Nitrogen Flux from Lake Michigan into Terrestrial Dune Ecosystems at Sturgeon Bay

REU Project at UMBS in 2013
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Abstract

Little is known about the movement of nutrients and its effects from water to land and how far inland these nutrients penetrate. For the most part, studies look at the unidirectional flow of organisms, nutrients, and energy from the terrestrial to the adjacent aquatic ecosystems (Hasler 1975, McClelland and Valiela 1998, Smith 1998 in Vander Zanden and Sanzone 2004). Dune systems in northern Michigan are part of the largest freshwater dune systems in the world and are found along the water to land ecotones (Haas and Cline 1972). Dunes are characterized in being nitrogen poor environments, so in this study we will be determining the flux of nitrogen from the aquatic Lake Michigan ecosystem, to the terrestrial Sturgeon Bay dune ecosystems at Wilderness State Park in Michigan. To find out how far inland nitrogen is penetrating we sampled *Ammophila breviligulata* as a distance from shore (to look at its δ^{15}N) , looked at the δ^{15}N of aquatic herbivore insects, how far back aquatic herbivore insects are moving via sticky traps, and tested insect removal rates along the shore of the aquatic herbivores. We found that both the δ^{15}N of *Ammophila breviligulata* as well as the percent aquatic herbivore insects decreased with distance from shore, and that there were 100% insect removal rates along the shore of the aquatic herbivores. Our findings indicate that aquatic herbivore insects provide precious nitrogen to the dune ecosystems, and that this is reflected in the δ^{15}N of *Ammophila breviligulata.*

Introduction

The movement of nutrients, prey, energy, and consumers across habitat boundaries can be dynamically and energetically important to the receiving ecosystem; and consequences of such movement can range in its effects from population dynamics to influences on broader ecosystem processes (Vander Zander and Sanzone 2004). The flow of these nutrients, prey, energy, and consumers between land and water occurs in both directions; and although land and water exist separately and are easily recognized as distinct biological communities, they are actually very real extensions of each other (Polis and Sánchez-Piñero et al. 2004). The extent of this influence of aquatic materials is potentially extraordinary, since most of the land's surface is adjacent to reticulated water sources (Hanschel 2004, Power et al. 2004, Polis and Hurd et al. 1997 in Polis and Sánchez-Piñero et al. 2004).

Nutrients, specifically in the form of nitrogen, are supplied into bodies of water by three major sources; river discharge, atmospheric deposition, and nitrogen fixation by blue-green algae. These nitrogen inputs are increasing at an accelerated rate, especially the inputs via river and stream discharge; due to urbanization and increasing fertilization of farmlands. It is important to understand that streams act as conduits of land derived nutrients, while lakes (or large bodies of water) typically serve as sinks for nutrients and contaminates (Hasler 1975, McClelland and Valiela 1998 in Vander Zander and Sanzone 2004). This nitrogen resource in the aquatic habitat enters the terrestrial food webs via three major conduits; on shore drift (including algae and aquatic prey insects), via predators that feed on this drift but also use land-based resources, and via windblown sea spray (Polis and Sánchez-Piñero et al. 2004). Once these materials enter the food web they flow through three food web channels to enrich terrestrial consumers: algae and animals along the shore, marine vertebrates that come to land, and fertilized plants (Polis and Sánchez-Piñero et al. 2004). How much and how far these nutrients penetrate inland are determined by terrestrial factors such as coastal topography, location, efficiency of the shore biota in converting marine input to terrestrial tissue, and consumer mobility (Polis and Sánchez-Piñero et al. 2004).

Dune systems in northern Michigan are part of the largest freshwater dune systems in the world and are found along the water to land ecotones (Haas and Cline 1972). Because nitrogen is a limiting factor for the survival and growth of life, its distribution across the dune ecosystem can portray nutrient transfer between the systems. This transfer, whether by onshore drift, insects, or windblown spray is deposited in the soil and eventually taken up by plants (Vander Zanden and Sanzone 2004). Stable isotope analysis can be used to quantify the transfer of nutrients across ecosystems (Polis and Sánchez-Piñero et al. 2004), and this technique has become increasingly popular in the investigation of diets, especially among organisms that are difficult to study directly because of either their size or habitats (Grant and Vande Kopple 2009). Carbon isotope values ($\delta^{13}C$) are indicators of an organism's diet because consumers tend to reflect the carbon isotope values of the foods they eat (DeNiro and Epstein 1978 in Grant and

