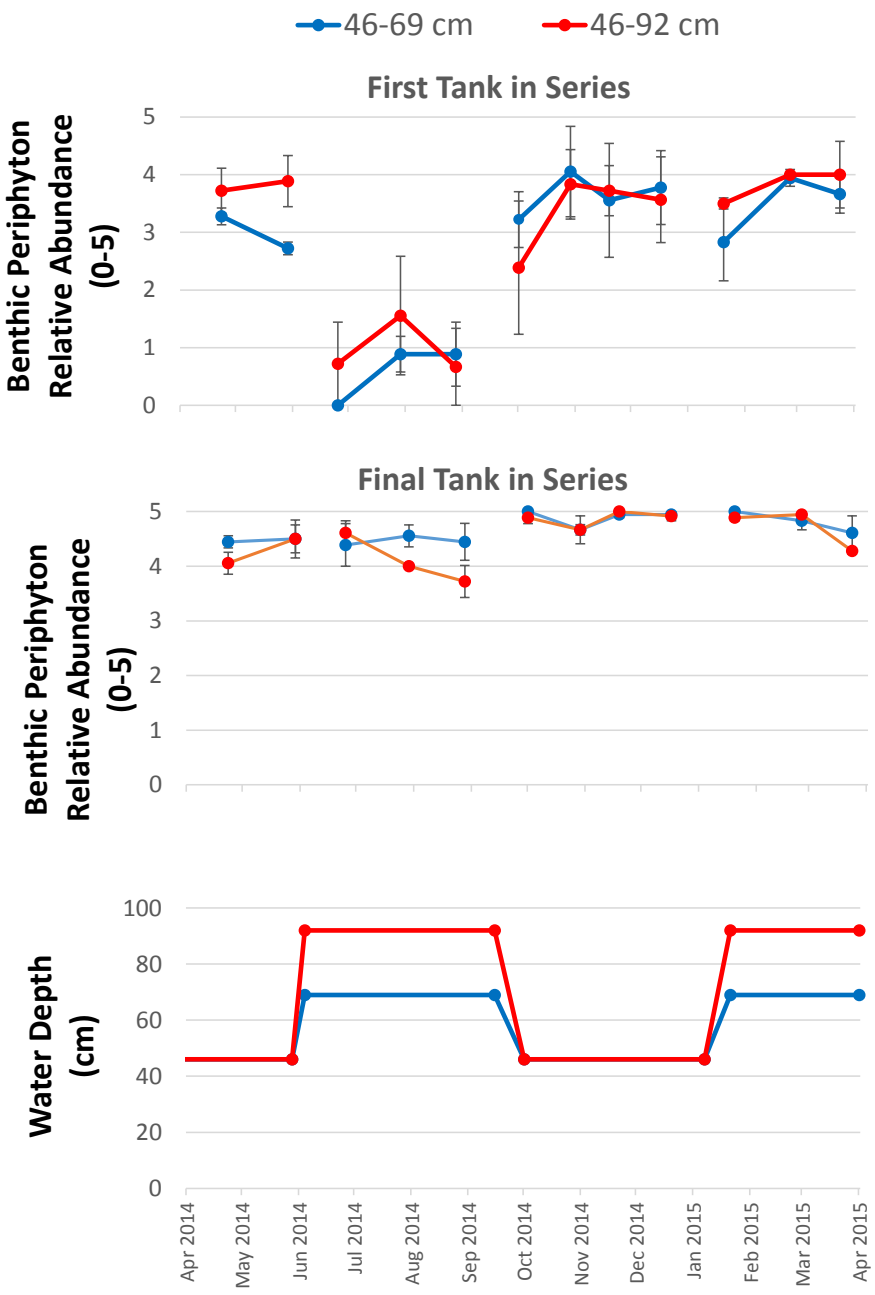

Figure 12. Relative density of benthic periphyton in the first tank in series (top panel) of process trains operated at variable water depths of either 46-69 cm or 46-92 cm. (bottom panel). Values represent the average (± SE) of triplicate mesocosms under each depth treatment, and 9 measurements within each tank for each date.

