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ECOSYSTEM PROCESSES OF EPILITHIC AND EPIXYLIC PERIPHYTON IN A BACKWATER OF THE UPPER MISSISSIPPI RIVER

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ECOSYSTEM PROCESSES OF EPILITHIC AND EPIXYLIC PERIPHYTON IN A BACKWATER OF THE UPPER MISSISSIPPI RIVER

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We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Master of Science in Biology - Aquatic Science

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ABSTRACT

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In large lotic aquatic systems like the upper Mississippi River (UMR), the majority of primary production is most often attributed to pelagic (free-floating) biological communities, or macrophyte communities. However, the ecological significance of benthic microbial communities is critical when assessing the quality of these aquatic habitats. Benthic systems teem with aquatic microbes, many of which inhabit matrix-enclosed biofilms (i.e., periphyton) that contribute to key ecological processes such as primary production, community respiration, nutrient cycling, and secondary production. The objective of this study was to culture periphyton on three different types of artificial substrates (unglazed ceramic tile and two types of wood: poplar and pine) situated inside exclosures developed specifically to inhibit colonization by large macroinvertebrate grazers. The exclosures were deployed in Target Lake, a backwater lake in Pool 8 of the UMR, for three week incubations throughout the summer of 2010. Periphyton growth on the substrates was subsequently analyzed for biomass (i.e., chlorophyll *a* and ash-free dry mass), primary production, and secondary production. Data from early summer (late May through early July) exclosure deployments showed significant (ANOVA, $p < 0.0001$) differences in biomass patterns among treatments, and indicated that there may have been more heterotrophic associations with the poplar substrates than with either the tile or pine. Despite the differences in biomass patterns, data from both primary and secondary production were not significantly different among treatments. Complications from flooding at the study site left all substrates from late summer (early August through late September) exclosure deployments unusable. Results from this study suggest that heterotrophic organisms constitute the majority of periphytic biomass, as well as play an important role in the structure and function, of periphyton communities on hard substrata in the UMR. In addition, the novel exclosure design functioned as intended, and could potentially be used for future studies to better understand how periphyton interacts with and influences other attributes of backwater ecosystems.

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INTRODUCTION

Large rivers are among the most ecologically and economically valuable ecosystems in the world, providing civilizations with fresh water, food, transportation routes, and power (Johnson *et al.* 1995). Unfortunately, anthropogenic modification of river morphology (e.g., dams and canalization) has severely altered the natural state of many large river floodplains (Ligon *et al.* 1995). Many rivers require an annual flood-pulse to distribute organisms and nutrients across a lateral gradient of habitat types (Bayley 1995). Dams change the natural flow regime of river systems and disrupt this necessary redistribution of resources (Poff *et al.* 1997; Tockner *et al.* 2000). In addition, the elimination of natural off-channel habitats through changes in discharge pattern affects the stability and integrity of the entire floodplain ecosystem (Gore and Shields 1995). In response to the problems listed above, restoration efforts focusing on ecosystem management and recovery have been initiated in some river systems and their floodplains (Sparks 1995). A better understanding of the ecological processes of biota inhabiting various off-channel habitats within a large river floodplain would be important to both conservation and restoration efforts.

The upper Mississippi River (UMR) is an extensive ecosystem of diverse habitats that harbor vast communities of fish and wildlife. In addition, the system provides a natural flyway for migratory birds that use the floodplain as resting grounds on their yearly migrations. Despite being of considerable ecological value, the UMR has been significantly altered from its natural state by the construction of extensive lock and dam

complexes. This effectively segments the river into a series of navigation pools which each contain different habitat types including the main channel, an impounded area, and several side channels and backwaters. Another consequence of the lock and dam system has been a decrease in the intra-annual variation in discharge (Alonso-Gonzalez *et al.* 2008). The level of connectivity between the various habitats is dependent on the annual flood-pulse, which extends the main channel onto the floodplain allowing for the transfer of biotic (e.g., fish and invertebrates) and abiotic (e.g., nutrients and sediment) components between habitats (Johnson *et al.* 1995). The transfer of these components can drive important processes such as off-channel primary productivity via nutrients from the main channel, as well as providing key life cycle cues for invertebrates and fish (Junk *et al.* 1989). The flood-pulse is especially important to backwater systems that may have very limited to no connection with the main channel throughout the majority of the year.

Backwater ecosystems include areas of the floodplain found beyond the shoreline of the main and secondary channels, and can be contiguous (i.e., connected) or isolated from the surface flow of the main channel (Wilcox 1993). These systems are typically shallow with high macrophyte abundance, and offer several important ecological services to the river community. Backwaters provide habitat for aquatic invertebrates (Tronstad *et al.* 2007), and areas of ample nursing habitat for native fish (Zigler and Jennings 1993). The high rate of productivity and low water velocity in these systems allow highly-organic sediments to accumulate. These organic-rich sediments provide ideal redox conditions for biogeochemical processing (e.g., nitrogen cycle) making backwater areas especially important for processing nutrients that enter the river system (Richardson *et al.* 2004; Strauss *et al.* 2004; Strauss *et al.* 2006; Houser and Richardson 2010). Backwater

ecosystems are also home to assemblages of autotrophic and heterotrophic biota (e.g., microbial biofilms) that are influenced by, and interact with, the surrounding environment as they undergo several important ecosystem processes.

In large aquatic systems like the UMR, ecological research efforts often focus on pelagic (free-floating) biological communities. Benthic (bottom-dwelling) communities are usually considered only within the context of being a source or sink of pelagic energy (Vadeboncoeur *et al.* 2002). However, the ecological significance of benthic communities is critical when assessing the quality of aquatic habitats. Benthic systems teem with aquatic microbes, many of which inhabit matrix-enclosed biofilms that contribute to key ecological processes such as energy flow and nutrient cycling (Battin *et al.* 2003). Evaluation of periphyton (i.e., microbial biofilms or Aufwuchs) has long been used to observe and monitor the effects of water chemistry, nutrient dynamics, hydraulic conditions, habitat availability, and food-web structure on benthic communities (Larned 2010). The term periphyton refers to an assemblage of both autotrophic (e.g., algae and cyanobacteria) and heterotrophic (e.g., bacteria, fungi, and protozoa) organisms residing in a polysaccharide matrix. The extreme biodiversity within periphytic communities can create hot spots of biological activity that can be of significant ecological importance (Larned 2010). Periphytic communities provide researchers with excellent model systems for studies in freshwater ecology because of their rapid developmental responses, small size, taxonomic diversity, and ubiquitous distribution (Lowe *et al.* 1996).

The diversity of substrate types within an aquatic ecosystem can lead to natural heterogeneity in periphyton community development and assemblage (Lowe *et al.* 1996). Variability in periphytic community structure is a result of differences in the chemical

and physical properties of several substratum types including rocks, macrophytes, wood, sand, etc. (Vadeboncoeur and Lodge 2000). Chemical properties of substrata are crucial because some substrate types (e.g., macrophytes) can release nutrients, primarily nitrogen (N) and phosphorus (P), that are essential for algal growth, while other substrates (e.g., rock and wood) do not release appreciable N or P (Vadeboncoeur and Lodge 2000). Physical drivers also correlate with periphyton biomass as algal accrual has been shown to increase with both an increase in substrate surface roughness and a more horizontal orientation of the substrate (Murdock and Dodds 2007). Although many studies still examine periphyton collected directly from natural substrata (Potapova and Charles 2005; Vadeboncoeur and Lodge 2000; Vadeboncoeur *et al.* 2006), the use of artificial substrates has become increasingly popular to assess periphyton community structure, ecosystem processes, and response to environmental stimuli (Biggs 1989; Lowe *et al.* 1996; Murdock and Dodds 2007).

The use of artificial substrates allows for the control of heterogeneity among replicates, thus reducing sampling error among samples from the same substrate type. The most common artificial substrate used is unglazed clay or stone tile, which is a surrogate for rock surfaces to examine epilithic communities. In a study by Lowe *et al.* (1996), periphytic community structure and function was highly similar between artificial-tile and natural-cobble substrates. The use of wood as an artificial substrate is less common. However, results from some studies using natural-wood substrates indicate that periphyton growth and function on wood substrates is similar to that of rock substrates (e.g., Vadeboncoeur and Lodge 2000). Another advantage in using artificial substrates is that they can be deployed in systems where sampling natural substrates

would be impractical. This can be done by placing the artificial substrates in mesocosms or exclosures, which can then be suspended at predetermined depths in the water column allowing for the control of light availability.

Light availability is an important limiting abiotic factor for the photoautotrophic members of the periphyton community (Larned 2010). In shallow aquatic ecosystems, such as streams, light availability is primarily dependent on riparian (i.e., near-shore) shading. However, in larger aquatic systems, like lakes and large rivers, light availability is dependent on a combination of depth, turbidity, and shading by macrophytes. Hepinstall and Fuller (1994) showed that periphyton biomass (as chlorophyll *a*) was significantly lower in shaded sites when compared to open canopy sites, suggesting that light can limit the growth of periphyton. Light availability also varies seasonally with photoperiod, and changes in riparian foliage and macrophyte abundance.

Periphyton can also be limited by nutrient availability, which is one of the most-studied areas of periphyton ecology (Larned 2010). Relationships between nutrient availability and algal growth in periphyton have been well documented (Tank and Dodds 2003), and can lead to inferences about the limiting condition of the ecosystem. Limitation within a system is dictated by Liebig's Law, which suggests that growth in a system is limited by the scarcest resource available relative to need. While limiting resources can include abiotic factors other than nutrients, such as light (Hepinstall and Fuller 1994), growth in freshwater systems is typically considered to be limited by the availability of P, and less commonly N (Tank *et al.* 2006). The objective of most nutrient limitation studies is to determine whether the availability of N or P limits algal growth in periphyton, although some studies have shown that periphyton can be co-limited by both

N and P (Larned 2010). Co-limitation occurs when different taxa within the periphyton community are limited by different nutrients, resulting in the supply and demand of those nutrients to be in close balance (Davidson and Howarth 2007). The degree of nutrient limitation also varies seasonally as fluctuating environmental conditions can affect the input of nutrients and subsequent uptake by organisms (Francoeur *et al.* 1999).

Periphyton communities are able to sustain long-term growth by obtaining N and P from several different sources (Larned 2010). Influxes of nutrient rich groundwater have been shown to increase periphyton growth, suggesting that algal accrual and even macrophyte growth can be regulated by groundwater discharge (Hagerthey and Kerfoot 2005). Other studies have shown that a higher water-column nutrient concentration can result in an increase in benthic-algae accrual (Blumenshine *et al.* 1997; DeNicola *et al.* 2006). Certain members of the periphyton community can also carry out luxury consumption when nutrient concentrations are high, providing a readily-available nutrient reservoir to reduce nutrient limitation when inputs are low (Havens *et al.* 1999). However, excessive inputs of N and P from anthropogenic pollution can result in the severe eutrophication, and subsequent degradation of freshwater habitats (Smith and Schindler 2009). Dodds (2003) suggests that the effects of nutrient loading could be dampened by the construction of periphyton-dominated wetlands that would act to filter P out of the ecosystem. Thus, uptake and retention of nutrients by periphyton can provide an important ecological service to aquatic habitats.

Another ecosystem process that is critical for the health of aquatic habitats is the production of oxygen by actively photosynthesizing algae within periphyton. Oxygen production by periphyton peaks at intermediate levels of nutrient availability where a

decrease in nutrients would cause nutrient limitation and an increase in nutrients would result in light limitation from eutrophic shading (Liboriussen and Jeppesen 2006). Primary production by periphyton also provides the system with oxygen that can be used by both autotrophic and heterotrophic organisms for respiration. Community respiration has been shown to follow the same light and nutrient requirement trends as primary production (Liboriussen and Jeppesen 2006). Although most periphyton studies focus on the autotrophic portion of periphyton, it is also important to consider contributions of heterotrophic organisms, especially bacteria, to the overall function of periphyton.

Bacteria play an important role in aquatic food chains (Fuhrman and Azam 1982), as well as several ecosystem processes such as the conversion and processing of nutrients and organic matter (Olapade and Leff 2004). These bacterial processes are essential for the development of healthy periphytic communities, where bacterial densities typically far outnumber all other biofilm members. The taxonomic composition of bacteria within periphyton varies seasonally (Rier and Stevenson 2001; Olapade and Leff 2004), which is similar to algal assemblages. Also, several studies have shown that bacterial production and densities are positively correlated with algal biomass (Hepinstall and Fuller 1994; Rier and Stevenson 2001). This suggests that the relationship between the autotrophic and heterotrophic components of periphyton is important, particularly when considering effects of biotic and abiotic shifts in environmental conditions.

The diversity of organisms that inhabit periphytic communities, and as a result a wide array of ecological processes, has led to the use of periphyton as a water quality biomonitor. A study by Biggs (1989) in shallow, swift New Zealand rivers, suggests that the biological effects of organic discharges can be monitored using periphyton. In

addition, periphyton have acted as a reliable bioindicator of environmental stress exerted by acid mine drainage (Pena and Barreiro 2008). Biofilms are also used by the water industry to improve the efficiency of water treatment (Sabater *et al.* 2002), making periphyton both ecologically and economically significant. The near ubiquitous distribution of periphytic communities across spatial and temporal scales means that contributions to periphyton ecology could have widespread implications for the management, conservation, and restoration of freshwater ecosystems.