Vande Kopple 2009), while nitrogen isotope values ($\delta^{15}N$) tend to indicate the trophic level because the heavier nitrogen isotope accumulates in the consumer with each trophic transfer (DeNiro and Epstein 1981 in Grant and Vande Kopple 2009). The usefulness of these stable isotopes to trace the material flow between spatially segregated ecosystems requires that the different subsystems have distinct isotopic signatures (Schindler and Lubetkin 2004). Conveniently, as found by Peterson and Fry (1987), the marked differences in stable isotopic signatures of carbon, nitrogen, and sulfur between aquatic and terrestrial resources make the use of stable isotope analysis suitable to the study of trophic connections at water to land interfaces (1987 in Polis and Sánchez-Piñero et al. 2004).

Sand dunes tend to be very sensitive to many pressures, including habitat loss, climate change, seal level rise, tourist pressures, agricultural improvement, over-stabilization, and a lack of management. Increased nitrogen deposition is thought to be a major contributor to the over-stabilization and species decline in the UK dune systems (UKREATE). Energy production through combustion of fossil fuels and food production emit pollutants in the forms of NO_x, SO_2, NH_3, and N_2O, which are transported into the atmosphere affecting rainfall chemistry and air quality (UKREATE). The deposition of these pollutants has resulted in the acidification of soils and waters in acid-sensitive areas and has contributed to nitrogen enrichment, or eutrophication, of semi-natural areas. This eutrophication can unfortunately result in the loss of biodiversity, and over-stabilization in dune systems, which are especially susceptible to changes in their ecosystem (UKREATE). Native dune vegetation (i.e. *Ammophila breviligulata*) protects human developments by stabilizing soil, and will be a very important factor in mediating negative impacts of global climate change that are already occurring, such as rising sea levels, severe storms, and greater erosion (Cochard et al. 2008 in Emery and Rudgers 2009). As such, dunes provide the physical substrate for maintaining ecosystem integrity along the aquatic – terrestrial border (Arun et al. 1999, Albert 2000 in Emery and Rudgers 2009). Because of this, it is imperative to give greater recognition of trophic connections across boundaries of these study systems, because they have clear and implicit implications for our understandings of human effects on both natural and managed ecosystems (Vander Zander and Sanzone 2004).

The boundaries between terrestrial and aquatic ecosystems are often thought of in terms of how the terrestrial ecosystems mediate the transfer of energy and nutrients into the aquatic ecosystem (Helfield and Naiman 2001). Similarly, most research performed at the terrestrial–

aquatic ecotone has traditionally focused on the unidirectional flow of organisms, nutrients, and energy from the terrestrial ecosystems to the adjacent aquatic ecosystem (Hasler 1975, McClelland and Valiela 1998, Smith 1998 in Vander Zanden and Sanzone 2004). Thus, exchanges of these materials, etc. from water to land are poorly understood, and their consequences for the dynamics of populations and ecosystems are even less well known (Jansson 1988, Wilson and Halupka 1995, Polis and Hurd 1996, Polis and Anderson et al. 1997; Ben-David et al. 1998 in Vander Zanden and Sanzone 2004).

Accordingly, in this study, we will be determining the flux of nitrogen from the aquatic Lake Michigan ecosystem, to the terrestrial Sturgeon Bay dune ecosystems at Wilderness State Park in Michigan. Specifically, we ask: what organisms are moving aquatic nitrogen from Lake Michigan to the Sturgeon Bay dune ecosystems, what is the gradient of aquatic nitrogen movement into the dune systems and how much nitrogen movement is occurring from water to land, and what effect does this movement of aquatic nitrogen have on the biomass of the dune grass *Ammophila breviligulata*? More specifically, our predictions based on the above questions are as follows respectively: 1) aquatic herbivore insects act as the primary mechanism for the movement of aquatic nitrogen from Lake Michigan into the terrestrial Sturgeon Bay dune ecosystems, 2) the aquatic nitrogen signature and the abundance /density of aquatic herbivore insects will decrease with distance from shore, therefore decreasing aquatic nitrogen movement inland, and 3) *Ammophila breviligulata* will show supplemented growth closer to shore with the increase in availability of aquatic nitrogen.

Materials and Methods

Study System:

In this study, we examined 30 *Ammophila breviligulata* within three transects, and had 10 sites to measure and observe aquatic prey insects (midges, mayflies, and caddisflies), and terrestrial predator insects (tiger beetles, ground beetles, ants, shore bugs, and grasshoppers) in the Sturgeon Bay dune ecosystems at Wilderness State Park in Emmet County, Michigan. All of our plant transects and insect sites were just north of Big Sucker Creek, and south of Little Sucker Creek as to avoid as much human interference as possible (Figure 1).

