

DEVELOPING FUNCTIONAL INDICATORS OF COASTAL WETLAND HEALTH

Graduate Dissertation Improvement Grant

Final Report

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Executive Summary

Nearly all monitoring efforts currently underway in Great Lakes coastal ecosystems are based on structural indicators (e.g., community composition, nutrient concentrations) whereas functional indicators have not been developed or tested for these ecosystems. During this project, a suite of candidate functional indicators were developed with the dual goals of identifying human impacts to Great Lakes wetlands and characterizing the functional nature of these systems. We focused on indicators that 1) represent meaningful ecosystem processes; 2) provide straightforward results that are readily comparable among sites; and 3) capture the complexities of coastal wetlands. A secondary objective was to make indicators cost-effective and simple enough to be easily and routinely monitored. Six functional processes were evaluated as potential indicators: periphyton biomass accrual, macrophyte biomass production, nutrient limitation, water column metabolism, organic matter decomposition, and sediment oxygen demand. Functional indicators were tested during the summer of 2011 at 20 sites that were also sampled as part of a larger GLRI and Michigan Department of Environmental Quality-funded coastal wetland monitoring program. Sites were chosen to span a gradient of surrounding human land use (agriculture and urbanization) and multiple wetland types were included.

Each candidate functional metric was evaluated for its ability to discriminate wetlands based on either human disturbance or natural variability in wetland function. Human disturbance gradients were constructed using chemical/physical and land-use data collected as part of the larger GLRI and MDEQ-funded monitoring program. Other gradients being used to evaluate candidate metrics include total and effective fetch, latitude, and depth. Metrics that appeared to respond to these gradients were retained for further analysis and inclusion in a preliminary functional index.

Our expectation is that, once fully developed and tested, functional indicators will be added to ongoing wetland assessment programs to identify particularly valuable or sensitive wetland habitats, identify and track human impacts that often go undetected with traditional methods, and measure the success of coastal wetland restoration. Though additional analyses must be completed before our functional index is ready for use, this document reports on the progress of indicator development based on work conducted in 2011 with support from the Illinois-Indiana Sea Grant College Program.



Photos (left to right): A wave-swept wetland in Lake Huron, MJC pulling decomposition bags, a fringing wetland in Lake Michigan.

Research Methods and Results

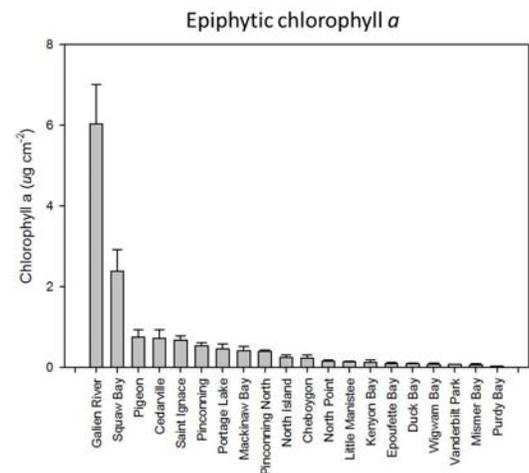
Sites and Sampling Stations

We selected 20 coastal wetland sites on Lakes Huron and Michigan. Sampling sites were chosen from a pool of 52 sites that were being sampled in 2011 for other monitoring efforts (GLRI and Michigan DEQ-funded projects). The 20 sites were selected to span gradients of human disturbance based on surrounding land use and natural gradients in hydrologic energy based on fetch and wave exposure. The 20 sites also represent 3 wetland types—open lacustrine, protected lacustrine, and drowned river mouth—which are the most common coastal wetland types in Lakes Michigan and Huron (Albert et al. 2005). Within each site, we randomly selected an area within the emergent vegetation to place a 23-m transect parallel to the shoreline. Sample points ($n=24$ per wetland) were located along this transect at 1-m intervals. For functional variables requiring *in situ* incubation (e.g., decomposition, periphyton growth, etc.) substrates were deployed July 19-23 and were retrieved September 22-29. Substrates were retrieved in the same order they were deployed in an effort to keep incubation intervals consistent across sites.