Objectives and Hypotheses

The primary objective of this research project was to better describe the function and growth patterns of periphyton in backwaters of the UMR, with a focus on trophic structure and ecosystem processes. Periphyton was cultured on three different types of artificial substrates (tile, poplar, and pine) situated inside exclosures developed to inhibit the colonization of large macroinvertebrate grazers. Periphytic communities were quantitatively analyzed for primary production, community respiration, and secondary production. The data obtained from these ecosystem processes may have important implications for understanding how periphyton interacts with and influences other attributes of a backwater ecosystem. In addition, an analysis of biomass, direct bacterial counts, and community composition among the periphyton communities provided a general understanding of how different substrate types influenced the growth and trophic nature of the periphyton.

Total periphyton biomass (autotrophic + heterotrophic) was not expected to significantly differ among the three artificial substrate types. However, it was hypothesized that the algal biomass would be greatest on the tile substrates indicating

more autotrophic associations on non-wood substrates. It was further hypothesized that primary production would then have been higher on the tile substrates, and the analyses correlated with heterotrophic activity including community respiration, direct bacterial counts, and secondary production would have been higher on the two wood substrates.

An ancillary objective of this research project was the design of a novel periphyton growth chamber. Development of new and innovative methods has always been an important aspect of field biology, and the acrylic chambers used in this study were designed specifically for deployment in large aquatic ecosystems. This research project provided a unique opportunity to assess the effectiveness of the exclosure design, and work towards the development of a reliable method for measuring periphyton growth in lakes and large rivers. Therefore, an important component of this study was to establish an efficient technique for monitoring periphyton growth in the UMR and other aquatic systems.

MATERIALS AND METHODS

Study Site

The UMR stretches from Minneapolis, MN, south to Cairo, IL at the confluence with the Ohio River. Unlike the lower Mississippi River, the UMR is segmented into a series of navigation pools by a system of locks and dams. In each pool, a portion of the main channel is dredged to a minimum depth of 2.7-m to facilitate commercial and recreational travel. Prior to the construction of the lock and dam system, the UMR was shallower and the majority of the floodplain was dominated by riparian forests. However, when the lock and dam complexes were constructed, the impounded water permanently flooded much of the riparian forest resulting in a regulated flow regime within each pool. This change in hydrology produced and sustained several distinct ecosystem types including the main channel, secondary and tertiary channels, marshland, and backwaters.

Navigation Pool 8 of the UMR extends from Lock and Dam 7 located near Dresbach, MN, downstream to Lock and Dam 8 located near Genoa, WI, and rests in a limestone bedrock floodplain surrounded by large limestone bluffs. The floodplain of Pool 8 contains several backwater ecosystems including floodplain depression lakes which are characterized by a shallow basin, relatively even depth, and limited shoreline development (Wilcox 1993). Target Lake (N 43° 47.39', W 91° 16.41'), located south of La Crescent, MN, on the west side of the wetted channel, is an example of a floodplain depression lake and was the primary location for this research project (Fig. 1). Field

work was completed in open areas of the lake, free of submerged and emergent macrophytes.

Substrate and Exclosure Design

Periphyton communities were cultured *in situ* on three different types of artificial substrates. Unglazed clay tiles (ca. 2.5-cm x 2.5-cm) were used to replicate stone surfaces for epilithic periphyton communities. The surface area of each individual tile was calculated from two measurements taken on each side of the tile approximately 5-mm from each of the corners. The surface area of the tile was recorded and each tile was labeled with a corresponding ID number. Wood dowels and pine boards were used to replicate two types of wood surfaces for epixylic periphyton communities. One cm thick discs cut from poplar dowels (2.86-cm diameter) were used to replicate a soft wood surface, and 1-cm thick pine squares (ca. 2.5-cm x 2.5-cm) were used to replicate a pine wood surface. An average surface area for each wood substrate type was determined using a caliper to measure the surface area of twenty randomly selected poplar discs or pine squares, and the mean surface area was then used as the representative surface area for the corresponding substrate type.

Several days prior to field deployment six of each of the three artificial substrate types were glued in a random order to a sheet of PVC (ca. 6.3-cm x 30.5-cm) using a water-proof adhesive (Fig. 2). After the adhesive had dried for 48 hours, the PVC sheets with attached substrates were soaked in reverse osmosis water for at least 24 hours prior to deployment to condition the substrates. Prior to substrate deployment, the

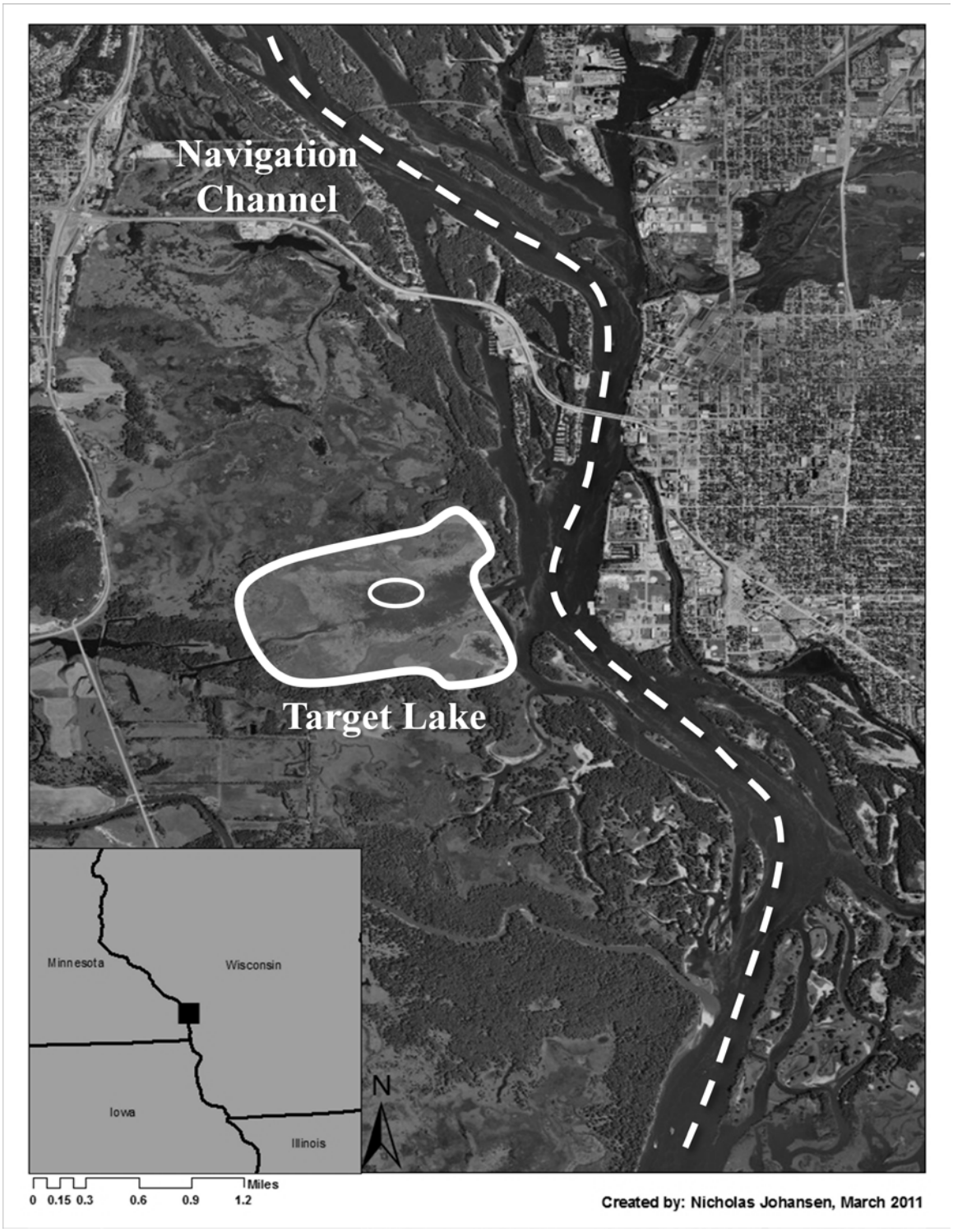

Fig. 1. Target Lake study site location on the UMR near La Crosse, WI. The circle in Target Lake is the approximate exclosure deployment area.

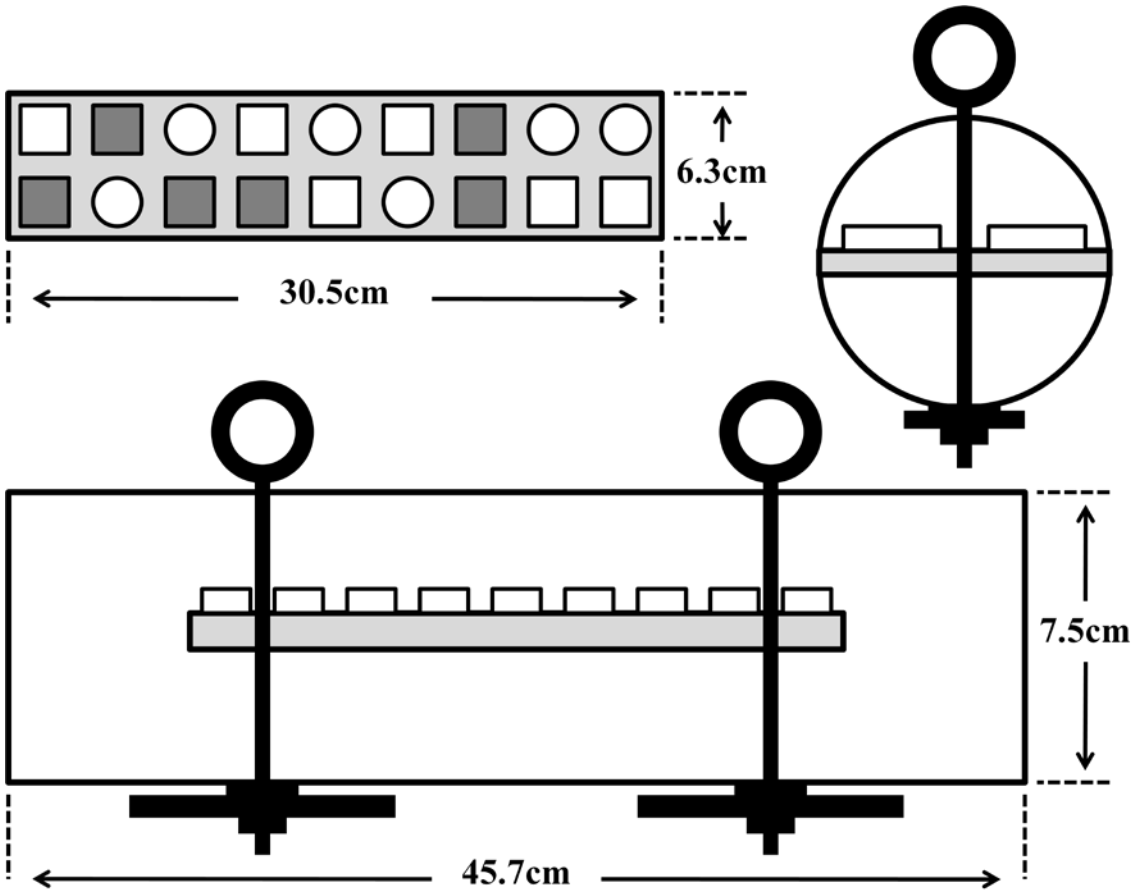

Fig. 2. Dimensional diagrams of the PVC substrate sheets and completed acrylic exclosures. Upper left, six of each of the three artificial substrate types (clay tiles □, poplar discs ○, and pine tiles ■) glued to a sheet of PVC. Upper right and lower, end and side views of a deployment ready exclosure. The fiberglass mesh window screen that would cover the ends of the acrylic tubes is not diagramed.

exclosures were constructed by securing each sheet of PVC with attached substrates into a clear acrylic tube (7.5-cm OD) using two zinc-plated eyebolts, one near each end of the PVC sheet (Fig. 2). A small metal washer and steel weight (7.62-cm x 2.54-cm x 0.32-cm; 65.0-g) were slid onto the threaded end of each eyebolt and secured with a hex nut. The steel weights were added to keep the artificial substrates facing upwards and to limit movement from wave action. The open ends of the acrylic tubes were covered with a circular piece of fiberglass window screen (15.3-cm diameter, 2.2-mm^2 mesh size), held on with a rubber band, to discourage large macroinvertebrate colonization, but still allow for the passage of water and colonizing microorganisms into the exclosure. Excluding large macroinvertebrates from the exclosures was important to inhibit top-down grazing pressure on the periphyton communities (Rosemond et al. 1993; Hillebrand 2002; Sabater et al. 2002). Finally, a piece of braided polypropylene rope (approx. 60-cm in length) was used to connect the two eyebolts on each exclosure, and the knots were melted with a heat gun to ensure that they would not come apart during the deployment.