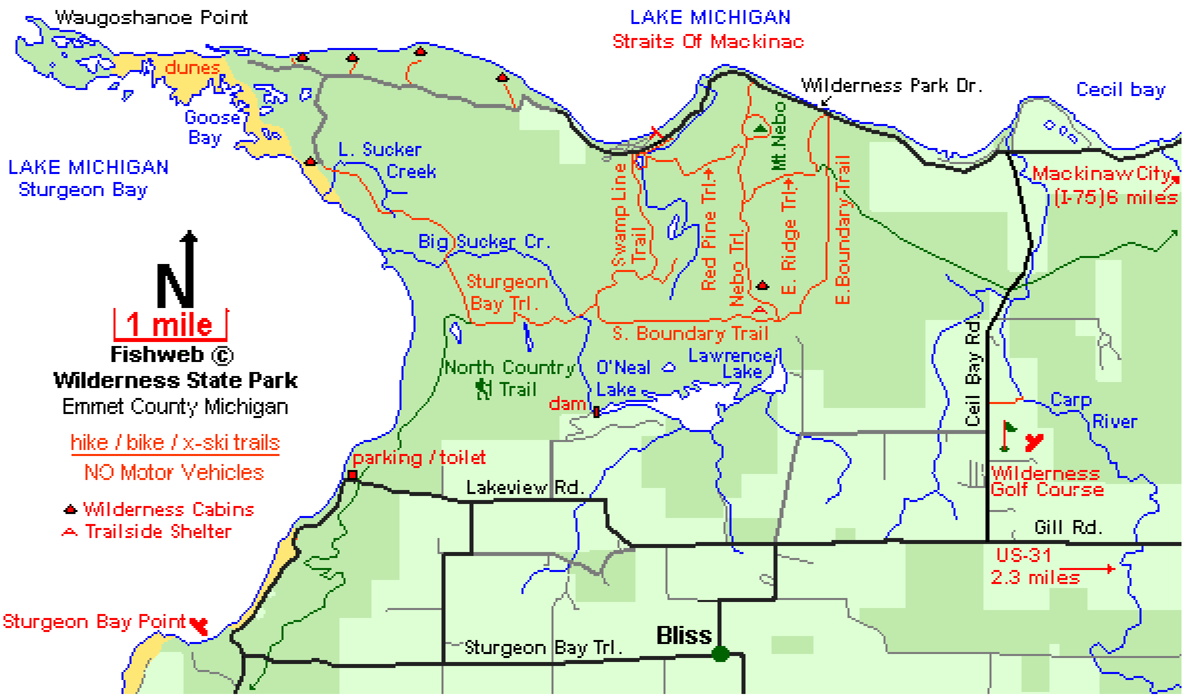

Figure 1: Map of Wilderness State Park, showing Sturgeon Bay and the location of our site between Little Sucker Creek and Big Sucker Creek.

Determining who is moving nitrogen from aquatic (Lake Michigan) to terrestrial (Sturgeon Bay dunes) habitats:

To determine who is moving precious aquatic nitrogen from Lake Michigan into the terrestrial Sturgeon Bay dune ecosystems, we set up 10 removal sites (at 8, 12, 35, 105, 115, 119, 142, 161, 176, and 197 meters) along a 200 meter range parallel to the beach (where the vegetation starts) of Sturgeon Bay, as determined by a random number generator, to measure the removal rates of aquatic herbivore insects (Figure 2). At each site, air temperature (°C), humidity (%), wind velocity (m/s), and GPS coordinates were recorded with Pen-Type Thermo-Hygrometer, Turbo Meter wind speed indicator, and Garmin GPSmap 62 equipment respectively. In order to set up these sites, we collected aquatic herbivore species (mayflies, caddisflies, midges) at night with a BioQuip light trap and dry ice and then placed them in a chest freezer overnight, and sorted them appropriately the next day. We then scattered the aquatic herbivore insects (grouping like with like; midges, caddisflies, mayflies) in each of our replicate sites. We placed 10 individuals of each of the midges, mayflies, and caddisflies in each of the 10 replicate sites. Replicate sites were circular plots, with a one foot diameter, to limit edge effects. Sites were left out for three hours, from approximately 8:00am to 11:00am, two of which were directly observed for a total of 1 hour each as to observe and record what predator insects are taking the aquatic herbivores and any other observations. We recorded the time at each site when all aquatic herbivores were removed by predator insect species.