Functional Variables Evaluated in 2011

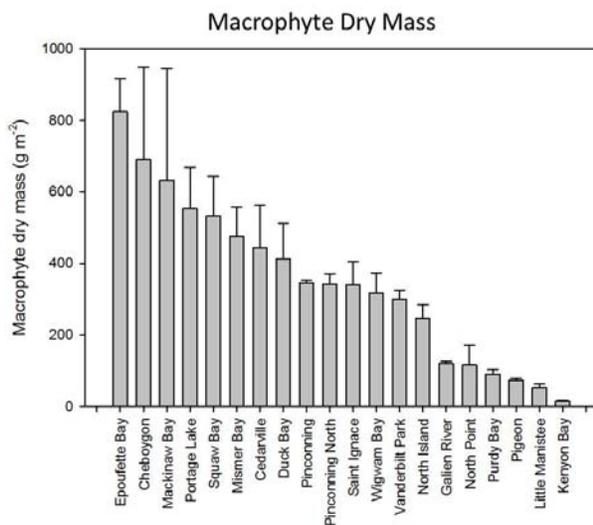
Periphyton Biomass Accrual – Periphyton (algae, bacteria, fungi, and microzoans held within a polysaccharide matrix) forms a critical energy base for aquatic ecosystems (Lamberti et al. 1996) and periphyton biomass accrual provides information on the potential energy available for consumers. In coastal wetlands, macrophyte stems provide substrate for attached organisms and this type of periphyton, known as *epiphyton*, is an important resource for invertebrate consumers. We measured chlorophyll *a* accrual on acrylic rods as a surrogate for epiphytic biomass. At each site, abraded acrylic rods (1.5-cm diameter; $n=5$ per site) were hammered into the substrate and left in place for 6 weeks. Upon retrieval, the bottom 15 cm of each rod was scrubbed with a nylon bristle brush (Photo at right) to dislodge biofilm and contents were collected on GF/C filters and frozen. Chlorophyll *a* was analyzed fluorometrically in the lab.

Epiphytic chlorophyll *a* was highly variable within and among wetlands sites (Figure at right). Mean chlorophyll *a* ranged from 0.03 to $6.0 \mu\text{g cm}^{-2}$. Epiphytic chlorophyll appeared to correlate negatively with wave exposure rather than ambient nutrient concentrations. Epiphytic chlorophyll will likely be included in the functional index for Great Lakes coastal wetlands because of its importance in wetland foodwebs and its apparent response to natural hydrologic conditions. We are conducting additional analyses to explore these relationships further.



Macrophyte Biomass – Macrophytes are an important structural feature in Great Lakes coastal wetlands. Macrophytes also represent approximately half of the primary production occurring in these systems (Cooper 2009). While few organisms feed on living macrophytes directly, macrophyte tissue enters the detrital pool after senescence. Additionally, macrophytes attenuate hydrologic energy by dampening waves and slowing currents which can lead to much clearer water within emergent macrophyte stands compared to water outside the vegetation stands (Cooper et al. 2012). Aboveground plant biomass was measured at three points in each wetland. Stem densities were counted in 1-m quadrats at each station and 5 stems were randomly selected from each quadrat to determine biomass. Collected stems were rinsed to remove epiphytic organisms, dried at 60°C until constant weight, cooled in a desiccator, and weighed. Average stem dry mass at each sampling point was multiplied by stem density to yield macrophyte standing stock (Dickerman et al. 1986, APHA 2005).

Macrophyte standing stock varied considerably within and among the 20 wetland sites (Figure below). The highest macrophyte standing stock that we measured occurred at Epoufette Bay in northern Lake Michigan and this was the only site in which *Typha* sp. was the dominant plant species. The three lowest macrophyte standing stocks occurred at sites dominated by *Nuphar* sp. and *Nymphaea* sp. (water lilies) while bulrush-dominated wetlands tended to have intermediate standing stock. Macrophyte production—inferred from our standing stock



estimates—appears to be a function of both dominant plant species and wave exposure. For example, the most sheltered bulrush-dominated wetlands (e.g., Cheboygon, Mackinaw Bay, and Portage Lake) tended to have higher standing stocks than wave exposed bulrush wetlands (e.g., Purdy Bay, North Point, and North Island). Macrophyte standing stock will likely be included in the functional index for Great Lakes coastal wetlands because of its apparent response to both species composition and hydrologic energy. We are conducting additional analyses to explore these relationships further.