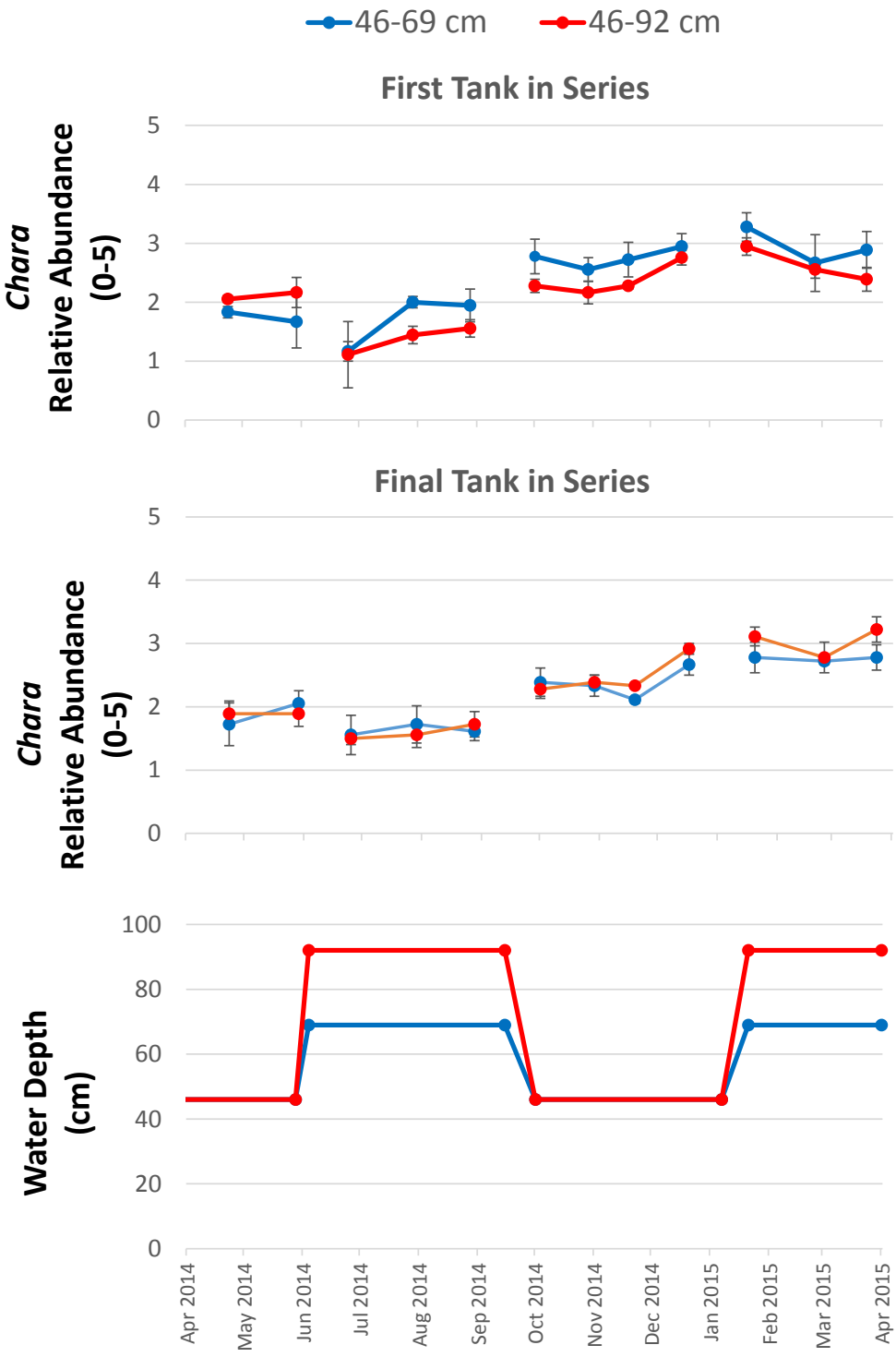

Figure 13. Relative density of *Chara* in the first tank in series (top panel) of process trains operated at variable water depths of either 46-69 cm or 46-92 cm. (bottom panel). Values represent the average (± SE) of triplicate mesocosms under each depth treatment, and 9 measurements within each tank for each date.