To complement the above process, we also set out 5, one square-foot sticky traps along three transects chosen randomly via a random number generator along the 200 meter transect for the insect removal rate sites, but perpendicular to the same 200 meter range (Figure 2). The three distances for the sticky trap transects along the 200 meter range were 0 (T1), 154 (T2), and 183 (T3) meters. Each transect had the same five distances plotted along it; 0, 18, 53, 74, and 92 meters, as measured through the use of a Newcon Optic Laser Range Monocular. Where the vegetation starts on the beach is what counted as distance 0m along each transect, and where the tree line started counted as the distance 92m along each transect. Traps were set out near dusk, with wind velocity, temperature, humidity, and GPS coordinates all recorded as previously done and collected early the following morning. The traps were then analyzed under a microscope to identify what insects were on them. This was done to measure and make note of what insect species (aquatic herbivores) were present at those various distances, in what abundances (%), and to determine what species are active at night since the removal sites described above only measure daytime removal rates.

We recorded into a Microsoft Office Excel spreadsheet to analyze at a later date.

Figure 2: Aerial map of Sturgeon Bay showing the aquatic herbivore insect removal rate site transect (vertical transect SBT4S1-SBT4END), and the three sticky trap/Ammophila breviligulata transects (horizontal transects, SBT1S1-SBT1S5, SBT2S1-SBT2S5, SBT3S1-SBT3S5).

Determining the gradient of aquatic nitrogen from Lake Michigan into the dune ecosystems and how much nitrogen movement is occurring from water to land:

To measure the gradient of aquatic nitrogen moving into the dune ecosystems and how much, we used the same three transects as the sticky traps, with the same five distances plotted along it, measured as previously done (Figure 2). We also measured, as previously done, the wind velocity, temperature, humidity, and GPS coordinates at each site along each transect. At each distance along each transect we collected two separate *Ammophila breviligulata* plant samples and bagged and labeled them appropriately.

These plant samples and bags were cleaned using deionized water, and put into a chest freezer for 24 hours. After freezing they were transferred into small white envelopes to be freeze-dried in a Lyophilizer for 24 hours. Plant samples were then ground in the 8000D Mixer/Mill for 6 minutes each and transferred into their own scintillation vials that were appropriately labeled; and then placed in a desiccator overnight to preserve them for further analysis to determine each samples' δ^{15}N. We examined the movement of aquatic nitrogen into the dunes using a two source mixing model (source one being insects, and source two using *Ammophila breviligulata* tissue).

To determine how much nitrogen movement is occurring from water to land we determined the δ^{15}N of the *Ammophila breviligulata* and aquatic herbivore insect samples. Because we were using a two source mixing model, the insect samples were necessary, as they are the source of the aquatic nitrogen. The *Ammophila breviligulata* samples were collected and prepared from the previous step in determining the gradient of nitrogen. The only additional step required in preparing the samples was to weigh out (in dry weight) the ground *Ammophila breviligulata* so that each sample weighed in-between 1.5-2.0mg. We weighed out the samples in small tin cups on a Mettler Toledo balance, folded them into small balls, and recorded their weight and what slot they were put in a tray.

To prepare the samples of the aquatic herbivore insects, we first set out BioQuip light trap as previously done. Once the aquatic herbivore insects were captured, we sorted them out, samples were cleaned using deionized water, transferred into glass vials (labeled appropriately), put into a chest freezer for 24 hours, and then freeze-dried in a Lyophilizer for 24 hours. Each insect sample was then ground in vials with a glass rod, and then weighed out (in dry weight) so that each sample weighed in-between 0.5-1.0mg as previously done with the *Ammophila breviligulata* samples.

Once all samples were prepared, they were given to the chemist at the University of Michigan Biological Station's Chemistry Lab, and were run through the Stable Isotope Mass Spectrometer to acquire the C/N ratio, %N, as well as the δ^{15}N of each sample.

We recorded into a Microsoft Office Excel spreadsheet. SPSS was then used to perform a descriptive analysis, a multiple correlation analysis, and a stepwise regression to quantify the data as found from our experiment.

Effect of aquatic nitrogen movement on biomass of *Ammophila breviligulata*:

To determine the effect of aquatic nitrogen movement on biomass of *Ammophila breviligulata*, we calculated the estimated percentage of aquatic nitrogen for each of the distances along the *Ammophila breviligulata* transects using a two source mixing model (source one being the δ^{15}N value of aquatic midge insects, and source two being the most un-enriched δ^{15}N value of *Ammophila breviligulata* tissue, which were the samples taken at site 53m along transects T1, T2, and T3).