Stationary exclosure rigs were constructed at the field site by positioning a schedule-80 PVC tube (1.5-m in length) at the surface of the water by tying each end of the tube to a submerged cinder block. A piece of foam swim-noodle (0.12-m diameter x 0.6-m in length) was threaded down each rope prior to tying it off to the PVC tube to keep the tube situated at the surface. Four exclosures were then secured to the PVC tube approximately 30-cm apart by threading a 20-cm plastic zip tie through the braid of the polypropylene rope and fastening it through pre-drilled holes on the PVC tube (Fig. 3).

Each deployment consisted of eight exclosures suspended near the surface of the water (approx. 20-cm depth) for a 21-day incubation period. The acrylic tube housing the

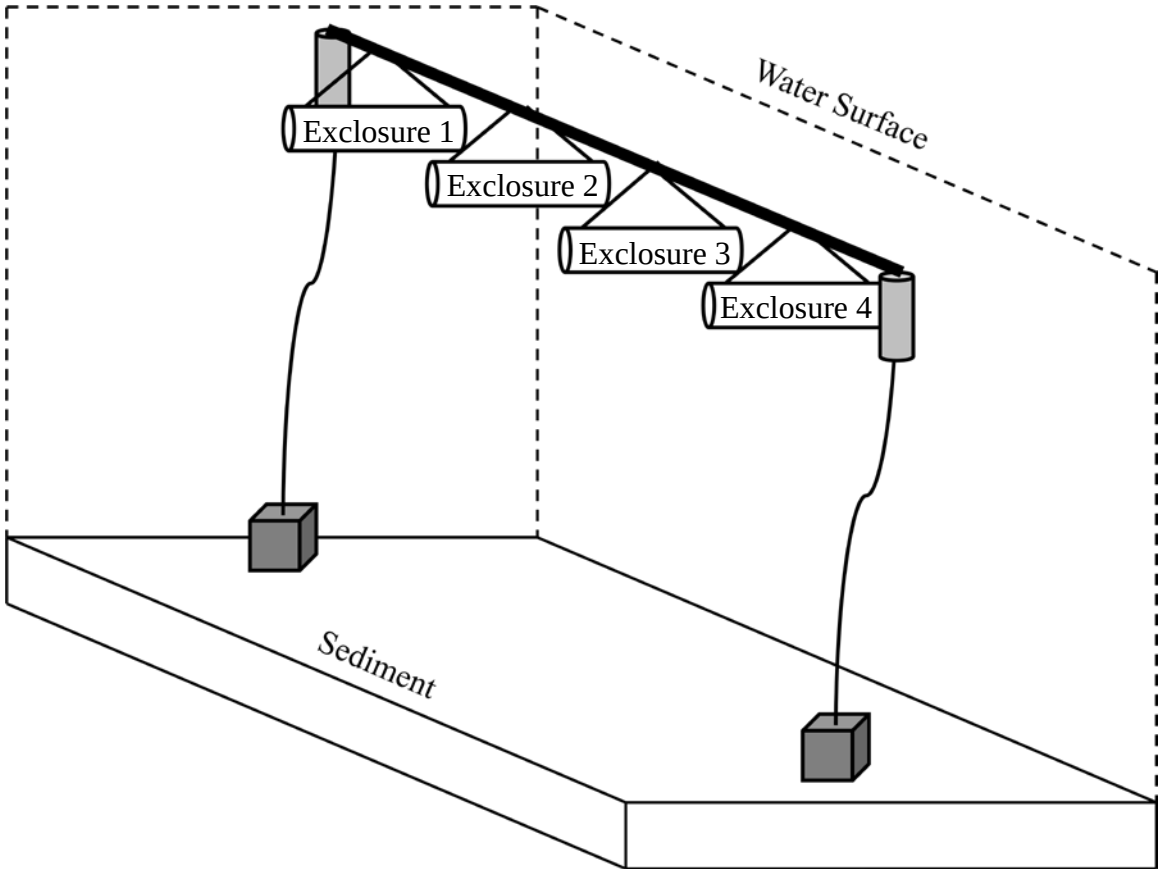

Fig. 3. A conceptual diagram of the construction and relative position of an exclosure rig during deployment in Target Lake. The four exclosures were secured just below the PVC pole (approx. 20-cm depth), which was positioned at the water's surface by two pieces of foam swim noodle. The entire rig was tied to two cinder blocks to keep it in place.

substrates was exchanged with a clean tube approximately every seven days throughout each incubation period to insure that organisms colonizing the acrylic tube would not interfere with the amount of light reaching the substrates. Only one of each type of substrate from each exclosure was used for a specific analysis. Exclosure deployments were based on a randomized complete block design with two time periods (T_1 and T_2; time factors) each including two to four sets (blocks). Eight exclosures were deployed during each set, resulting in eight possible replicates per substrate type for each analysis. The net primary production (NPP), community respiration (CR), and acridine orange direct count (AODC) analyses each included two sets (S_1 and S_2; Fig. 4), and the chlorophyll *a*, ash-free dry mass (AFDM), and autotrophic index (AI) analyses each included four sets. Diatom diversity and secondary production were analyzed only once during each time period.

The four T_1 set deployment periods for chlorophyll *a*, AFDM, and AI were May 28th through June 19th, June 3rd through June 24th, June 10th through June 29th, and June 16th through July 7th. The two T_1 set deployment periods for NPP and CR were May 28th through June 19th and June 3rd through June 24th. The two T_1 set deployment periods for AODC were June 10th through June 29th and June 16th through July 7th. The single T_1 deployment period for diatom diversity and secondary production was May 28th through June 19th and June 16th through July 7th, respectively. The T_2 sets for all analyses were deployed August 6th and August 12th, and again September 2nd and September 9th, but complications from extensive precipitation events rendered all T_2 exclosures unusable for lab analyses.

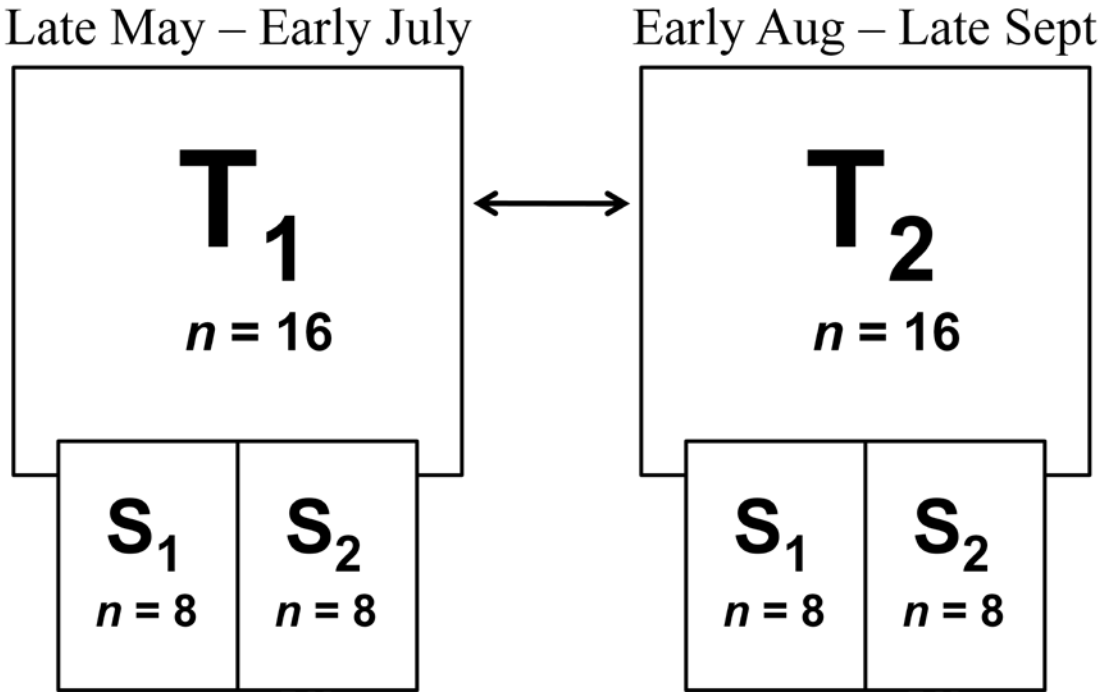

Fig. 4. A diagram outlining the randomized complete block design for the NPP, CR, and AODC analyses, including the number of replicates (*n*) of each treatment for each time (T_1 and T_2; time factors) and set (S_1 and S_2; blocks), and the time factor deployment periods (2010).

A Hydrolab multiparameter probe was used during each trip to the field site to characterize the backwater habitat by measuring several physical and chemical components of the water including; temperature, pH, specific conductivity, salinity, dissolved oxygen (DO) concentration, and DO percent saturation. The probe was also used to record changes in exclosure depth due to fluctuations in the stage of the UMR. In addition, during each trip to the field site, a 1-L sample of site water was collected in a 1-L Nalgene bottle and filtered (Whatman GF/B) for laboratory analysis of ammonium, nitrate, and soluble reactive phosphorus (APHA 2005).

After the 21-day incubation period for each set of exclosures, the PVC sheets with attached substrates were removed and placed into Rubbermaid containers filled with site water. The substrates designated for *in situ* analyses were processed immediately at the site, while all other substrates designated for *ex situ* analyses were transported back to the lab in the Rubbermaid containers and stored in site water for no longer than 12 hours before processing. The processing procedure for all analyses included scraping the periphyton from the substrates using a razor blade. The majority of periphyton biomass was removed with three consecutive scrapes in one direction followed by three scrapes perpendicular to the first three. A brief wash of the razor blade and substrate with analysis-specific solution (e.g., 90% acetone, formalin, etc.) removed any loose biomass. Once in the appropriate container, the periphyton was analyzed for one of several different biomass estimates or ecosystem processes. In addition, multiple samples of the same substrate type were not combined to increase the biomass of an individual replicate, so each replicate represents the periphyton community from a single artificial substrate.

Biomass Analyses

Phaeophytin-corrected chlorophyll *a* content of the periphyton, a surrogate for algal biomass, was measured spectrophotometrically (Steinman *et al.* 2006). The periphyton was scraped off of each substrate into 20-mL glass scintillation vials, which were then brought to a total volume of 10-mL with 90% aqueous acetone solution. The vials were mixed on a vortex for five seconds, and left to steep in the dark at 4ºC for a maximum of 96 hours (Biggs 1987). After the incubation period, the samples were analyzed for chlorophyll *a* concentration using a spectrophotometer (Varian Cary 50 UV-Vis Spectrophotometer) (Steinman *et al.* 2006). Three mL of each sample was transferred to a 1-cm quartz cuvette, and the optical density (OD) was read at 750 and 664-nm. The sample was then acidified with 0.1-mL of 0.1N HCl, inverted three times, and after 90 seconds the OD at 750 and 665-nm was read. The OD's for each sample were then used to determine the chlorophyll *a* concentration ($\mu g/cm^2$) on each substrate.

Determination of AFDM was used as a second measure of the biomass of the periphyton (Steinman *et al.* 2006). The periphyton was scraped off of each substrate onto aluminum weighing dishes, which were then placed in a drying oven at 105ºC for at least 48 hours. After the appropriate time in the drying oven the aluminum dishes were cooled in a desiccator for at least two hours and weighed on an analytical scale. The dishes were then ignited in a muffle furnace for one hour at 500ºC, cooled in a desiccator for 24 hours, and again weighed on an analytical scale. The AFDM was determined using the pre-ash and post-ash weights. The AI, which indicates heterotrophic associations or poor water quality, was calculated by dividing the AFDM (mg/cm^2) by the chlorophyll *a*

concentration (mg/cm^2; converted from $\mu g/cm^2$), and was used to estimate the trophic nature of the periphyton communities (APHA 2005).

Primary Production and Community Respiration

Analysis of primary production and community respiration was performed *in situ* immediately after the 21-day incubation for each set. Periphyton from each of the eight replicates per substrate type was scraped into four light and four dark 300-mL glass biochemical oxygen demand (BOD) bottles, which were then filled with filtered (Whatman GF/B) site water and capped. The DO in two of the light and two of the dark BOD bottles for each substrate type was fixed immediately per the azide modification of the Winkler method (APHA 2005) for initial DO concentration. Fixing the samples stabilized the DO in each bottle until titration and quantification of DO could be completed. The DO was fixed by adding 1-mL of $MnSO_4$ solution and 1-mL of alkali-iodide-azide reagent, inverting several times and waiting for the precipitate to settle, and then adding 1-mL of concentrated H_2SO_4 and inverting several times until the precipitate had completely dissolved (APHA 2005). The remaining light and dark BOD bottles were suspended at the same depth (approx. 20-cm) and location as the original exclosures for five hours (Wetzel and Likens 1991). After the five hour incubation the BOD bottles were retrieved, the DO in each BOD bottle was fixed, and all initial and final BOD bottles were transported back to the lab for titration and quantification of DO (Wetzel and Likens 1991).