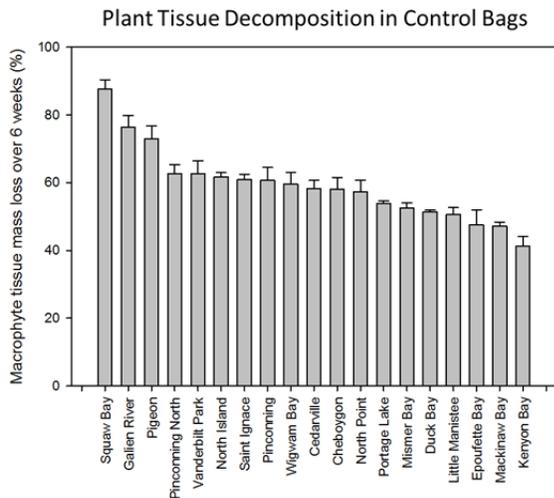
Decomposition – In addition to primary production, the other source of energy to food webs in coastal wetland ecosystems is the decomposition and mineralization of organic matter (OM). Like primary productivity, the decomposition of OM is a critical ecosystem process, but it is not known how this process responds to natural hydrologic and human-induced stressor gradients in Great Lakes coastal wetlands. Decomposition rates of organic matter were evaluated at each of the sites using two approaches. First, we used pre-weighed and dried bulrush (*Schoenoplectus pungens*). We recognize that bulrush may not be the dominant plant species at all coastal wetland sites but it was important that we standardized plant tissue species in order

Litter Decomposition Bag

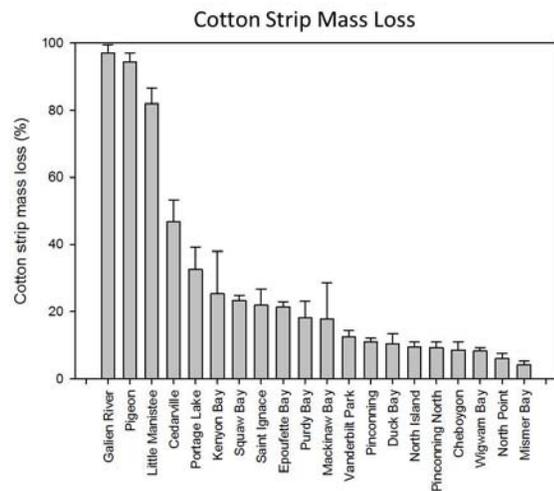


to compare decomposition rates across sites. Bulrush stems were collected from one site (Vanderbilt Park, Lake Huron), dried, and 5 stems (approximately 10 g dry mass) were placed in marked, mesh bags. At each site 5 mesh bags of dried OM were secured to the benthos in 25-50 cm of water using metal stakes (Photo previous page). Bags were retrieved after 6 weeks and the remaining OM was dried and weighed to determine mass loss.

We also used mass loss of cotton canvas to determine decomposition rates based on the methods of Maltby (1988) and Mendelssohn et al. (1999). Briefly, 12 cm x 30 cm cotton strips ($n=5$) were placed directly on the sediment surface in mesh bags and left to incubate for 6 weeks. Upon retrieval, strips were rinsed in the field and returned to the lab for drying and weighing. We are also planning to test tensile strength of remaining cotton as an alternative index of microbial decomposition (Maltby 1988, Mendelssohn et al. 1999).



Decomposition of plant tissue over the 6-week incubation period ranged from 41 to 88% in control bags and within-site variability was lower than anticipated (Figure at left). It is unclear at this point what is driving the variability in decomposition rates that we measured since no clear correlations with either human disturbance or wave/current energy were evident. For example, sediment redox conditions, available nutrients, and the activity of shredding invertebrates all likely influence plant tissue decomposition rates.

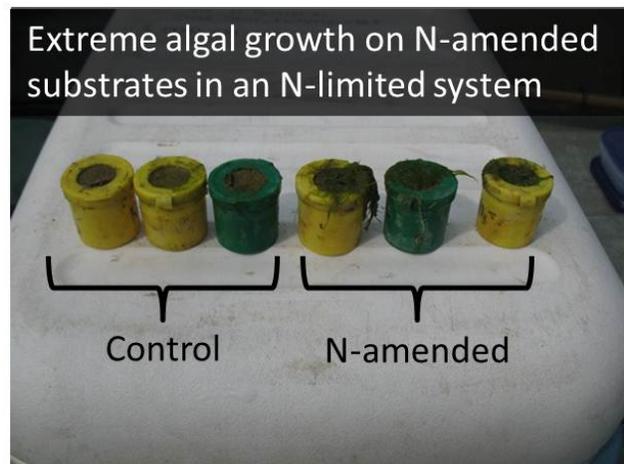
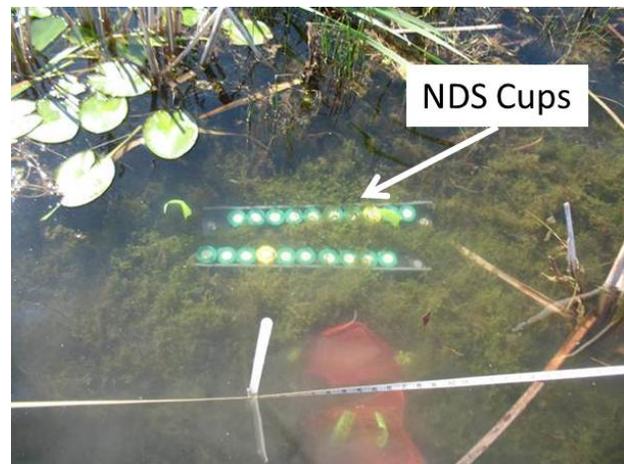