Results

Determining who is moving nitrogen from aquatic (Lake Michigan) to terrestrial (Sturgeon Bay dunes) habitats:

At the aquatic herbivore insect removal rate sites along the 200 meter transect on the beach of Sturgeon Bay, we found that there were 100% removal rates of the aquatic herbivore insects (midges, caddisflies, mayflies) for all 10 sites. Complete removal of the aquatic herbivore insects was done within two hours, with the exception of site distances 12, 35, 105, 115, and 119 meters. At these sites, it took 3 hours for complete removal to be observed because there were two significantly larger caddisfly individuals placed in these sites as opposed to the other five. It should be noted, that all other individuals were removed by this time, and had the two larger caddisfly individuals been the average smaller size, complete removal would have been achieved in approximately two hours, as was true for the other sites. We observed that all of the aquatic herbivore insect removals were done by local ants and were taken to nearby colonies within one meter of each removal site, and that removal of the aquatic herbivore insects was occurring immediately; even before all aquatic herbivore insects were placed in each site. An individual ant was noted to be able to take one aquatic herbivore insect itself, whereas it was necessary for multiple ants to break apart and then carry away the larger caddisflies therefore requiring more time for removal.

We found the percentages of all insects that were aquatic herbivores from shore to tree line along sticky traps for T1 to be 50%, 32%, 11%, 38%, and 24% for the distances of 0, 18, 53, 74, and 92 meters along the transect respectively. We also noted that this sample was typical of the abundance of aquatic herbivore insects that emerged on a standard emergence night. We found the percentages of all insects that were aquatic herbivores from shore to tree line along sticky traps for T2 and T3 to be 64.5%, 83%, 76.5%, 70.5%, and 83% for the distances of 0, 18, 53, 74, and 92 meters along the transects respectively. The sticky traps for T2 and T3 were set out over the same night, and we noted that this sample was typical of the abundance of aquatic herbivore insects that emerged on a large emergence night (specifically midges) when compared to the sticky traps from T1 (Figure 3). For each set of sticky traps on each night, it was noticed that the primary, and most abundant by far, aquatic herbivore insect moving along the transects were midges.

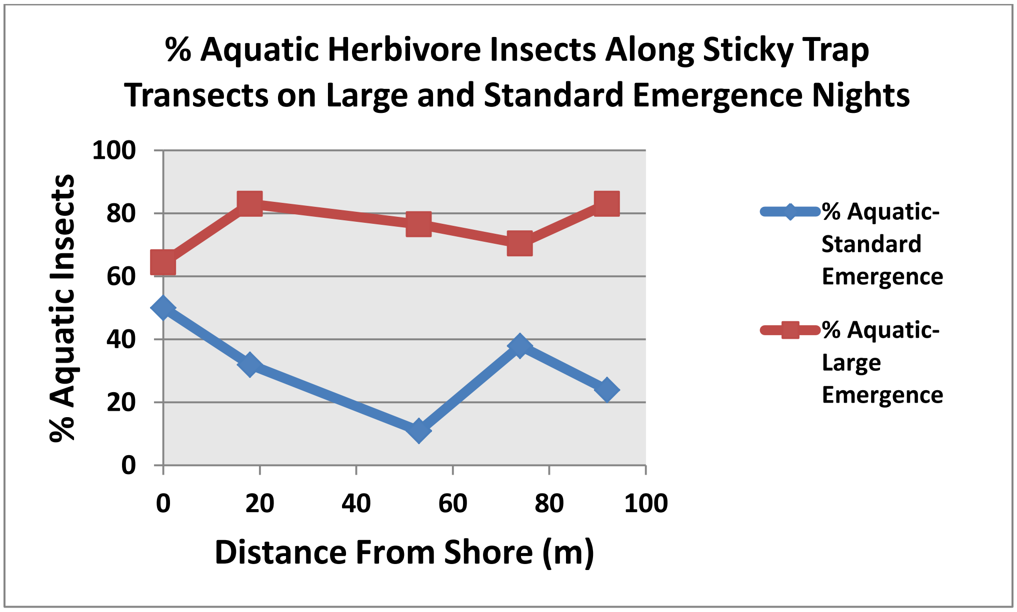

Figure 3: Percentage of aquatic herbivore insects at five distances along three transects starting at the beginning of the vegetation (0m) on the beach and ending at the beginning of the tree line (92m), as found through the use of sticky traps. The blue line represents the percent of aquatic herbivore insects on a standard emergence night along the sticky trap transect T1. The red line represents the averaged percentage of aquatic herbivore insects on a large emergence night along the sticky trap transects at 154 and 183 meters.