The change in DO between initial and final BOD bottles was used to calculate the NPP and CR ($\mu gO_2\ cm^{-2}\ hr^{-1}$) for each substrate. Rates were converted to net biomass production (NBP) and biomass consumed during respiration (RB) ($\mu gC\ cm^{-2}\ hr^{-1}$) using

the following conversions; NBP = 0.375*NPP/1.2 and RB = 0.375*CR (Brower *et al.* 1997). The contribution of the filtered site water was -33.6 ± 17.3 µgC L^{-1} h^{-1} for NBP and 38.7 ± 21.4 µgC L^{-1} h^{-1} for RB, and was determined by filling light and dark BOD bottles (n = 3 for both) with filtered site water only, and administering them to the procedure described above.

Secondary Production and Bacterial Enumerations

The radiolabeled thymidine incorporation technique, modified from Fuhrman and Azam (1980), was used to estimate the rate of secondary production occurring within the periphyton on each substrate type. Four replicate periphyton samples chosen at random from each substrate type were scraped into separate sterile 50-ml polypropylene conical centrifuge tubes, which were then filled to 50-ml with filter sterilized (0.2-µm pore size) site water and mixed on a vortex for 30 seconds. Immediately after mixing, 5-mL of sample from each tube was transferred to a sterile 15-mL culture tube. Two of the samples were transferred in duplicate for a total of six tubes per substrate type. The tubes were labeled, and 0.5-mL of 10% formaldehyde was added to one tube from each duplicate sample to serve as a killed control. The killed controls were mixed on a vortex for 30 seconds to encourage the seepage of formaldehyde into the periphyton.

Samples were labeled by adding 10-µL of [methyl-^{3}H] thymidine (TdR) (82.2 Ci/mmol isotope specific activity; GE Healthcare) to each tube. The tubes were vortexed for 30 seconds in 3 ten-second intervals, and the exact time was noted on a table. The tubes were then incubated for exactly 20 minutes at room temperature. After the incubation was complete, 0.5-mL of 10% formaldehyde was added to each live tube. The tubes were immediately mixed on a vortex for 30 seconds, and placed on ice for 10

minutes. To lyse the cells precipitate the nucleic acid macromolecules, 5.0-mL of ice cold 10% trichloroacetic acid (TCA) was added to each tube, and left on ice to incubate for 30 minutes.

The TCA treated samples were filtered (0.2-µm Nucleopore filter on a 0.45-µm Millipore cellulose membrane filter, both on a 25-mm Millipore filtration base) and rinsed with two 3-mL ice cold 5% TCA washes and one ice cold 5-mL 70% ethanol wash. The 0.2-µm Nucleopore filters were then placed into 20-mL plastic scintillation vials. Once all of the samples had been filtered, 10-mL of scintillation cocktail (3a20; Research Products International Corp.) was added to each vial including three vials without filters that were used to determine the background radiation. The disintegrations per minute (dpm) for each sample was determined using a liquid scintillation counter (Beckman Coulter LS 6500), and the values were then used to calculate the rate of secondary production for each substrate type using the following conversion; cells produced / assay = substrate dpm*(1 Ci / $2.2x10^{12}$ dpm)*(1 mmol TdR/ specific activity Ci)*(1 mol TdR / 10^3 mmol TdR)*($2x10^{18}$ cells produced / 1 mol TdR).

An isotope dilution assay was also performed to determine if there were elevated levels of non-labeled thymidine in the samples. The isotope dilution was completed using one sample from each substrate type chosen at random from the four remaining replicates not used for the secondary production procedure. Periphyton was scraped off of the substrates into 50-mL polypropylene conical centrifuge tubes, which were then filled to 50-ml with filter sterilized (0.2-µm pore size) site water and mixed on a vortex for 30 seconds. Immediately after mixing, 5-mL samples were transferred to five sterile 15-mL culture tubes for each substrate type. Then, prior to adding the [methyl-^{3}H]

thymidine, various volumes of 1-µM non-labeled thymidine solution were added to the five tubes to final concentrations of 0, 5, 15, 25, and 35-nM non-labeled thymidine. After the non-labeled thymidine was added, the procedure followed the same steps as the secondary production procedure described above. The results were graphed, and a linear regression was used to determine if there was isotope dilution in the samples.

Bacterial enumerations were completed using the AODC method (APHA 2005). Duplicate periphyton samples from each substrate type were scraped into sterile 15-mL culture tubes containing 2.0-mL of sterile 50-mM phosphate buffer and 1-mL of 5% glutaraldehyde, and stored in the dark at 4ºC. On the day of analysis, 6-mL of 50-mM sterile phosphate buffer was added to each sample, and the tubes were mixed on a vortex for 30 seconds. Immediately after mixing, three 10-mL serial 1/10 dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were prepared in phosphate buffer for each sample. Once the serial dilutions were complete, 0.3-mL of 0.025% acridine orange stain was added to each sample and the samples were left to incubate for 10 minutes. Samples were filtered (black 0.2-µm Nucleopore filter on a 0.45-µm Millipore cellulose membrane filter, both on a 25-mm Millipore filtration base) and rinsed with one 5-mL sterilized 50-mM phosphate buffer wash. After rinsing, the black 0.2-µm Nucleopore filter was mounted on a microscope slide with a single drop of low fluorescence immersion oil. Another drop of oil was added on top of the filter followed by a cover slip and stored in the dark at 4ºC.

The slides were observed with an epifluorescent microscope with an acridine orange filter set. A Whipple ocular micrometer (i.e., Whipple grid) was calibrated and inserted into the ocular of the epifluorescent microscope. A total bacterial count of at least 400 cells per sample was recorded by counting at least 20 bacterial cells per field in

20 randomly selected fields. The data from this analysis was used to determine the total number of bacterial cells per unit of surface area for the periphyton communities on the different substrate types.

Community Composition

Samples from each substrate type were preserved in formalin for an algal survey to characterize the periphyton community. High amounts of microbial biomass and detritus in the samples prohibited a simple analysis of green algae and cyanobacteria genera, so instead the samples were cleaned by oxidizing the organic matter in the samples until only the silica frustules of the diatoms remained (Lowe and LaLiberte 2006). Diatoms were then identified to genera by their frustules (Needham and Needham 1962; Lowe and LaLiberte 2006). The substrate types were analyzed in triplicate, and a total of six-hundred diatom frustules were identified for each sample. The diversity of diatom genera within each periphyton community was determined using the Shannon-Wiener Biodiversity Index (H') (Krebs 1985).

Data Analysis

Due to extensive precipitation events (explained in detail in results), substrates from T_2 could not be used for analysis. Remaining data from T_1 were analyzed with a randomized complete block design to assess the effects of different substrate types on periphyton biomass and ecosystem processes. The blocks were represented by the exclosure deployment periods within T_1. The assumptions for parametric analyses were evaluated using the Shapiro-Wilk test to assess the normality of the data, and the Levene's Test to assess the equality of variances in the data. Analysis of variance (ANOVA; *F*-test) was used to identify significant differences among factor levels

(substrate types). Data from the deployment sets were pooled for each analysis, and sum of squares 4 was used in the ANOVA model to account for possible block effects and missing values. If significant differences were found, a Tukey's HSD test was used to determine where among the factor levels the differences occurred. In addition, data from the three substrate types were collapsed to calculate Spearman's rank correlation coefficients in order to explore relationships among the different variables. All comparisons and correlations were evaluated at $\alpha = 0.05$. All statistical analyses were conducted using the statistical software package "R" (R Development Core Team 2006) or SAS statistical software (V. 9.31; SAS Institute Inc. 2004).

RESULTS

Exclosure Deployments

The data collected were from four exclosure deployment periods during the early summer time period (T_1; late May thru early July, 2010). Exclosures were deployed for two separate late summer time periods (T_2; early August thru late September, 2010), however both deployments were cut short due to precipitation events that resulted in considerable rises in the stage of the UMR as well as significant flooding (Fig. 5). Flooding during the T_2 time period deposited sediment on the substrates, rendering them unusable for analyses. In addition, the exclosures could not be retrieved within the 21 day incubation period because of unsafe boating conditions.

Fluctuations in the stage of the UMR also affected the physical and chemical characteristics measured at the Target Lake study site. The mean (± SD) values of the physiochemical measurements taken from the surface waters during the study were; exclosure depth 0.475 ± 0.32 m, temperature 23.1 ± 1.9 °C, pH 7.76 ± 0.48, DO 7.61 ± 2.3 mg/L, DO saturation 86.2 ± 28 %, specific conductivity 437 ± 38 µS/cm, salinity 0.221 ± 0.02 ppt, TSS 3.06 ± 1.3 mg/L, TDS 349 ± 50 mg/L, turbidity 3.55 ± 1.5 NTU, ammonium 55.9 ± 45 µg NH_4^+-N/L, nitrate 509 ± 735 µg NO_3^--N/L, and soluble reactive phosphorus 11.6 ± 11 µg P/L. The complete weekly data for the physiochemical measurements can be found in Appendix A.

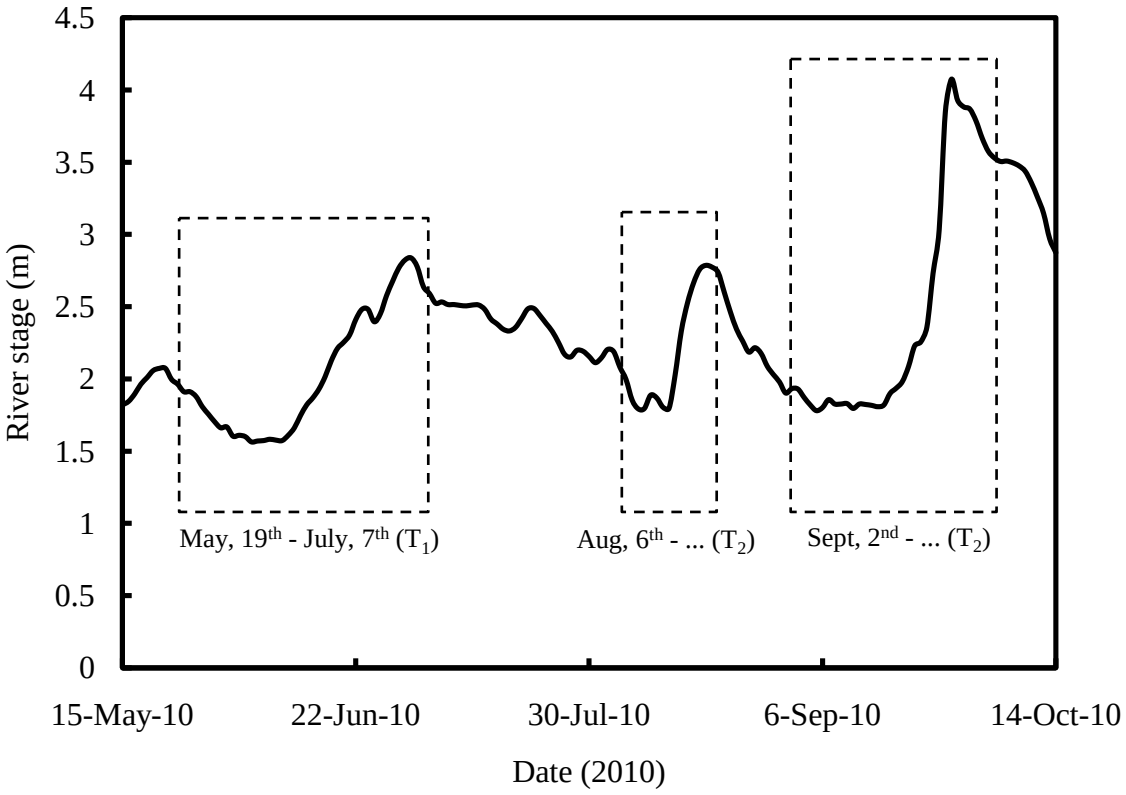

Fig. 5. Hydrograph of the Mississippi River at La Crosse, WI (river mile 696.9; N 43° 83.33', W 91° 25.00') between May 15th and October 14th, 2010. The dashed boxes outline the T_1 exclosure deployment period, and the two attempted T_2 exclosure deployments. Data obtained from the US Army Corps of Engineers Water Control Center, St. Paul District.

Periphyton Biomass and Community Composition

There was a significant difference among treatments for both chlorophyll *a* concentration ($F_{(2,83)} = 15.66$; $p < 0.0001$) (Fig. 6) and AFDM ($F_{(2,87)} = 18.33$; $p < 0.0001$) (Fig. 7). However, the biomass patterns among treatments were not the same between the two analyses as the mean chlorophyll *a* concentration was significantly higher on the pine substrate, while the mean AFDM was significantly lower on the tile substrate ($p < 0.05$ for both). In addition there was a significant difference among treatments for AI ($F_{(2,87)} = 32.40$; $p < 0.0001$), as the poplar substrate was significantly higher than the tile and pine substrates ($p < 0.05$) (Fig. 8). This indicated that there may have been more heterotrophic associations with the poplar substrates than with either the tile or pine substrates.