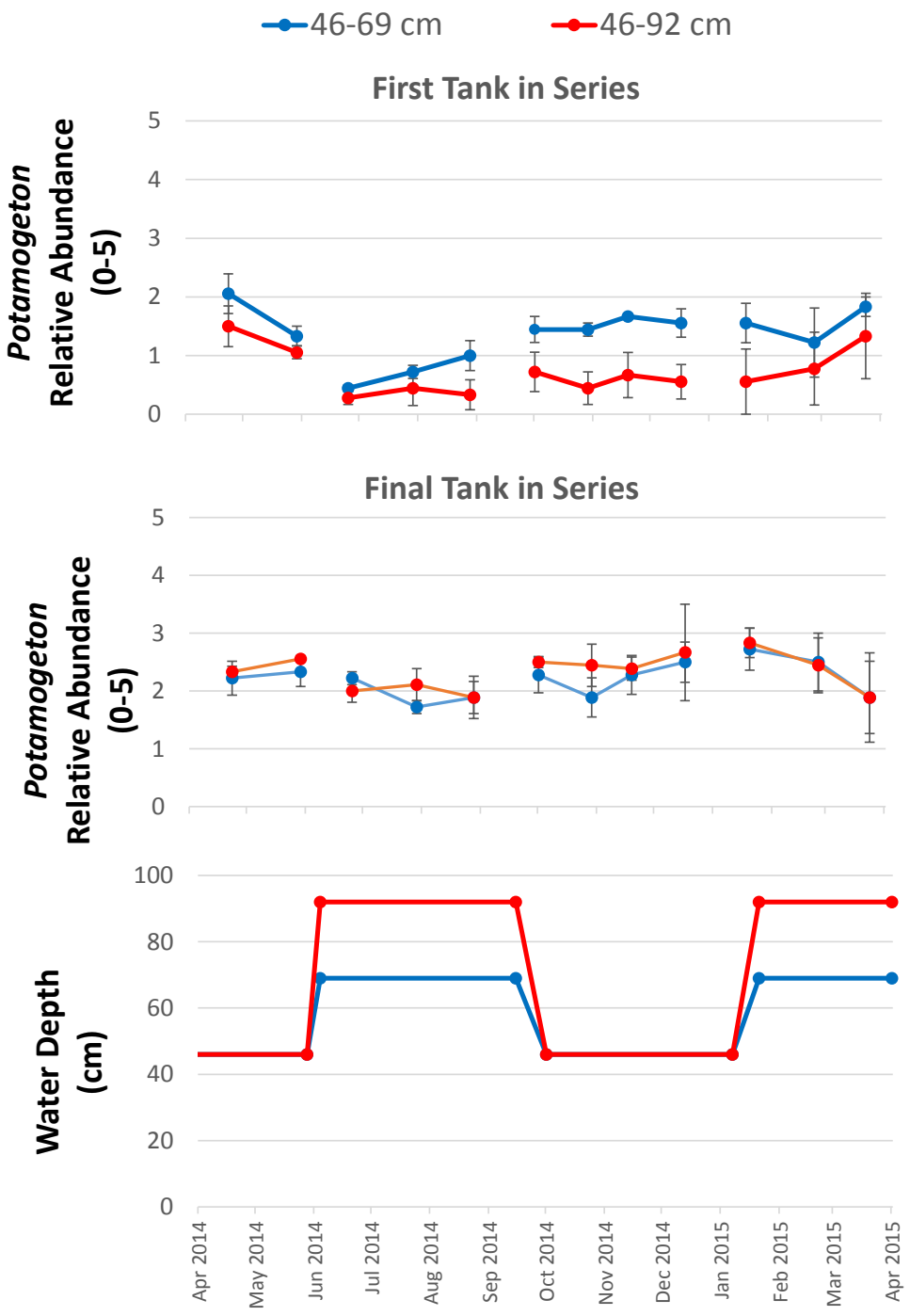

Figure 14. Relative density of *Potamogeton illinoensis* in the first tank in series (top panel) of process trains operated at variable water depths of either 46-69 cm or 46-92 cm. (bottom panel). Values represent the average (± SE) of triplicate mesocosms under each depth treatment, and 9 measurements within each tank for each date.

Synopsis of Initial Findings

Biofilm development occurred sooner and closer to the inflow in mesocosms with shallow water depths (23 cm). Benthic mat relative density was strongly affected by the deep water conditions that occurred in variable-depth treatments between May and September 2014, but this effect was evident only in the upstream tanks. At the beginning of the second deep-water period (January – April 2015), no such effect was observed.

Periphyton enzyme activity on a relative weight basis was higher at the midpoint than at the outflow. Periphyton biomass per unit area of the benthic surface was greatest near the outflow, however, which resulted in the highest enzyme activity per unit benthic surface area in the outflow region.

Potamogeton and *Chara* were able to persist across a range of water depths (23-92 cm), but did not achieve high relative biomass density values in these mesocosms. *Chara* density was similar across each of the four tanks in series of static depth treatments, while *Potamogeton* exhibited higher biomass in the inflow region, as compared to the outflow region, suggesting nutrient limitation to plant growth on the low-nutrient rock substrates.

References

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