Cotton strip mass loss was much more variable than plant tissue decomposition and ranged from 4 to 97% mass loss over the 6-week incubation period (Figure at left). Interestingly, macrophyte tissue decomposition did not seem to correlate with cotton strip mass loss. For example, the sites with the lowest rates of macrophyte tissue decomposition had intermediate cotton strip mass loss rates and the site with the 3rd highest cotton strip mass loss (Little Manistee) had a low macrophyte decomposition rate. These differences suggest that the two methods are accounting for different aspects of microbial activity and both may be important functional variables. Both decomposition variables will be analyzed further for possible inclusion in the functional index.

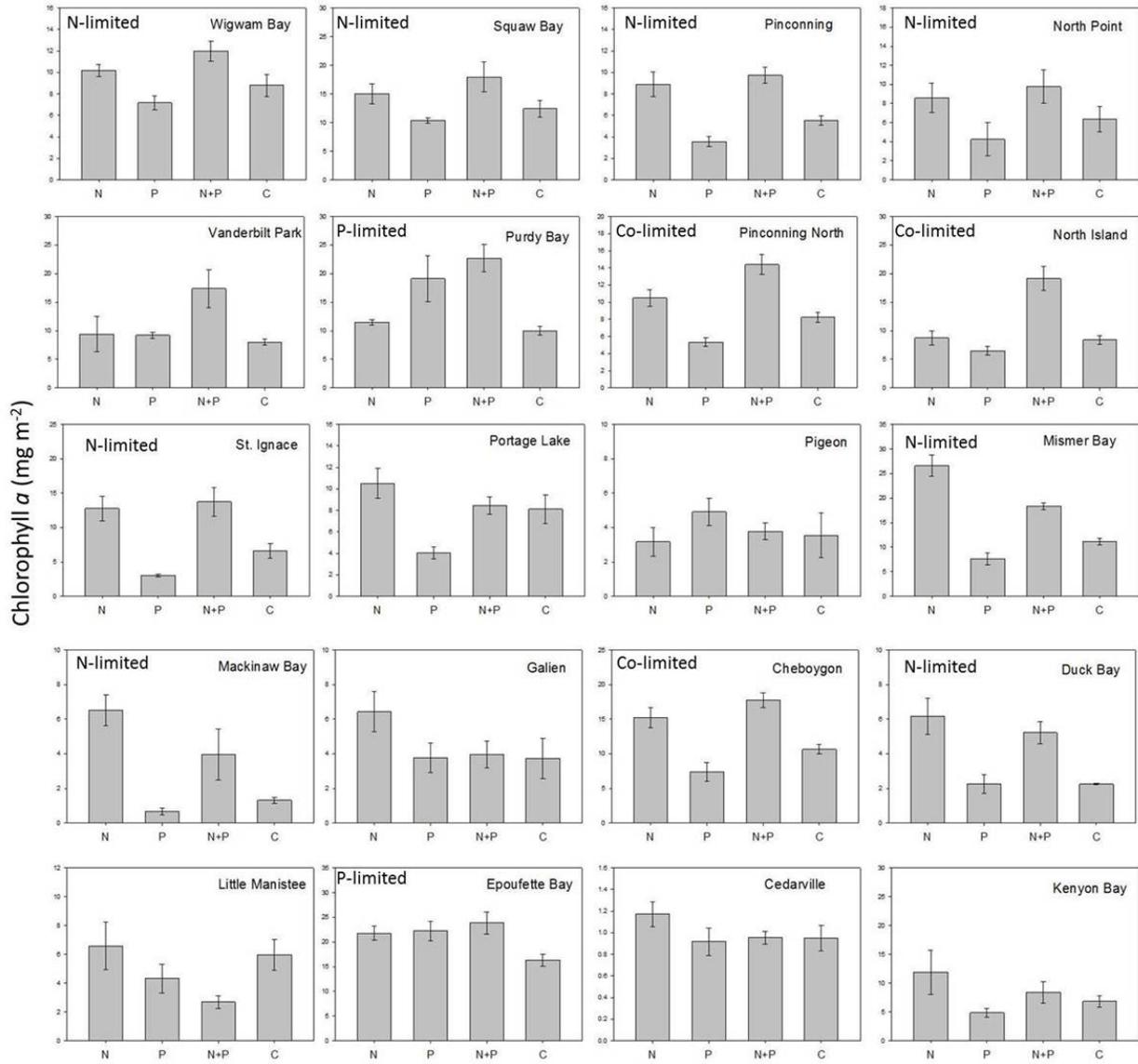
Nutrient Limitation – Nutrients play a critical role in both structural and functional attributes of aquatic communities and nutrient conditions can be strongly influenced by human activities. However, in our previous research in Great Lakes coastal wetlands, we consistently found very low concentrations of dissolved N and P, likely due to the high productivity of these systems that results in rapid uptake of nutrients. An alternative to using dissolved nutrient concentrations alone to monitor nutrient conditions is to conduct assays in which N and P are added separately or in combination to determine whether these macronutrients limit a particular biological function. We conducted a series of nutrient amendment assays to test whether water column gross primary productivity (GPP) and community respiration (CR), periphyton production, or macrophyte decomposition rates were limited by N, P, or the combination of N and P.