The AI did not have a strong correlation with chlorophyll *a* concentration (Fig. 9), however there was a significant positive correlation between AI and AFDM ($r = 0.6294$, $p = 0.0283$) (Fig. 10). These results indicated that the heterotrophic portion of the periphyton, which is accounted for by the AFDM analysis, may have had an important influence on the overall trophic structure of the periphyton community. In addition, AFDM was positively correlated with AODC ($r = 0.9429$, $p = 0.0048$) (Fig. 11), and exclosure depth ($r = 0.7773$, $p = 0.0029$) (Fig. 12).

The three most dominant diatom genera regardless of substrate type were *Cocconeis* sp., *Navicula* sp., and *Gomphonema* sp. (Table 1). However, the mean Shannon-Weiner diversity indices (H') were significantly different among treatments ($F_{(2,6)} = 40.75$, $p = 0.003$). The mean H' for tile and pine were not significantly

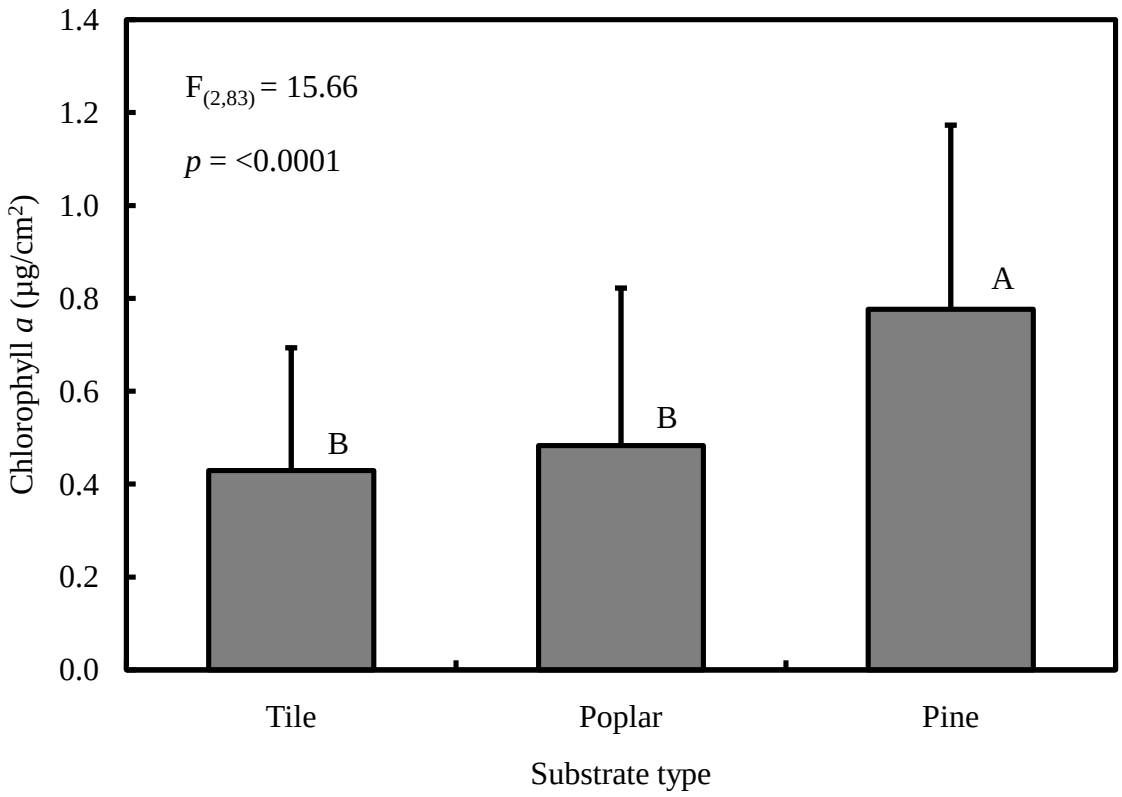

Fig. 6. Mean (± 1 SD) phaeophytin-corrected chlorophyll *a* concentrations on three artificial substrate types. Data were pooled from four exclosure deployment periods in Target Lake between May 28th and July 7th, 2010. *F*-test results shown are for the randomized complete block ANOVA model. Different letters associated with the treatment means signify significant difference ($p < 0.05$; Tukey's HSD test) among the treatments.

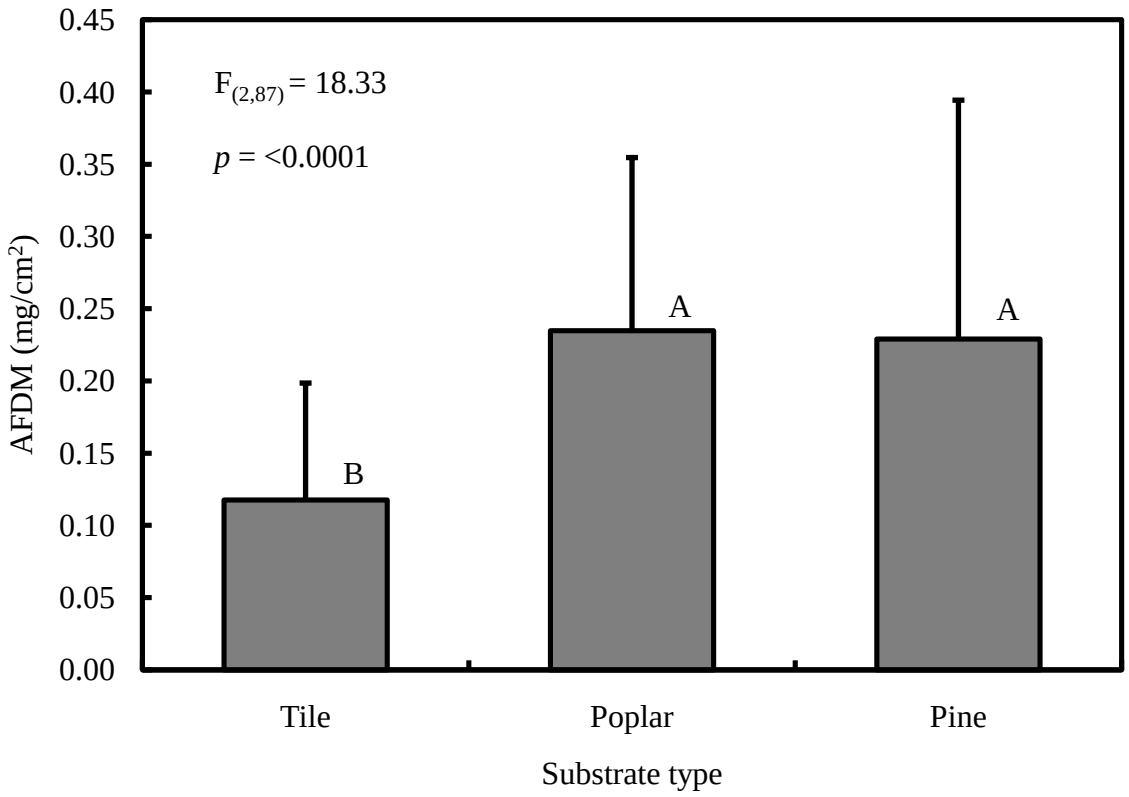

Fig. 7. Mean (± 1 SD) ash-free dry mass (AFDM) on three artificial substrate types. Data were pooled from four exclosure deployment periods in Target Lake between May 28th and July 7th, 2010. *F*-test results shown are for the randomized complete block ANOVA model. Different letters associated with the treatment means signify significant difference ($p < 0.05$; Tukey's HSD test) among the treatments.

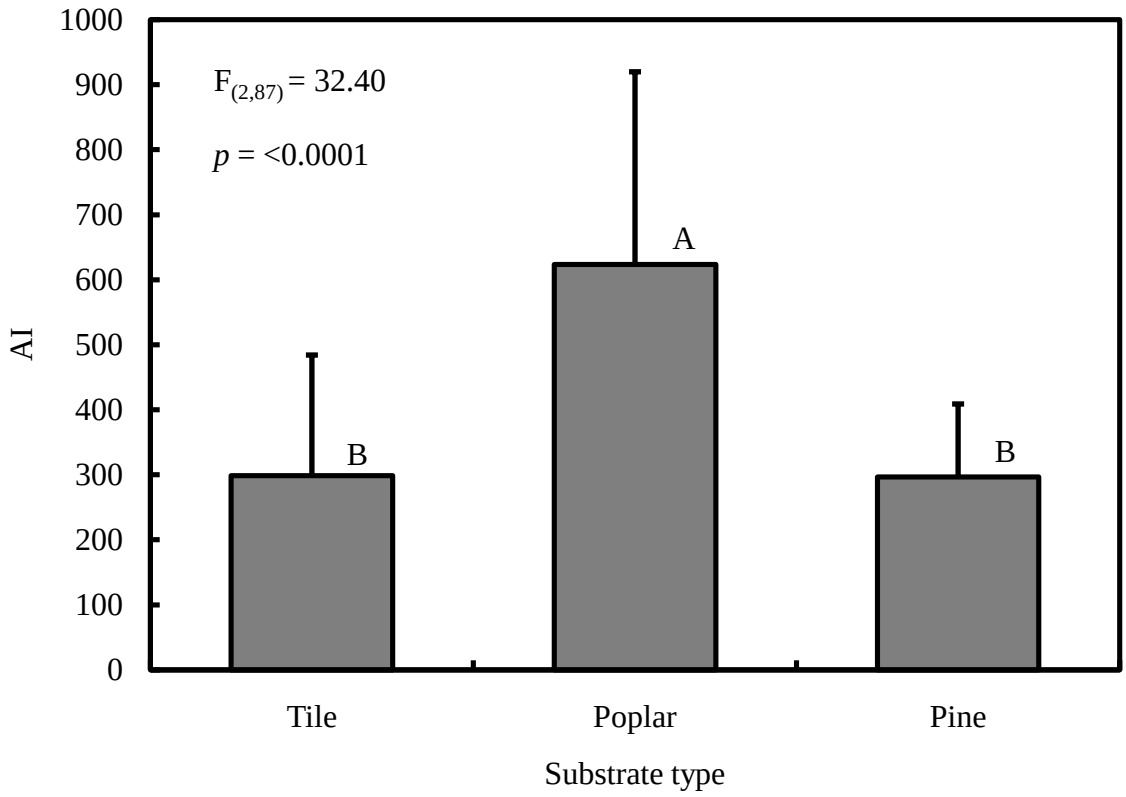

Fig. 8. Mean (± 1 SD) autotrophic index (AI) values on three artificial substrate types. Data were pooled from four exclosure deployment periods in Target Lake between May 28th and July 7th, 2010. *F*-test results shown are for the randomized complete block ANOVA model. Different letters associated with the treatment means signify significant difference ($p < 0.05$; Tukey's HSD test) among the treatments.

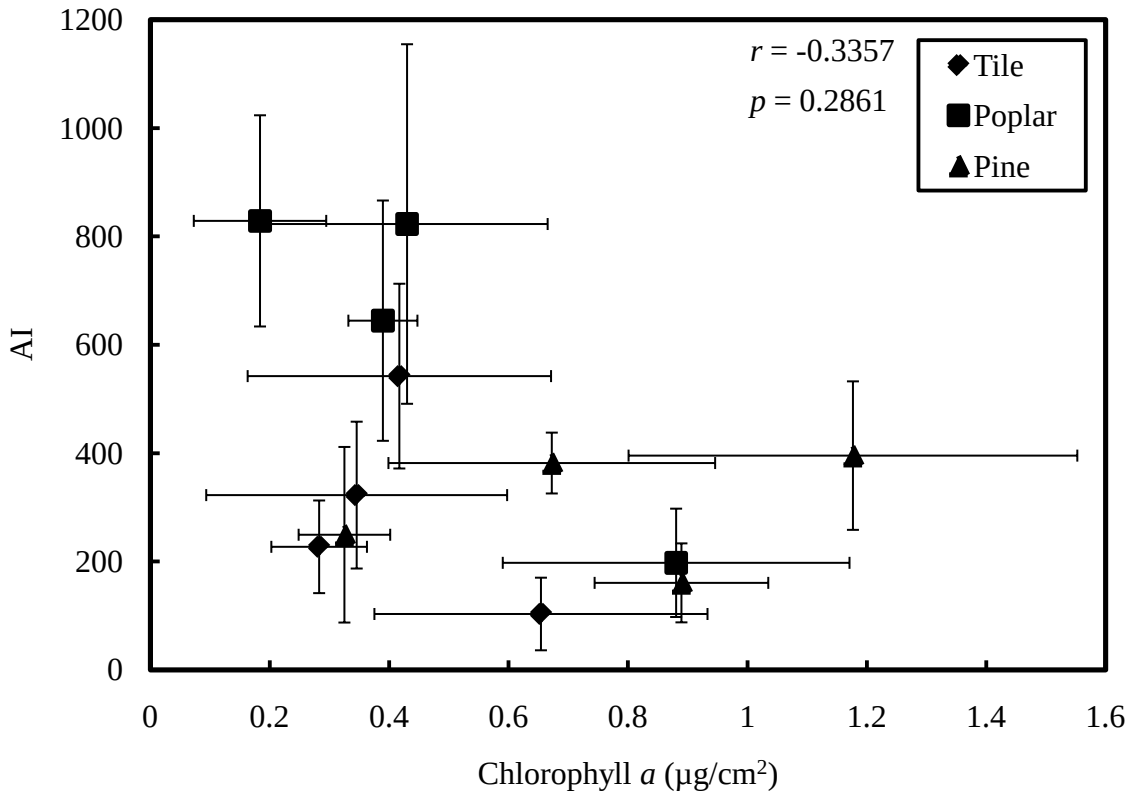

Fig. 9. Mean phaeophytin-corrected chlorophyll *a* concentrations in relation to the mean autotrophic index (AI) on three artificial substrate types from four exclosure deployment periods in Target Lake between May 28th and July 7th, 2010. Correlation and significance coefficients shown are for the Spearman's rank correlation among all values independent of substrate type. Error bars = ± 1 SD.