Determining the gradient of aquatic nitrogen from Lake Michigan into the dune ecosystems and how much nitrogen movement is occurring from water to land:

We performed a descriptive analysis test in SPSS on the dependent variable, δ^{15}N and the independent variable, distance from shore, for the three *Ammophila breviligulata* transects (T1, T2, and T3), and found that the data are normally distributed. For the six samples at each of the five sites along the three transects, we found the mean δ^{15}N to be -2.72 ± 0.99, -4.27 ± 0.31, -4.95 ± 0.50, -4.28 ± 0.80, and -4.80 ± 1.47 for the distances of 0, 18, 53, 74, and 92 meters respectively (Figure 4). The percent nitrogen for all of the plant samples was found to be between 1.02-2.02%.

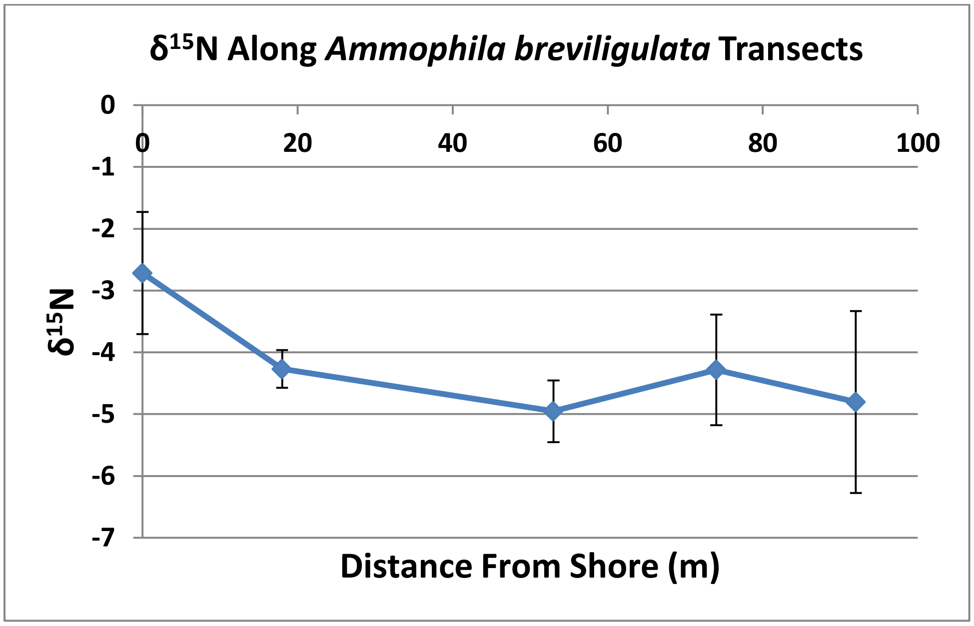

Figure 4: Mean δ^{15}N of *Ammophila breviligulata* with standard deviation (as error bars) for each site along the three *Ammophila breviligulata* transects (T1, T2, and T3), starting at the beginning of the vegetation (0m) on the beach and ending at the beginning of the tree line (92m), as found through the use of a Stable Isotope Mass Spectrometer.

For the aquatic herbivore insects caught in the BioQuip light trap, we found δ^{15}N to be 5.40, 3.51, and 5.77 for midges, mayflies, and caddisflies respectively.

Through the use of a multiple correlation analysis in SPSS we were able to determine that the variables, δ^{15}N, distance from shore along transects T1, T2, and T3, as well as the percent aquatic herbivore insects on the sticky trap transects for small and large emergence nights are all strongly correlated. We ran a stepwise regression next to have a better look at the causation of these correlations with the dependent variable being δ^{15}N, and the independent variables being distance from shore along transects T1, T2, and T3, and the percent aquatic herbivore insects on the sticky trap transects for small and large emergence nights. We found that the small emergence night for percent aquatic herbivore insects explains the largest portion of variation for δ^{15}N, and that it is highly significant ($t=4.14$, $p<0.001$). When the mean δ^{15}N of *Ammophila breviligulata* along the three transects is graphed with the percent aquatic herbivore insects on a standard emergence night as found through the sticky traps along T1, this significance is visible

(Figure 5). However, we found that the large emergence night for percent aquatic herbivore insects and distance from shore are not significant (t=-1.04, p>0.3, and t=-1.55, p=0.13 respectively).