Periphyton — Nutrient diffusing substrates (NDS) were used to determine whether algal growth was nutrient-limited and to determine which macro-nutrient was most limiting to growth (Tank et al. 2006). At each site, NDS consisting of four treatments—N only, P only, N+P, and control (no nutrients)—were deployed for 8 weeks at 0.5-m depth (n=5 per treatment; Photo at right). The NDS were prepared according to Tank et al. (2006) in 30-ml plastic cups with snap-on lids fitted with fritted glass disks for algal colonization. After ~56 days, chlorophyll *a* was measured for each substrate to determine nutrient limitation. Two-way analyses of variance (ANOVA) with *post-hoc* Tukey's tests were used to determine nutrient effects on chlorophyll *a* at each site.

Periphyton chlorophyll *a* varied considerably within and among the 20 wetland sites (Photo at right and figure next page). Nutrient limitation was evident at over half of the sites, with N-limitation occurring at 8 sites, P-limitation occurring at 2 sites, and co-limitation occurring at 3 sites. The magnitude of limitation was also highly variable and represents an additional metric of nutrient conditions. We plan to include both categorical and magnitude-based nutrient limitation metrics in a final functional index.



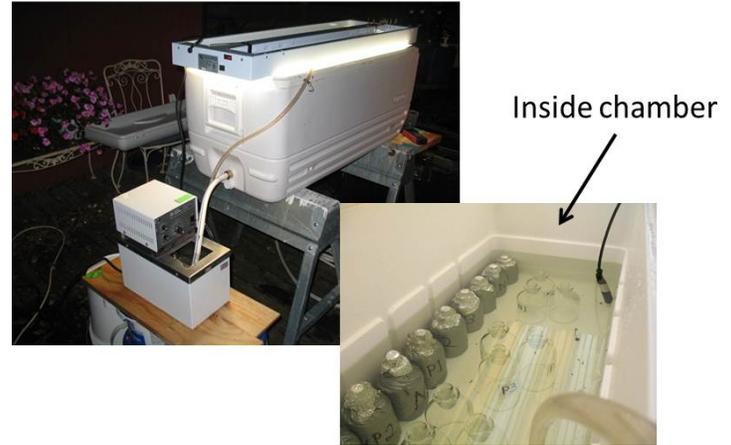
Biofilm Chlorophyll on Nutrient Diffusing Substrates



Water Column Metabolism — Nutrient limitation on water column GPP and CR was measured by incubating light and dark bottles under light-saturating conditions at 20°C. We selected 20°C as an incubation temperature based on observations in similar wetlands over the past 10 years (M. J. Cooper, unpublished data). A portable growth chamber consisting of a large cooler, a 4-tube fluorescent grow light (T4 bulbs), and a thermostatic water bath was used for incubations (Photo at right). Photosynthetically active radiation (PAR) within the chamber was maintained at $360 \mu\text{mol m}^{-2} \text{s}^{-1}$ which we determined to be saturating but not inhibitory for algae based on Wetzel (2001). The portable growth chamber was set up at a shore station near each wetland, which allowed us to run incubations immediately after collecting water samples. Each nutrient treatment (N, P, N+P, and control) was represented by 3 light and 3 dark bottles. Nitrogen bottles were spiked with a combination of KNO_3 and NH_4Cl to elevate ambient dissolved N concentrations by 0.05 mg L^{-1} . Phosphorus bottles were spiked with a solution of KH_2PO_4 to elevate ambient dissolved P concentrations by 0.05 mg L^{-1} . The combination bottles received both nutrients. Incubations were run for 5-7 hours. Initial and final dissolved oxygen (DO) concentrations were measured with a Hach Corporation LDO meter. Dissolved oxygen flux was used to calculate GPP and CR rates hour^{-1} . Two-way analyses of variance (ANOVA) with *post-hoc* Tukey’s tests were used to determine nutrient effects on GPP and CR at each site. Nutrient uptake velocity was also determined for each treatment at each site but final rates have not yet been calculated.