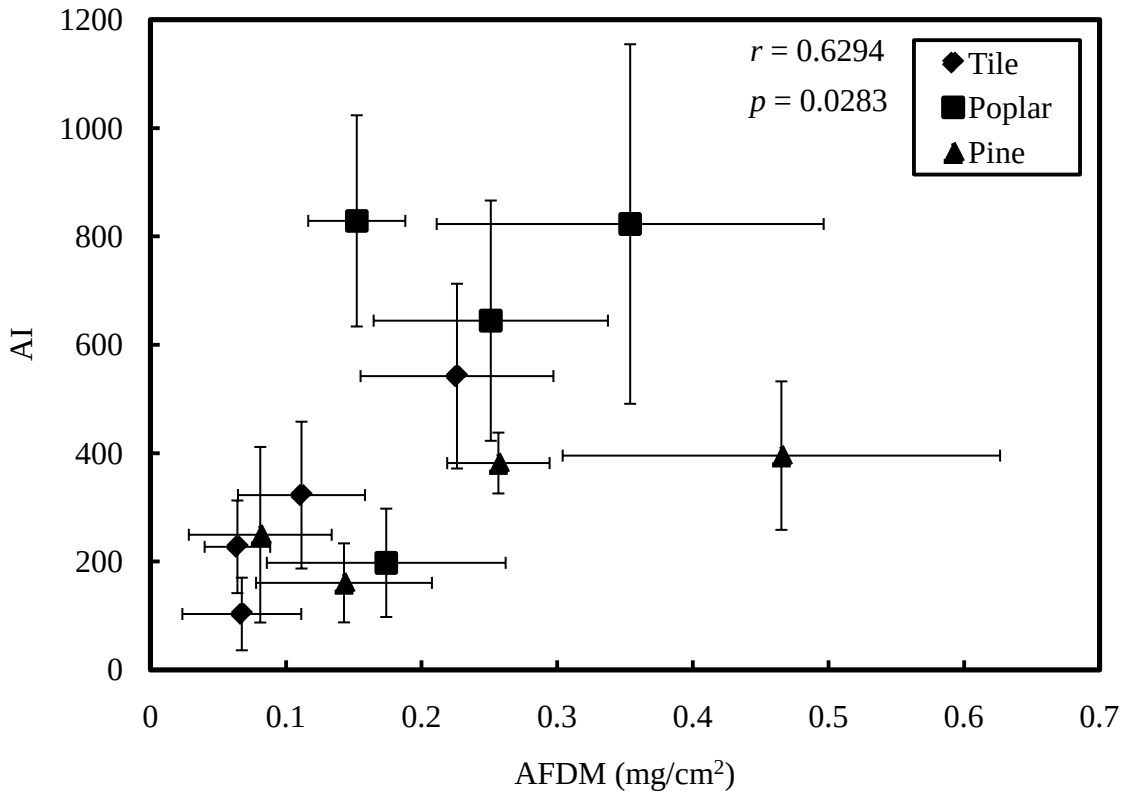

Fig. 10. Mean ash-free dry mass (AFDM) in relation to the mean autotrophic index (AI) on three artificial substrate types from four exclosure deployment periods in Target Lake between May 28th and July 7th, 2010. Correlation and significance coefficients shown are for the Spearman's rank correlation among all values independent of substrate type. Error bars = ± 1 SD.

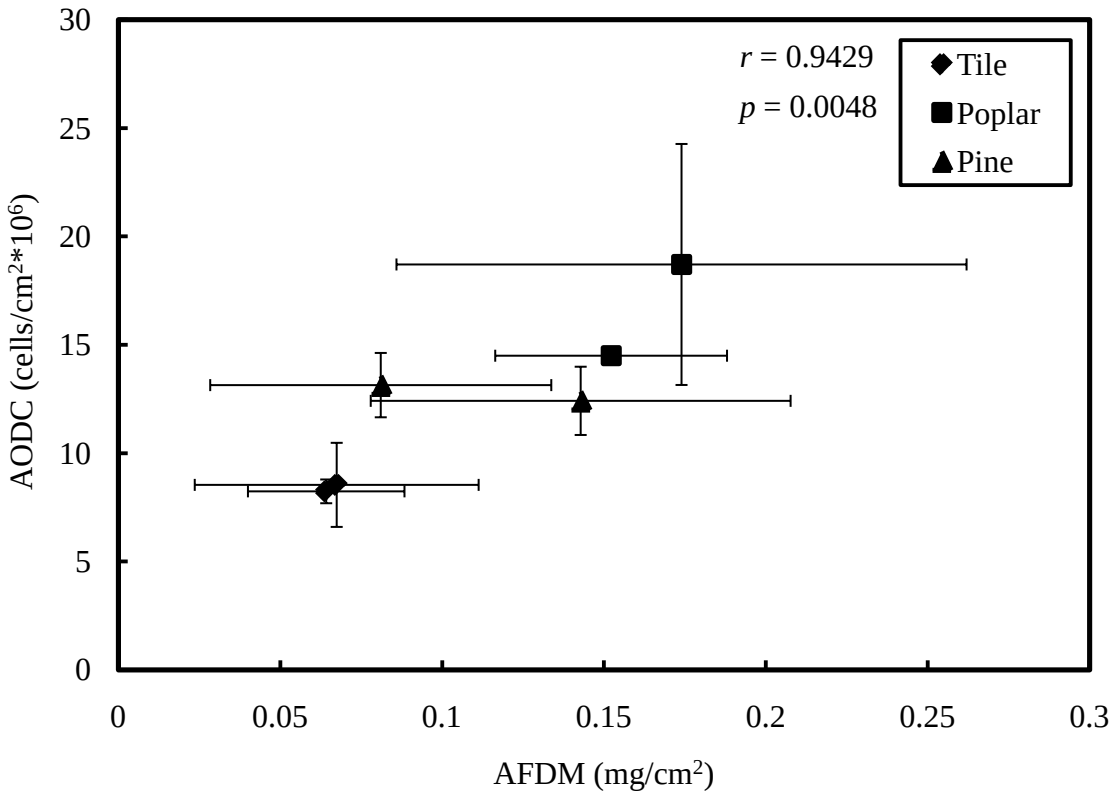

Fig. 11. Mean ash-free dry mass (AFDM) in relation to the mean number of bacterial cells on three artificial substrate types from two exclosure deployment periods in Target Lake between June 10th and July 7th, 2010. The number of bacterial cells was determined using the acridine orange direct count method (AODC) with a 10^{-1} dilution for all samples. Correlation and significance coefficients shown are for the Spearman's rank correlation among all values independent of substrate type. Error bars = ± 1 SD.

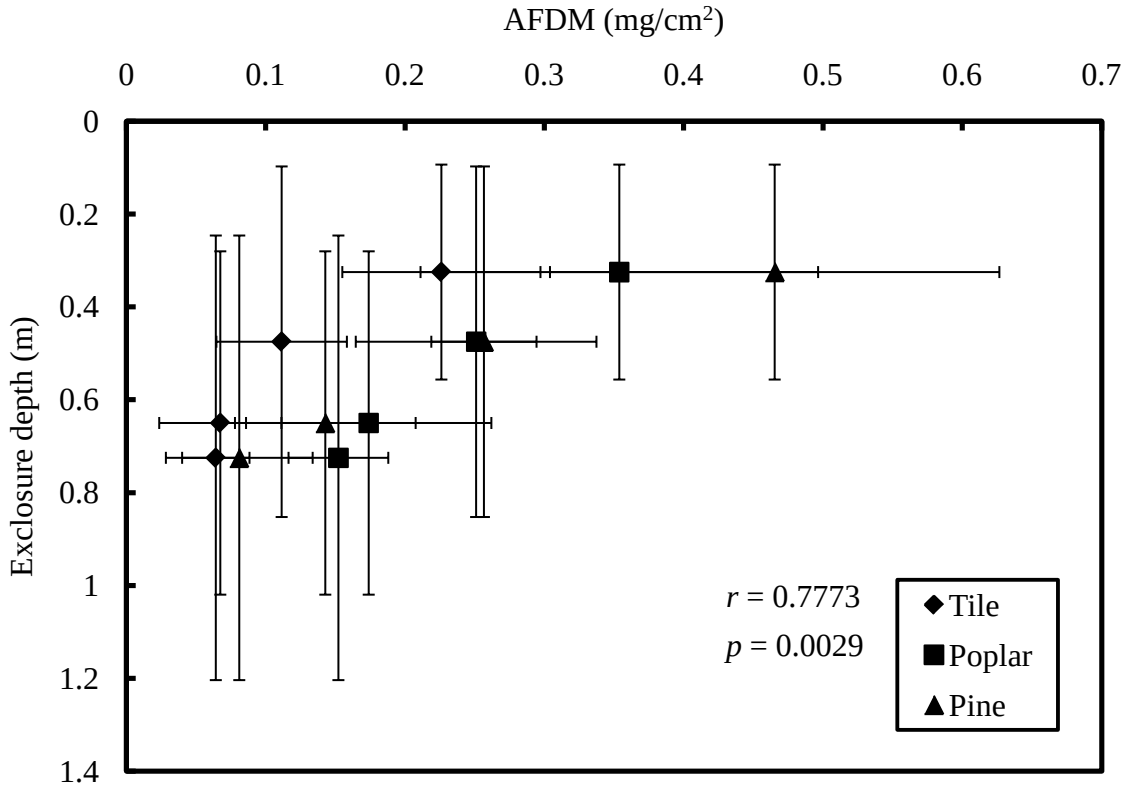

Fig. 12. Mean ash-free dry mass (AFDM) in relation to mean exclosure depth on three artificial substrate types from four exclosure deployment periods in Target Lake between May 28th and July 7th, 2010. Correlation and significance coefficients shown are for the Spearman's rank correlation among all values independent of substrate type. Error bars = ± 1 SD.

Table 1. Mean (±1 SD) values for Shannon-Wiener diversity indices (H') and percent relative abundance (%RA) of diatom genera on three artificial substrate types (n = 3 for each substrate type) from an exclosure deployment between May 18th and June 19th, 2010 in Target Lake. F-test results from the overall one-way ANOVA model indicate a significant difference between the H' means ($F_{(2,6)}$ = 40.75, p = 0.003), and H' means that share a superscript letter are not significantly different from one another (Tukey's HSD test, $p < 0.05$).

Substrate type	Mean H'	Genus	Mean % RA
Tile	2.11 ± 0.08^{A}	*Cocconeis* sp.	34.2 ± 2.02
		Navicula sp.	28.1 ± 0.54
		Gomphonema sp.	26.5 ± 2.29
		Epithemia sp.	5.23 ± 0.94
		Cymbella sp.	1.80 ± 0.20
		Rhoicosphenia sp.	1.58 ± 0.21
		Fragilaria sp.	1.20 ± 0.69
		Synedra sp.	0.71 ± 0.06
		Cyclotella sp.	0.27 ± 0.11
		Rhopalodia sp.	0.11 ± 0.06
		Amphora sp.	0.11 ± 0.03
		Melosira sp.	0.11 ± 0.06
		Nitzschia sp.	0.05 ± 0.03
		Cymatopleura sp.	----
Poplar	1.55 ± 0.10^{B}	*Cocconeis* sp.	61.5 ± 0.94
		Navicula sp.	25.1 ± 0.63
		Gomphonema sp.	8.83 ± 0.56
		Epithemia sp.	0.66 ± 0.10
		Cymbella sp.	1.15 ± 0.15
		Rhoicosphenia sp.	1.15 ± 0.10
		Fragilaria sp.	----

Table 1. continued

Substrate type	Mean H'	Genus	Mean % RA
Poplar	1.55 ± 0.10^{B}	*Synedra* sp.	0.27 ± 0.08
		Cyclotella sp.	0.49 ± 0.15
		Rhopalodia sp.	----
		Amphora sp.	0.16 ± 0.05
		Melosira sp.	0.38 ± 0.11
		Nitzschia sp.	----
		Cymatopleura sp.	0.22 ± 0.08
Pine	2.06 ± 0.05^{A}	*Cocconeis* sp.	53.1 ± 0.56
		Navicula sp.	20.4 ± 1.14
		Gomphonema sp.	12.0 ± 1.87
		Epithemia sp.	2.17 ± 0.19
		Cymbella sp.	4.46 ± 0.60
		Rhoicosphenia sp.	0.98 ± 0.11
		Fragilaria sp.	----
		Synedra sp.	0.49 ± 0.00
		Cyclotella sp.	0.33 ± 0.05
		Rhopalodia sp.	5.65 ± 1.25
		Amphora sp.	0.05 ± 0.03
		Melosira sp.	0.22 ± 0.08
		Nitzschia sp.	----
		Cymatopleura sp.	0.11 ± 0.03

different ($p = 0.1603$), but were both significantly higher than that on poplar ($p < 0.05$ for both).

