Carbon Export from Great Lakes’ Coastal Wetlands: Quantification, Mechanisms and Implications
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Statement of Work and Methods

Great Lakes coastal wetlands are unique habitats along the outer margins of the Laurentian Great Lakes. These habitats are differentiated from adjacent near shore habitats by warmer temperatures, decreased wave energy, specialized macrophyte vegetation and shallow water depths (Jude and Pappas 1992; Wei et al. 2004). Despite making up less than 1% of the total Great Lakes’ surface area, coastal wetlands support critical spawning, nursery and foraging habitats for over 80 species (>80%) of Great Lakes’ fishes in addition to diverse assemblages of birds and amphibians (Jude et al., 1992; Wei et al., 2004 and Cvetkovic and Chow-Fraser, 2011). It is believed that these small expanses are able to support such diverse communities due to their structural complexity and high primary productivity being fueled by nutrient and sediment deposition (Brazner et al., 2000 Jude and Pappas 1992; Wei et al. 2004). Consequently, coastal wetlands have been referred to as “metabolic hotspots” and “centers of organization” and their productivity has been compared to that of tropical rainforests and coral reefs (Brazner et al., 2000).

While the productive nature of Great Lakes coastal wetlands is generally accepted amongst professionals, the fate of the resulting organic carbon is another consideration. Fundamentally, there has been debate over whether coastal wetlands are sinks or sources of organic carbon in aquatic systems. More specifically, relative dietary contributions from coastal wetlands to food webs of offshore habitats have not yet been quantified for the Great Lakes. This idea stems from previous work in marine coastal wetlands which proposed that if marsh primary production surpasses the needs of those food chains, organic compounds are exported to near shore habitats (Odum, 1968). Alternatively, professionals estimate that dietary carbon contributions from wetlands could insignificant due to the large proportion of Great Lakes’ surface area occupied by offshore habitats (greater than 99%) (Brazner et al., 2000; Syranta et al., 2010). Therefore, a quantification of wetland-based carbon in near shore diets is necessary for the Great Lakes region.

Research focused on revealing dominant sources of carbon in consumer diets is important because it can reveal important energy sources that may otherwise go unnoticed (Sierszen et al., 2006). The most recent technique for linking energy sources to consumers is the use of stable isotopes (Peterson et al., 1987). This method is a powerful tool for ecologists because carbon isotopes can be found and analyzed in a variety of sample types with remarkable precision utilizing isotope-ratio mass spectrometry (IRMS) (Peterson et al., 1987). In fact, organic carbon stable isotope analysis from the particulate matter in the water column of Lake Eerie demonstrated current-driven carbon connections between marshes and near shore habitats (Bouchard et al., 2007). However, the design of this study fell short of our research interests because it failed to estimate dietary contributions to offshore food webs since it never showed that wetland carbon was actually being integrated into the diets of consumers (i.e. fish) (Bouchard et al., 2007).
Alternatively, carbon isotopes obtained directly from tissue samples are useful in linking consumers to dominant food sources. This is because the $^{12}$C:$^{13}$C isotope ratio in consumers is conserved relative to their diet (Peterson et al., 1987). In fact, the stable carbon isotope ratio of fish consumers typically matches their food items within 0.5% (Vander Zanden and Rasmussen, 2001). Using this knowledge, stable carbon isotope measurements from all potential food sources can be compared with that of consumers to estimate the overall dietary contribution of each food source via multiple-source isotope mixing models (Sierszen et al., 2006). This methodology provides an estimate of biological, dietary carbon exchange. This has the potential to expose ecologically important energy sources by estimating the proportion of diet coming from discrete sources such as wetlands.