Water column metabolism (GPP and CR) rates were highly variable among sites. Control bottle GPP correlated strongly with water column chlorophyll *a* ($r=0.856$, $p<0.001$). Nutrient limitation of GPP was observed at 11 sites, with N and Co-limitation being most common (Table at right). Nutrient limitation of CR was observed at only 5 sites (Table at right).

Portable Growth Chamber

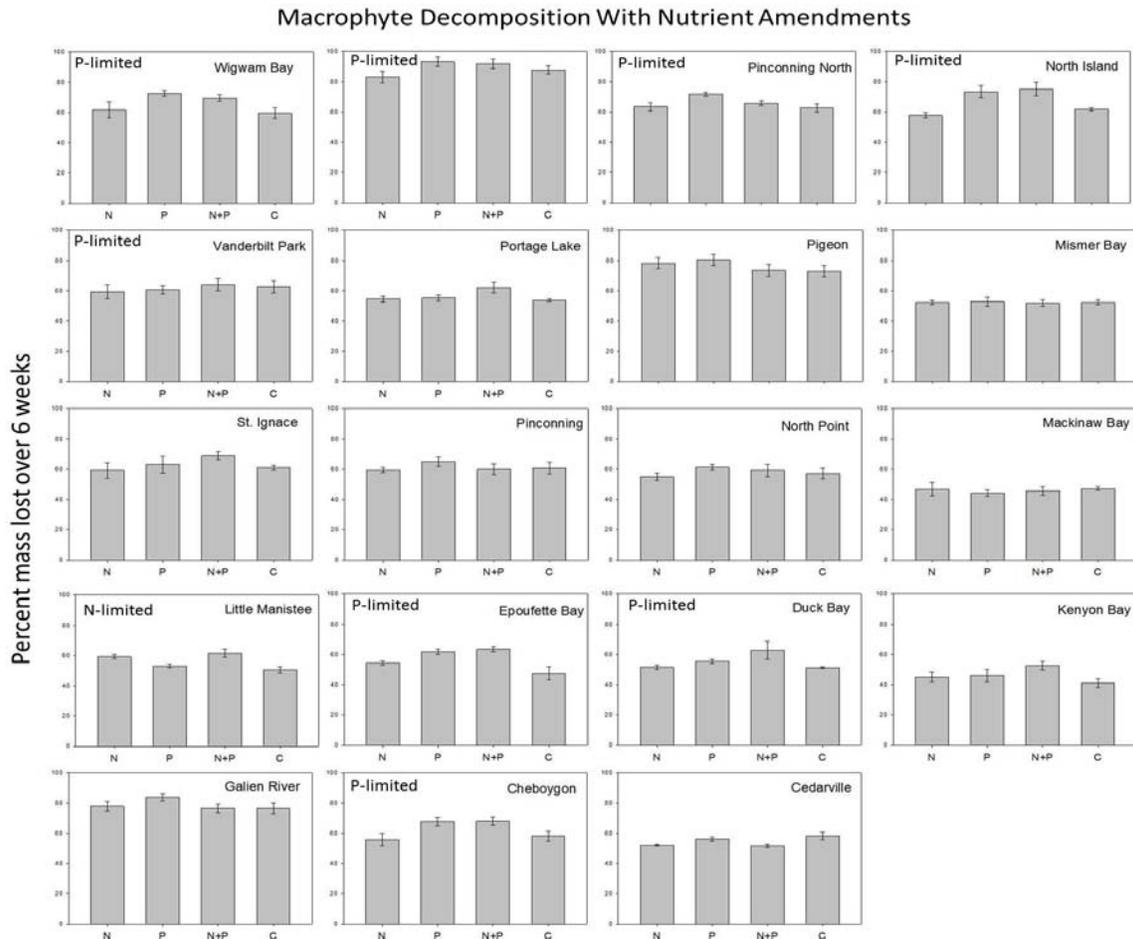


Summary of results for water column GPP and R assays to determine N and P-limitation.