There was a significant difference among treatments for AODC ($F_{(2,8)} = 10.16$; $p = 0.0064$) (Fig. 13). The AODC was significantly higher on poplar than on tile ($p < 0.05$), and pine was not significantly different from either tile or poplar.

Primary and Secondary Production

The mean rates for both NBP ($F_{(2,17)} = 3.02$; $p = 0.0756$) and RB ($F_{(2,17)} = 0.33$; $p = 0.7257$) were not significantly different among treatments (Fig. 14). There was not a correlation between NBP and RB ($r = 0.8571$, $p = 0.8717$).

Isotope dilution assays were not performed during the analysis of secondary production from the first exclosure deployment period, so those data were not used for statistical analyses. This also resulted in too few data points to provide meaningful correlations between AODC and secondary production. Isotope dilution assays from the second exclosure deployment period indicated there was a substantial pool of thymidine in the samples and therefore the original specific activity of 82.2 Ci/mmol for the [methyl-^{3}H] thymidine had to be corrected in the tile, poplar, and pine treatments to 43.4, 39.9, and 41.5 Ci/mmol respectively, prior to calculating the rates of secondary production. The rates of secondary production were not significantly different among treatments ($F_{(2,9)} = 4.15$; $p = 0.0528$), however the results did suggest a biological importance as the secondary production rate on the tile was noticeably less than the rate on either poplar or pine (Fig. 15).

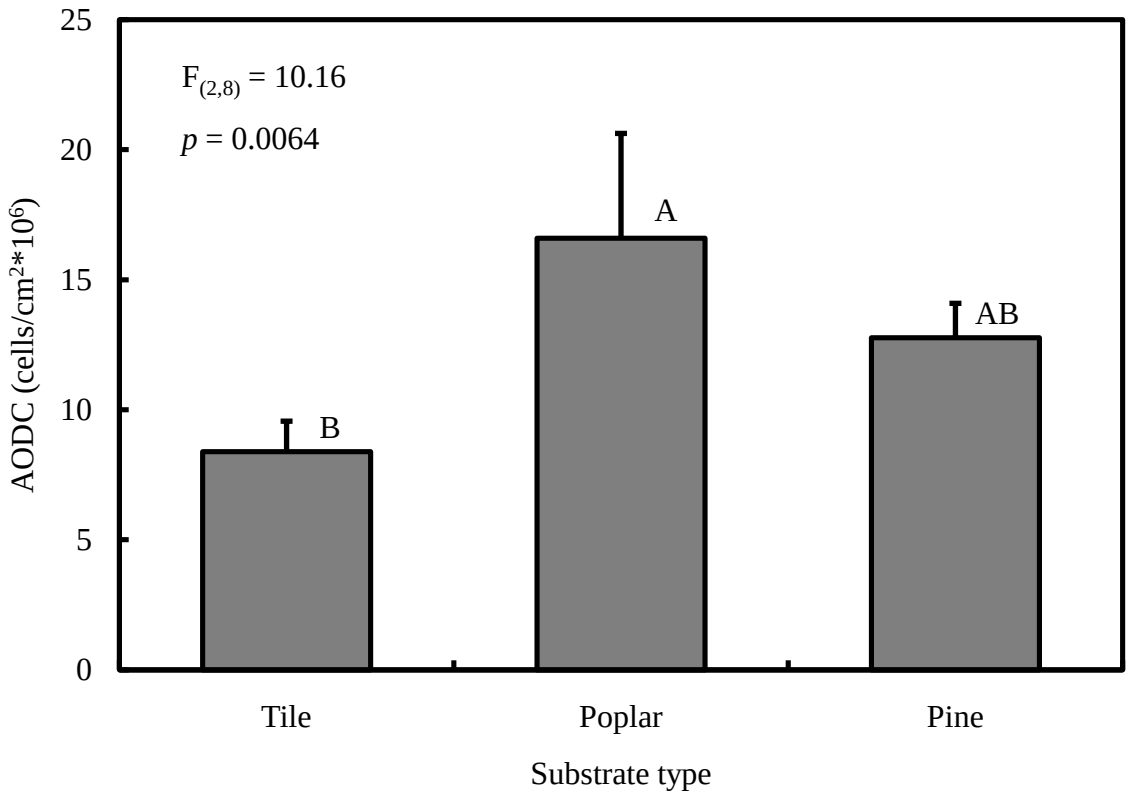

Fig. 13. Mean (± 1 SD) number of bacterial cells (determined by AODC with a 10^{-1} dilution) on three artificial substrate types. Data were pooled from two exclosure deployment periods in Target Lake between June 10th and July 7th, 2010. *F*-test results shown are for the randomized complete block ANOVA model. Different letters associated with the treatment means signify significant difference ($p < 0.05$; Tukey's HSD test) among the treatments.

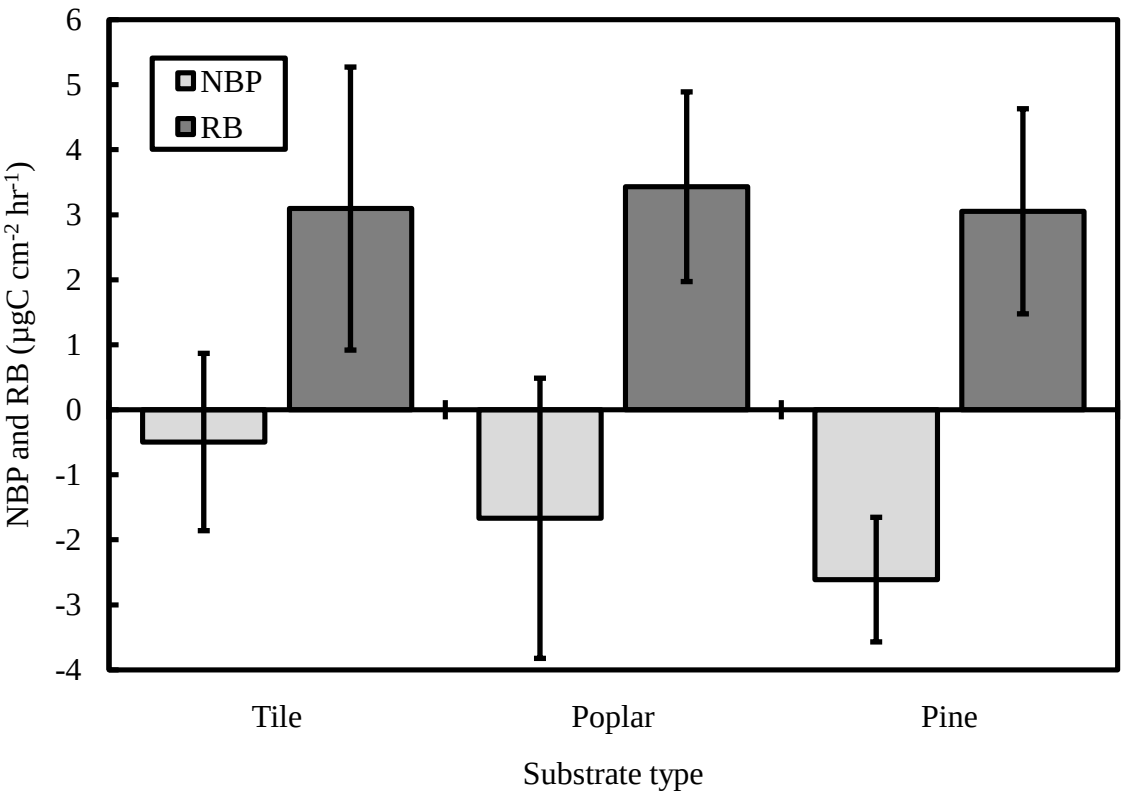

Fig. 14. Mean (± 1 SD) rates of net biomass production (NBP; $F_{(2,17)} = 3.02$, $p = 0.0756$) and biomass consumed during respiration (RB; $F_{(2,17)} = 0.33$, $p = 0.7257$) on three artificial substrate types. Data were pooled from two exclosure deployment periods in Target Lake between May 28th and June 24th, 2010. *F*-test results are for the randomized complete block ANOVA model.

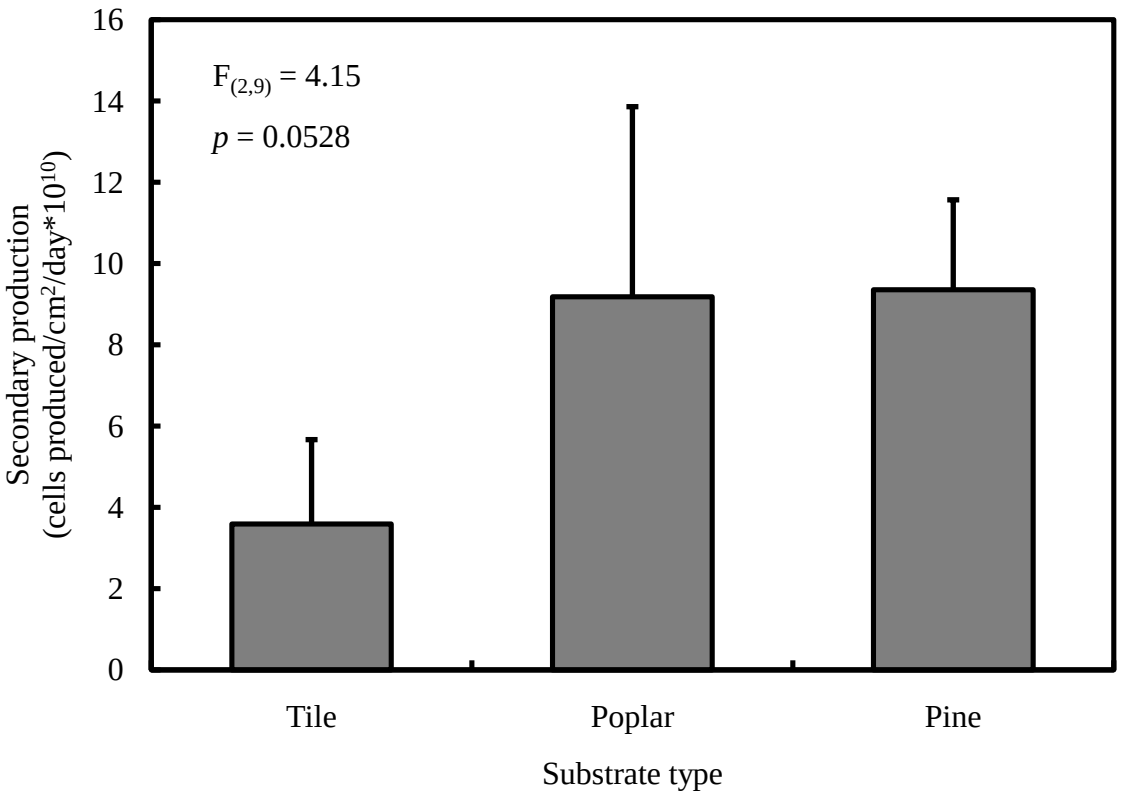

Fig. 15. Mean (± 1 SD) secondary production rates on three artificial substrate types from an exclosure deployment period between June 16th and July 7th, 2010 in Target Lake. *F*-test results shown are for the overall one-way ANOVA model.

DISCUSSION

Experimental Design: Exclosures and Substrates

The development of new and innovative sampling techniques has facilitated major contributions to periphyton ecology by giving researchers the ability to control for various environmental factors that influence the structure and function of periphytic communities including water velocity (Dodds and Biggs 2002), nutrient conditions (Fairchild *et al*. 1985; Tank and Dodds 2003), depth (Liboriussen and Jeppessen 2006), substrate type (Lowe *et al*. 1996), and invertebrate grazing (Wellnitz *et al.* 1996; Kiffney and Bull 2000). The substrate exclosures that were fabricated exclusively for this study successfully allowed for the control of substrate type as well as grazing pressure by macroinvertebrates, and the straightforward design made deployment and retrieval easy and time efficient. A major disadvantage of the substrate exclosure design, and more specifically the deployment strategy, was a susceptibility to sudden increases in the stage of the UMR (i.e., significant increases in exclosure depth) that likely caused significant block effects among deployment periods during the early summer time period, and rendered substrates completely unviable for analysis during the late summer time period. The exclosure rigs allowed for weekly depth adjustments in response to small fluctuations in exclosure depth at the study site ($\leq$ ~0.2-m), but were not designed to handle significant increases in exclosure depth that ultimately resulted in a high potential for light limitation from increased depth and turbidity, reduced water flow to the substrates due to fouling of the mesh screens, and in some cases prevented access to the

study site all together. Despite the difficulties that this study faced as a result of unusually high amounts of precipitation in the UMR valley during the summer of 2010, the exclosure design did function as intended and would likely be more effective if deployed in aquatic systems that do not experience regular fluctuations in water depth (e.g., lake littoral zones and non-flashy river systems).