One drawback of carbon stable isotopes is that dietary contributions from multiple sources is only applicable in situations where each food source (wetland and near shore) has unique carbon isotope ratios. This allows mixing models to estimate the proportion of support coming from each food source. These requirements are met in Great Lakes’ ecosystems where wetland phytoplankton and periphyton utilize dissolved inorganic carbon from the water column remarkably deficient in carbon 13 (Johnson et al., 2002). This deficiency is mostly attributed to the decomposition of macrophyte detritus in coastal wetlands (Keough et al., 1998). Therefore, isotopically-light planktonic and benthic food sources in wetland complexes allow comparisons to be made, presumably as long as the carbon sources (DIC) are not thoroughly mixed by hydrologic mixing of wetland and near shore waters.

In addition to addressing the extent of carbon exchange between wetlands and near shore food webs, a question that remains unanswered by isotope analysis is the probable mechanism behind the carbon exchange. Dietary exchanges of wetland/near shore carbon are likely exported biologically via fish immigration into or emigration from wetlands. Therefore, habitat use for fish of the Great Lakes is important to examine since it is generally accepted that wetland visits are limited to major events such as spawning, hatching or seasonal cross habitat foraging (Jude et al., 1991).

In order to track this habitat use, several methods have evolved including tag and recapture methods and physical jaw or fin tags (Pangle et al., 2010). While effective, these methods have logistical and financial limits in large scale studies such as between wetlands and near shore habitats of the Great Lakes (Pangle et al., 2010). A more appropriate approach for large scale movements is the use of trace elements in fish otoliths. Although composed mainly of calcium carbonate, various trace elements integrate into the crystalline structure of otoliths as a fish grows based upon the concentrations found in the water column (Kalish, 1989; Campana et al., 1999; Pangle et al., 2010). Due to differences in water chemistry between wetlands and near shore habitats, one study was able to estimate near shore and wetland habitat use in yellow perch within Lake Superior with 100% accuracy based upon one trace element (Brazner et al., 2004).

Furthermore, since otoliths actively grow throughout a fishes’ lifespan similar to rings found on a tree, the chronological order of fish movements between habitats is preserved in rings of growth (Brazner et al., 2004; Pangle et al., 2010). This order can be used to determine
the proportion of time spent in wetland and near shore habitats by selectively sampling growth increments. The method we will be using to sample specific increments of growth for trace elements is Laser Ablation Inductively Coupled Mass Spectrometry (LA-ICP-MS) increments (Figure 1) (Fowler et al., 1995; Gemperline et al., 2002).

Figure 1. Sagittal otolith sampled with laser ablation. All three ransects are 50 by 250 microns. Three lasers were directed to sample the core, the other two were aligned with the outer growth increments in order to determine trace element concentrations for different years of growth (Humphrey and Demartini, 2008).

Once wetland-based dietary carbon content is exposed and habitat usage is known, comparisons will be made between wetlands from distinct regions representing contrasting intensities of anthropogenic disturbance (i.e. high agricultural influences vs. undeveloped expanses of wetlands). This comparison is important since previous studies have shown that nutrient disturbances in riverine wetlands of Lake Superior have altered trophic pathways and reduced wetland efficiencies (Sierszen et al., 2006). Therefore, disturbance may be reducing their ability to provide crucial ecosystem services such as habitat and diet to offshore fisheries.

Methods

Wetlands will be chosen on the eastern coast of Lake Michigan and the north-western coast of Lake Huron including Saginaw Bay (see Figure 1). Selections will also be based upon the availability of collaborators with the Department of Natural Resources (DNR) and Grand Valley State University to sample near shore game fish with fyke nets or beam trawls. The
The minimum wetland size considered will be 10 hectares and a total of 10 wetlands from each Lake were chosen for a total of 20 sites. Wetlands will also be chosen to represent varying degrees of anthropogenic disturbance as well as to limit hydrologic mixing so that trace element and carbon isotope data from food bases would be distinct enough for comparisons to be made for habitat use and dietary composition.