| <u>Site</u> | <u>GPP limit</u> | <u>R limit</u> |
|----------------------|------------------|----------------|
| Vanderbilt Park | Co | P |
| Purdy Bay | none | none |
| North Island | none | none |
| Pinconning | N | none |
| Pinconning North | Co | Co |
| Wigwam Bay | N | N |
| <u>Galien River</u> | Co | none |
| Portage Lake | Co | none |
| Little Manistee | N | none |
| Pigeon River | none | none |
| Squaw Bay | none | none |
| North Point | none | none |
| <u>Cheboygon</u> | N | none |
| <u>Saint Ignace</u> | none | none |
| Duck Bay | none | N |
| Cedarville | none | none |
| Mackinaw Bay | N | none |
| <u>Mismer Bay</u> | Co | none |
| Kenyon Bay | none | none |
| <u>Epoufette Bay</u> | Co | P |

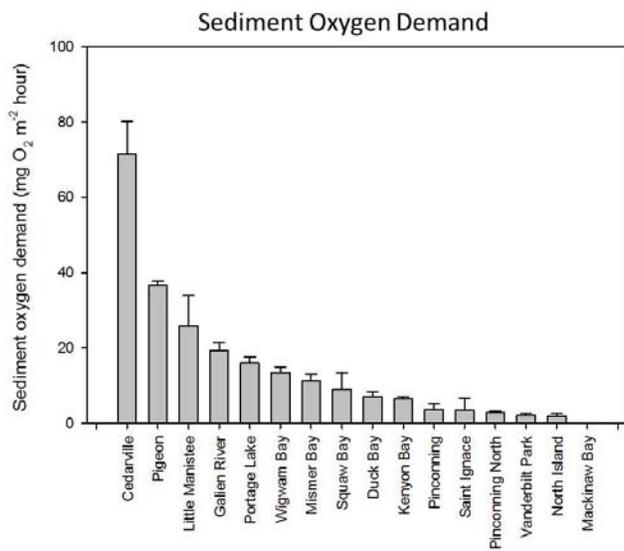
Macrophyte Decomposition — Decomposition of macrophyte tissue is an important mechanism by which carbon and nutrients bound in plant matter are recycled back into their respective labile pools for use by the wetland foodweb. Dissolved nutrients can affect decomposition rates especially during periods of high primary productivity when nutrient uptake rates are high and available nutrient pools are small. We tested whether macrophyte decomposition rates were nutrient limited using litter bags that were placed over permeable sacks of N, P, or N+P combined fertilizer, or neither (control). Urea fertilizer was used for the N treatment, triple super phosphate (i.e., rock phosphate) was used for the P treatment, the two fertilizers were mixed for the combination treatment, and rinsed pea gravel was used for the control treatment. Litter bags (n=6 per treatment, 24 per wetland) contained 5 pre-weighed dry *Schoenoplectus pungens* stems that were collected at the Vanderbilt Park site. Litter bags were incubated in 0.25-0.50 m of water and were placed over the nutrient sacks and pinned in place using wire flags. Litter bags were retrieved after 6 weeks, rinsed, and dried to determine percent mass loss. Two-way analyses of variance (ANOVA) with *post-hoc* Tukey’s tests were performed to determine nutrient effects on decomposition at each site.

Nutrient limitation of macrophyte decomposition was observed at 9 of the 20 sites (Figure below). Phosphorus-limitation was most common (8 sites), N-limitation occurred at 1 site, and co-limitation was not observed at any site. Plant tissue decomposition and nutrient limitation of decomposition will likely be included in the functional index if clear drivers of decomposition can be elucidated.



Sediment Oxygen Demand — Wetland sediments are often high in organic content, which can increase microbial respiration. Microbial respiration consumes oxygen (among other electron accepters) in proportion to the microbial community's metabolic rate. Therefore, oxygen uptake velocity or carbon dioxide production rate per area of sediment surface is an integrative measure of sediment respiration. This functional measure may be a useful indicator of both natural variability in sediment conditions as well as an indicator of human impacts (e.g., heightened organic sediment deposition caused by eutrophication).

We measured *in situ* sediment oxygen demand at each site using a combination of darkened sediment chambers and darkened water column respiration bottles. Having separate water column respiration estimates allowed us to account for water column respiration within the sediment chambers so that sediment respiration alone could be calculated. Sediment chambers consisted of large plastic funnels covered with aluminum foil to block light (Photo at right). Funnels were inserted approximately 3-5 cm into the sediment surface and held in place with metal stakes. Chambers were allowed to equilibrate for approximately 30 min before initial DO readings were taken. Final DO readings were made after 2-3 hours.