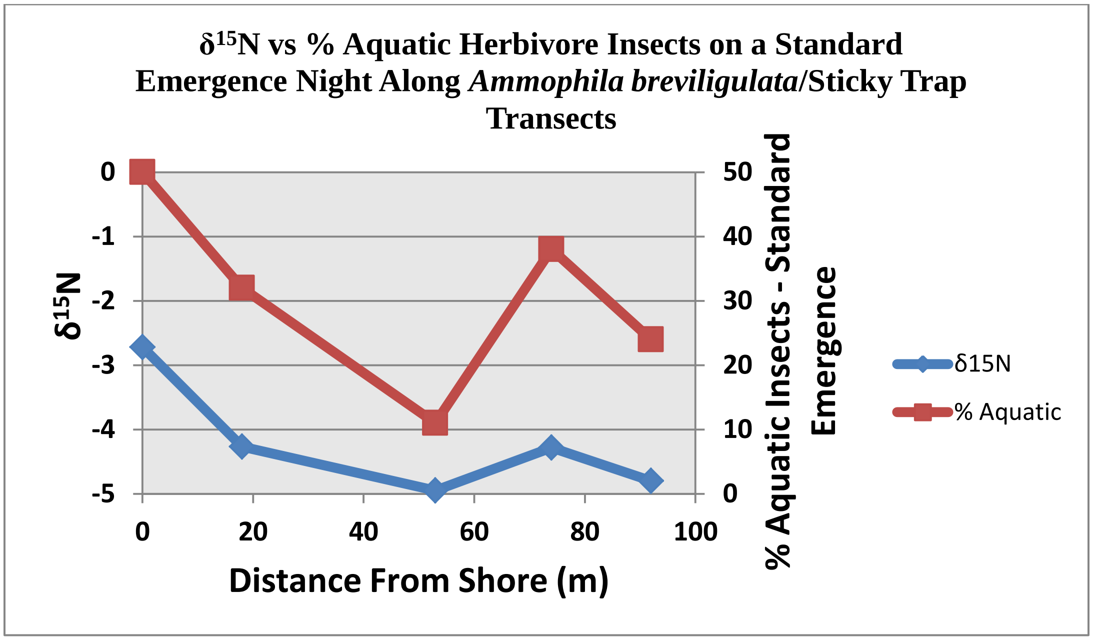

Figure 5: Mean δ^{15}N of *Ammophila breviligulata* (left y-axis) for each site along the three *Ammophila breviligulata* transects (T1, T2, and T3) plotted with the percent of aquatic herbivore insects on a standard emergence night along the sticky trap transect T1 (right y-axis).

Effect of aquatic nitrogen movement on biomass of *Ammophila breviligulata*:

Through the use of a two source mixing model (source one being the δ^{15}N value of aquatic midge insects, and source two being the least enriched δ^{15}N value of *Ammophila breviligulata* tissue (which were samples taken at site 53m along transects T1, T2, and T3) we calculated the percent of aquatic nitrogen in *Ammophila breviligulata* tissue to be 21.5%, 6.6%, 0.0%, 6.5%, and 1.4% for the distances of 0, 18, 53, 74, and 92 meters respectively (Figure 6).