The use of artificial substrates to culture and study periphyton is common because they allow for the control of heterogeneity among samples (Biggs 1989; Lowe *et al.* 1996; Murdock and Dodds 2007), but concerns have been raised about how well they compare to natural substrates. A study by Cattaneo and Amireault (1992) showed that artificial substrates may provide under (e.g., epilithic) or over (e.g., epiphytic) estimations of periphyton growth when compared to naturally occurring substrates from the same environment. Still, artificial substrates can be useful tools to discern differences in periphyton production among several different substrate types including the three that were used in this study (i.e., tile, poplar, and pine). Quantifiable periphyton growth was observed on all of the substrates retrieved for analysis, and differences among the results for various analyses could be due to the differing physical and chemical properties unique to each substrate type.

Periphyton Biomass and Ecosystem Processes

The heterogeneous composition of backwater habitats in the UMR presents a wide range of substrate types for periphytic communities to colonize and grow. The three artificial substrate types used in this study each represented a type of hard substrata that can be found throughout different UMR habitats, and periphyton growth on the substrates was reflected in biomass and productivity measurements. Several studies have

shown that periphyton growth can differ among various substrate types (Vadenboncoeur and Lodge 2000; Potapova and Charles 2005; Vadenboncoeur *et al.* 2006; Murdock and Dodds 2007). Periphyton growth can also change along depth gradients (Lowe *et al.* 1996), however, this factor was not intended to be accounted for in my study. Unfortunately record amounts of precipitation resulted in high variability in substrate depth, both within and among deployment periods, which correlated strongly with total periphytic biomass (i.e., AFDM) (Fig. 12).

Phaeophytin-corrected chlorophyll *a* concentration (Fig. 6) and AFDM (Fig. 7) were both significantly different among substrates, indicating that total periphytic biomass does differ among various types of hard substrata. These results suggest that studies which combine data from rock and wood substrates to represent a single group of hard substrata to be compared against various types of soft substrata (Potapova and Charles 2005; Vadeboncoeur *et al.* 2006) may be introducing error by assuming that periphytic biomass is the same among differing hard substrate types. In addition, AI and H' indicated that the poplar substrate had significantly more heterotrophic associations as well as a significantly lower diatom diversity (Fig. 8; Table 1, respectively), which provided further evidence that the periphytic communities differed greatly among the substrate types.

Differences in periphyton production may have been due to the unique chemical composition of each substrate type. None of the substrates used in this study supplemented the periphytic communities with nutrients (e.g., N and P) like has been observed with periphyton growth on macrophytes (Dodds 2003). However, the poplar and pine substrates may have exuded various chemical compounds produced by

colonizing fungi that discouraged or inhibited the colonization and growth of other microorganisms. A study by Heilmann-Clausen and Boddy (2005) showed that fungal exudates from colonized beech wood can act as defense mechanisms to inhibit colonization by competing fungi, and thus greatly affect the microbial communities in the wood. In addition, Heilmann-Clausen and Boddy (2005) expressed concerns regarding the potential for a change in the natural chemical composition of the wood substrates by means of autoclaving. The poplar and pine substrates used in my study were not autoclaved, but their chemical composition most likely was altered from its natural state during the kiln-drying process.

The physical characteristics of the substrates were also an important factor to consider when assessing differences in periphyton production. A study by Murdock and Dodds (2007) examined periphytic colonization of artificial substrates of differing roughness and determined that substrate texture can significantly affect the accrual of periphyton biomass. The relationship between substrate topography and biomass accrual was relevant to my study because the unglazed ceramic tiles were textured, the poplar substrates were cut to a cross grain (i.e., grain perpendicular to fibers in the wood), and the pine substrates were cut to a face grain (i.e., grain parallel to fibers in the wood). These physical differences in substrate topography and roughness may have promoted or inhibited periphyton colonization and growth, and may also have affected the efficiency of the scraping method used to quantitatively remove the periphyton from the substrates. Future studies looking more closely at effects of substrate type on periphyton growth should bear in mind the physical and chemical characteristics associated with different types of artificial substrates.

The lack of significant differences among treatments for NBP and RB (Fig. 14), as well as the error associated with the results, was most likely the result of changes in light intensity caused by different exclosure depths and water turbidity. In addition, the amount of living periphyton biomass per individual substrate may not have been enough to significantly influence changes in DO concentration, and instead the measured results may have been due to microorganisms (e.g., nanoplankton and bacteria) in the filtered site water used to fill the BOD bottles. The negative values for NBP may have indicated an excess of decaying organic material, which is often seen in aquatic systems following large inputs of allochthonous organic matter (Roelke *et al.* 2006). Since there were no extraneous inputs of organic matter into the BOD bottles after the periphyton was introduced, these results could indicate that the initial three week incubation period was too long and resulted in a significant amount of senesced periphytic matter that was broken down by microbes during the BOD bottle incubation.

Although the overall results from my analysis of periphyton primary production and community respiration were inconclusive, other studies have shown that area-specific production of oxygen by periphyton on rock and wood substrates is significantly less than on sediments (Vadeboncoeur *et al.* 2006), and that primary production increases with total periphytic algal biomass (Liboriussen and Jeppesen 2006). Oxygen produced as a result of primary production by periphyton, and the subsequent respiration of oxygen by microbes decomposing organic matter within the periphyton, both provide important ecological services to shallow aquatic ecosystems like those found throughout the UMR. Future studies measuring periphyton NBP and RB should consider using more biomass

(i.e., substrates with greater surface area or combining several substrate replicates per bottle) to increase quantifiable productivity and obtain more conclusive results.

The rate of secondary production was not statistically significant among substrate types (Fig. 15), but a $p = 0.0528$ implied a possible biological significance which might be confirmed with an increase in replicates. There were not enough data points to perform meaningful correlations between the rate of secondary production and any other factor, and an unidentifiable source of error in the data from either the rate of secondary production or bacterial enumerations (i.e., AODC) prevented the determination of reasonable results for the turnover time of the bacterial populations. There was a significant positive correlation between AFDM and AODC (Fig. 11), suggesting that bacteria represented a significant portion of the heterotrophic biomass associated with the periphyton. However, there was not a significant correlation between chlorophyll *a* concentration and AODC, even though several studies have shown that bacterial production and densities are positively correlated with algal biomass (Hepinstall and Fuller 1994; Rier and Stevenson 2001).

The conversion of dissolved organic carbon into bacterial biomass (i.e., secondary production) is an important part of aquatic food chains (Fuhrman and Azam 1982), but is usually measured in planktonic, rather than periphytic, samples. Since the procedure used in this study was designed for planktonic samples, an assumption was made that periphyton samples were sufficiently homogenized using a vortex (see secondary production and bacterial enumerations methods). This assumption was not tested, but should be prior to future studies to identify possible error associated with homogenizing or to determine an efficient homogenizing technique. In addition, isotope dilution assays

indicated the presence of high amounts of non-labeled thymidine associated with the periphyton samples which decreased the [methyl-^{3}H] thymidine specific activity by approximately 50% for each substrate type.

Conclusions

Although most studies in periphyton ecology focus on the autotrophic portion of periphyton, the results from my study including relatively high AI values, high rates of secondary production, and a significant positive correlation between AFDM and AODC, indicate that the heterotrophic portion of periphyton comprises the majority of the periphytic biomass and also plays an important role in the structure and function of the periphytic communities on rock and wood substrates in the UMR. Even though some of the results from this study were inconclusive, they can be used to infer several interesting relationships and correlations about the ecology of periphyton on different hard substrates in the UMR. In addition, the novel substrate exclosures used in this study functioned as intended, and would have provided more definitive results had it not been for the variability in exclosure depth throughout the incubation periods. The unpredictability of large aquatic ecosystems like the UMR can present numerous problems for experimental studies, as seen in this study by the effects of early and late summer flooding. However, a focus on furthering benthic research in the UMR by implementing new and innovative methods will be critical to the future management, conservation, and restoration of the ecosystem.

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APPENDIX A

TABLE 2. COMPILED DATA FOR THE PHYSICAL AND CHEMICAL PARAMETERS MEASURED IN TARGET LAKE, 2010

Table 2. Compiled data for the physical and chemical parameters measured in Target Lake, 2010 during each exclosure deployment, acrylic tube replacement, and exclosure retrieval.

		Physiochemical parameters			
Exclosure #	Date	Temperature (°C)	pH	Dissolved O_2 (mg/L)	Dissolved O_2 (% saturation)
1-4	May, 28	24.57	8.09	8.51	101.4
	June, 3	24.75	8.65	10.11	120.6
	June, 9	25.03	8.06	9.80	117.2
	June, 19	22.08	7.59	9.71	111.1
5-8	May, 28	24.47	7.83	8.02	97.7
	June, 3	25.23	8.45	8.78	104.0
	June, 10	20.57	7.74	5.43	60.2
	June, 19	21.82	7.11	7.49	85.4
9-16	June, 3	26.07	8.59	9.70	113.2
	June, 10	20.72	7.97	6.43	71.4
	June, 16	19.89	7.21	8.41	92.0
	June, 24	23.68	7.39	5.03	59.0
17-24	June, 10	21.05	7.70	5.52	61.8
	June, 16	19.92	6.75	7.77	48.8
	June, 25	24.35	7.69	6.31	75.0
	June, 29	22.81	7.44	5.51	64.6
25-32	June, 16	20.86	7.57	12.92	143.9
	June, 25	23.90	7.53	7.65	89.6
	July, 1	24.53	8.25	7.33	86.5
	July, 7	24.70	7.45	1.71	20.2

Table 2. continued

		Physiochemical parameters		
Exclosure #	Date	Depth (m)	Specific conductivity (µS/cm)	Salinity (ppt)
1-4	May, 28	0.2	426.4	0.21
	June, 3	0.2	359.9	0.18
	June, 9	0.2	459.2	0.23
	June, 19	0.7	397.7	0.20
5-8	May, 28	0.2	492.6	0.25
	June, 3	0.2	432.4	0.22
	June, 10	0.2	452.2	0.23
	June, 19	0.7	437.6	0.22
9-16	June, 3	0.2	400.0	0.21
	June, 10	0.2	413.5	0.21
	June, 16	0.5	465.4	0.23
	June, 24	0.9	418.9	0.21
17-24	June, 10	0.2	457.5	0.23
	June, 16	0.5	530.7	0.27
	June, 25	0.9	396.2	0.20
	June, 29	1.3	442.8	0.22
25-32	June, 16	0.2	459.2	0.23
	June, 25	0.6	396.3	0.20
	July, 1	0.9	435.9	0.22
	July, 7	0.5	467.2	0.24

Table 2. continued

Exclosure #	Date	Physiochemical parameters		
		Total suspended solids (mg/L)	Total dissolved solids (mg/L)	Turbidity (NTU)
1-4	May, 28	5.8	413.3	6.1
	June, 3	3.8	293.3	2.6
	June, 9	4.2	295.0	2.8
	June, 19	3.6	315.0	4.4
5-8	May, 28	2.0	410.0	5.8
	June, 3	2.2	296.7	6.1
	June, 10	3.2	360.0	3.0
	June, 19	5.0	416.7	6.1
9-16	June, 3	4.0	316.7	4.6
	June, 10	5.2	438.3	3.6
	June, 16	3.0	338.3	2.7
	June, 24	2.8	303.3	4.4
17-24	June, 10	2.6	445.0	2.2
	June, 16	2.8	328.3	3.2
	June, 25	2.2	371.7	2.1
	June, 29	2.0	325.0	2.1
25-32	June, 16	2.4	316.7	3.0
	June, 25	2.0	380.0	2.2
	July, 1	1.4	330.0	2.3
	July, 7	1.0	293.3	1.7

Table 2. continued

Exclosure # P/L)*	Date	Physiochemical parameters		
		Ammonia (µg NH_4^+-N/L)	Nitrate (µg NO_3^--N/L)*	Soluble reactive phosphorus (µg
1-4	May, 28	188.2	3.196	4.596
	June, 3	33.12	26.03	1.374
	June, 9	15.64	23.67	11.21
	June, 19	21.52	801.0	BD
5-8	May, 28	122.2	142.3	3.409
	June, 3	49.55	BD	26.47
	June, 10	55.11	BD	11.04
	June, 19	21.20	845.0	2.901
9-16	June, 3	55.83	BD	15.62
	June, 10	115.0	262.0	2.392
	June, 16	51.49	133.7	BD
	June, 24	10.49	161.8	BD
17-24	June, 10	125.9	291.6	BD
	June, 16	52.21	410.8	BD
	June, 25	25.55	78.49	BD
	June, 29	40.93	2173	29.72
25-32	June, 16	47.30	392.3	1.035
	June, 25	27.48	39.02	BD
	July, 1	24.02	2631	29.18
	July, 7	35.22	242.4	BD

* BD, negative concentrations were considered below the detection level.