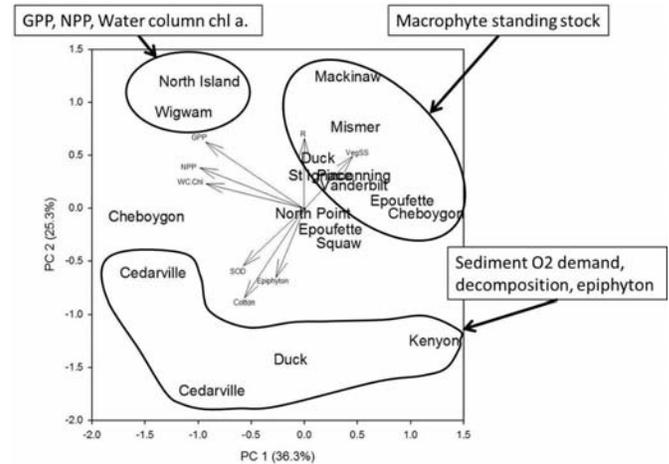


Mean sediment oxygen demand ranged from 2 to 71 mg O₂ m⁻² h⁻¹ (Figure at left). The highest rates occurred at sites that had noticeably thick layers of organic sediment. For example, the four drowned river mouth sites (Pigeon, Little Manistee, Gallien River, and Portage Lake) all had thick organic sediment layers and were among the five sites with the highest sediment oxygen demand. The Cedarville site also had a thick layer of benthic organic matter and had the highest sediment oxygen demand. Sites that were markedly wave swept (e.g., North Island, Vanderbilt Park, Pinconning North) had sandy sediments and tended to have low sediment oxygen

demand. Because Sediment oxygen demand appears to capture variability in hydrologic conditions and may also correlate with human disturbance, we anticipate that this metric will be important in the functional index.

Summary, Ongoing Work, and Future Directions

The suite of functional variables that we measured as part of this project puts us well on our way to developing a functional index of coastal wetland condition. At this writing, we have one additional set of metrics—nutrient uptake velocity—yet to calculate before the full suite can be compared to gradients of anthropogenic disturbance and natural disturbance gradients to identify the particular variables most useful in a functional index. In an effort to summarize the available data and characterize the 20 wetland sites, we conducted a principal components analysis (PCA) on 8 functional variables. The PCA suggested that wetland sites fit into 3 separate groups (Figure at right). Based on this analysis, sites could be described as having either particularly high water-column productivity and chlorophyll *a*, high macrophyte standing stock, or high decomposition rates. A number of sites did not fit into any of these categories, likely because they had approximately average values for most of these variables.



Principal components analysis of 8 functional variables measured at 20 Coastal wetland sites. Sites were grouped based on common functional characteristics (labels). Three groups were identified, though 4 sites did not fit into a particular group based on the PCA.

After our dataset is complete, we will compare each functional variable to gradients of anthropogenic disturbance as well as natural gradients such as wave exposure, wetland type, underlying geology, and latitude. These analyses will help us to identify functional attributes to include in a final index. After variables are selected, metrics will be derived and assembled into the final index. Our goal is to combine coastal wetland functional indicators with structural indicators that are currently being used in coastal wetland monitoring programs to identify particularly vulnerable or sensitive habitats, identify and track human impacts that are often not detectable with traditional methods, and measure the success of coastal wetland restoration projects. Functional indicators of coastal wetland health will benefit coastal restoration efforts by identifying candidate sites for restoration and tracking restoration outcomes. Functional indicators could also be used to assess water quality impacts in coastal habitats when traditional methods such as analyzing grab samples are inadequate. Furthermore, climate change is likely to have marked effects on Great Lakes coastal habitats by altering seasonal precipitation patterns and affecting coastal wetland hydrology, changes that will likely impact many wetland functions. Thus, a comprehensive coastal wetland monitoring plan that includes both structural and functional indicators is necessary for early detection of emerging and chronic human impacts on Great Lakes coastal ecosystems.

Publications, Presentations, and Dissertation Improvement

Our goal is to produce two manuscripts using data collected as part of this project. The first paper will be our preliminary functional index which will include specific methodology for measuring the functional variables included in the index as well as a scoring and interpretation scheme. Ideally, this paper will be useful for monitoring agencies as well as academic scientists

interested in testing and improving upon our methodology. The second paper will focus specifically on the nutrient assays conducted as part of this project. We feel that the set of nutrient-limitation assays that we conducted for water column metabolism, periphyton, and decomposition is very novel for Great Lakes coastal habitats and will provide substantially more information than nutrient concentration data currently being collected during routine monitoring. We plan to present the nutrient-limitation study at the 2012 International Association for Great Lakes Research meeting in May and will present the functional index development study at future conferences. Finally, this grant has greatly improved MJC's doctoral dissertation by providing data that is crucial to two of the chapters (aforementioned papers).

Acknowledgements

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