Fish selection and collection

Several Great Lakes fish species will be used in the dietary contributions study because similar small-scale studies have found astounding variation in littoral carbon content and habitat use based upon the species under examination. This variation has been shown to vary by up to 85% between lake trout and bluegill from the same lake in smaller Canadian lakes (Van Zanden et al., 2002). Fish will be chosen accordingly to fully represent near shore fish communities. The habitat use and growth analysis will be limited to yellow perch since because of financial constraints and because of its economic important to the Great Lakes, its abundance throughout Great Lakes wetlands and near shore habitats and because of its known use of both habitats throughout its lifecycle (Brazner et al., 2004).

Juveniles and adults will be sampled for each species within each wetland and near shore habitat. Fish less than 60mm total length will be classified as juveniles. Fish greater than 60mm will be considered adults. Species used include yellow perch, largemouth bass, smallmouth bass, rock bass, walleye, northern pike, bluegill, pumpkinseed, blunt nose minnow, and slimy and mottled sculpin. A minimum of three and maximum of five representatives from each species age group were required for otolith analysis and carbon stable isotope tissue analysis. The same fish samples will be used for the otoliths and isotope analysis in order to limit the effects of sampling.
Juvenile fish will be assumed to be residents, having never left the habitat in which they were collected; therefore, their otolith cores will reflect the trace element chemistry and relative carbon isotope signature of the habitat in which they were collected from. Their otolith chemistry will be used for comparisons with motile adult otoliths presumed to travel between near shore and wetland habitats. Adult tissues will be collected from near shore locales and will be compared with food sources from wetland and near shore habitats in multiple source mixing models.

Wetland fish samples will be collected with fyke nets set in all present vegetation zones with >20cm of water. All fish will be euthanized in a solution of excess buffered MS-222 (Tricaine Methanesulfonate) and placed in whorl-packs on ice. All samples will be frozen with 24 hours. Otoliths extraction and tissue sampling occurred within a year of collection. All samples are to be labeled with species, location, age group (juvenile/adult), date and the fish total length.

Near shore samples will be collected by collaborators with the Michigan DNR and Grand Valley State University via gill nets or beam trawls conducted between 1 and 2.5 miles from adjacent wetlands. All fish will be euthanized with MS-222 and placed in separate whirl packs with appropriate labels before being frozen. Adults will be collected similar to wetland collections and species, date of capture and total length will be recorded before preservation.

A. Carbon stable isotope tissue collection/preparation/analysis

A half inch cube (5mg) of dorsal filet will be acquired from each fish (juveniles and adults) for tissue carbon isotope analysis. Care will be taken to remove skin, bones and cartilage from the tissue before placing each tissue sample in a sterile container for stable isotope analysis. Juveniles with sufficient muscle tissue will be used to represent possible food sources from wetlands since they are presumed to be wetland residents. All samples are to be frozen as opposed to preservation in ethanol or formalin due to the deleterious effects of these chemicals on isotopes (Jardine et al., 2003). All utensils used in tissue extraction were stainless steel rinsed under 90% ethanol. Tissues are going to be stored in sterile petri dishes frozen until analysis. Extraction and isotope analysis are going to be conducted at Notre Dame Lamberti Lab under Mathew Cooper.

Primary and secondary consumer collections will be made to indicate the carbon isotope signature of potential food sources available for each habitat type. Fish and plankton/macro invertebrate sampling are to be conducted within 5 weeks of one another to avoid season shifts in community composition.

Wetland collections will consist of macro invertebrate d-net sweeps within all vegetation zones present in the wetland. Snails, mayflies, caddisfly larvae, and amphipods were used as an indicator of potential food sources in wetlands because of their feeding habits and because of their sessile behavior within habitat types. Five hundred micron sweep nets will be used and contents are going to be washed into 253 micron sieves. Macro invertebrates remaining in the sieve will then be sorted and three to five of each specimen will then be preserved on ice until they could be frozen in the lab in sterile petri dishes. Also, plankton tows
will be conducted with a 40cm diameter net within each wetland with sufficient open water. Care will be taken to avoid any detritus or surface water and three replicates were taken, 100 meters each within the wetland. Samples will then be compiled and placed in acid-washed Nalgene sample bottles and sieved through stacked 253, 150 and 80 micron sieves before acidification with 1N hydrochloric acid (HCl). HCl will be applied via a fumigation hood until all reactions with CaCO₃ stopped – minimum 8 hours. Size fractions will then be rinsed with DI water and vacuum-filtered onto 50 micron nitex before being placed into sterile petri dishes. Samples will be placed on ice and frozen within 24 hours of collection.

Near shore food sources will be sampled in the same locations as fish collections with confirmation from Global Positions System. Three plankton tows will be conducted, 100 meters each before compositing samples in an acid-washed Nalgene transport bottle. Samples will be placed on ice before sieving on shore. Plankton samples will then be acidified to remove CaCO₃ and rinsed with DI water before vacuum-filtering 253, 150 and 80 micron size classes on 50 micron nitex filter material. Plankton samples will then transferred to sterile petri dishes and placed on ice until freezing. Benthic macro invertebrate samples will be collected with a PONAR dredge sampler. Five samples will be collected and compiled before transporting back to shore. Replicate PONAR samples are to be collected at 10 meter intervals on transects parallel to shore. Samples will be placed on ice or frozen until they can be sieved through a 500 micron sediment sieve. Macro invertebrates will then be sorted into functional feeding guilds similar to the wetland macro invertebrate collections and they will be placed in sterile petri dishes for stable isotope analysis.

Zooplankton samples will be treated with HCl in order to dissolve CaCO₃ which can skew carbon stable isotope ratios. Although this has negative effects on nitrogen isotopes, no negative influences have been observed for isotopes of carbon (Jardine et al., 2003). Macro invertebrates will be frozen, un-acidified. All samples are to be frozen and delivered to the Lamberti lab at Notre Dame where tissue analyses will commence. Multiple source mixing models will be used on the resulting data to estimate wetland-based dietary contributions to fish communities under the guidance of Professor Hoffman and Professor Sierszen (EPA-Duluth).

**Otolith preparation/analysis**

Otolith collections will be conducted in the lab with the aid of dissecting microscopes, if necessary. All otoliths are going to be removed from yellow perch collected in near shore habitats within a year of collection. This will make use of the same fish used in tissue isotope analysis. Sagittal otoliths will be removed and handled according to a modified protocol (Campana et al., 2000). This included cleaning of any connective tissue with Super Q water and storage in coin envelopes. Sagittal otoliths from the right side are going to be used for trace element analysis. Otoliths will be extracted with clean stainless steel forceps and handled with acid washed plastic forceps and non-powdered nitrile gloves at the final stages of handling. Otoliths are going to be allowed to air dry before being stored dry in coin envelopes with appropriate labels. Otolith sections will be prepared by mounting otoliths directly to acid washed cover slips with thermoplastic glue according to Donohoe et al. (2010). After cooling
and drying, otoliths will be sanded to the plane of the core with 30 and 3 micron lapping paper by hand before polishing with 3 micron polishing paper.

Marked/scored cover slips with samples will then transferred to petrographic slides with silicon glue previously tested for trace elements (Jones et al., 2003). Mounting of several otoliths on one petrographic slide will follow a modified protocol by Donohoe (2010) which optimized sampling at Central Michigan University. Otoliths will then be sonicated with Milli-Q water prior to laser ablation to remove any contamination before analysis. Otolith margins will be used to reconstruct recent habitat use for adult fish. Juvenile fish will be used as a reference to compare concentrations of several trace elements since they are presumed to be residence for the habitats they are found in. Trace elements analysis will be selected for comparison based upon a publication by Brazner (2004) which used Strontium to discriminate between near shore (bay) and wetland habitats. All laser ablation will be conducted at the Center of Applied Research and Technology (CMU) under Professor James Student. The resulting data will also be interpreted with the guidance of Professor Brazer (Nova Scotia otoliths research laboratory), Professor Pangle (committee member, CMU) and James Student (committee member, CMU).

**Statement of Work Citations.**