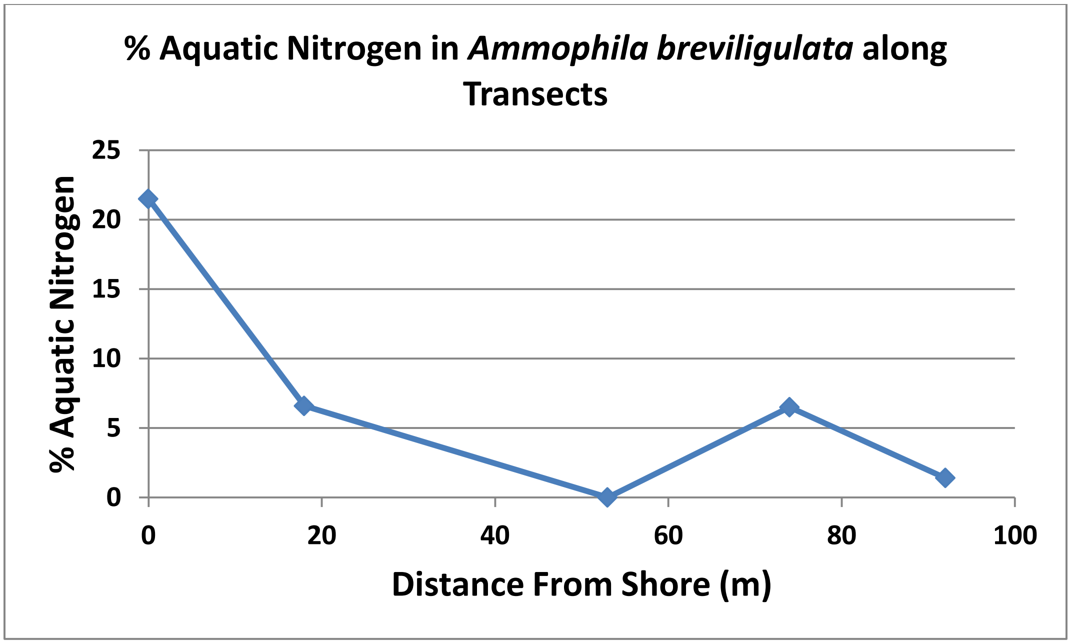

Figure 6: Calculated percent aquatic nitrogen in *Ammophila breviligulata* along transects (T1, T2, and T3) as found through the use of a two source mixing model (source one being the $\delta^{15}N$ value of aquatic midge insects and source two being the $\delta^{15}N$ value of *Ammophila breviligulata* tissue at site 53m along transects T1, T2, and T3).

Discussion

In this experiment we found that aquatic herbivore insects, specifically midges, act as the primary mechanism for the movement of aquatic nitrogen from Lake Michigan into terrestrial Sturgeon Bay dune ecosystems, as found by conducting aquatic herbivore insect removal sites, and sticky trap transects. Removal sites showed that the primary predator who physically moved these aquatic herbivore insects into the dune systems from shore were ants (terrestrial insects). We found that the gradient of aquatic nitrogen and the abundance/density of the aquatic herbivore insects did in fact show a decreasing trend with distance from shore, and a decrease in aquatic nitrogen movement inland as found through the *Ammophila breviligulata* transects, insect capture (midges, mayflies, and caddisflies), and the testing of these species for $\delta^{15}N$ values via a Stable Isotope Mass Spectrometer. This decreasing trend mirrored that of the aquatic herbivore insect standard emergence night captured on the sticky traps, but not that of the large emergence night. Lastly, *Ammophila breviligulata* was shown to have a higher percent of aquatic nitrogen in its tissues the closer to shore it was, hinting that the closer to shore these plants are the more supplemented growth they will have with the increase in availability of aquatic nitrogen.

The aquatic herbivore insect removal rates were as expected, although the time it took for complete removal and the terrestrial predator insect was not anticipated, although it makes sense. Ant hills were observed starting at the beginning of the vegetation on the beach and individuals were noticed scavenging along the shoreline for prey at all daytime hours. Therefore, it was accepted by us that they would be the primary mechanism for the removal of the aquatic herbivore insects that were washed up or dead on shore; and it made sense that removal happened quickly since nitrogen is poor in dune environments.

The sticky trap transects proved to be quite interesting. We were fortunate enough to capture snapshots of standard and large aquatic herbivore insect emergence nights, with midges being the most prevalent aquatic herbivore insect on each night, and at each distance. However, there were some marked differences between these nights in the percent aquatic herbivore insects present. On the standard emergence night the trend of percent aquatic herbivore insects was as expected, decreasing with distance from shore since aquatic herbivore insects develop in the water. On the large emergence night the trend of percent aquatic herbivore insects was that of a blanket effect, which was not expected. The sticky traps at each site on the large emergence night had percentages of aquatic herbivore insects ranging from about 65% to 83%, which was much different, and higher than percentages found on the standard emergence night with the highest percentage being 50% at water's edge and the lowest being 11% (Figure 3). We theorize that the standard emergence nights are the most common, based on our own personal observations of being at the site, and that the large emergence nights are rarer and don't alter the gradient of aquatic nitrogen moving back from shore as does the standard emergence night. Our findings for the mean δ^{15}N along the *Ammophila breviligulata* transects support the above statement, as it follows the same trend (Figure 4). Our results above seem congruent with other similar studies. Lundberg and Moberg (2003) as well as Gratton and Vander Zanden (2009) found an important vector of water to land resources to be aquatic herbivore insects. While midges were found to be the "mobile link" organisms that connect aquatic food webs to that of terrestrial food webs (Hoekman et al. 2011). Gratton et al. (2008) also found that midges can fuel adjacent terrestrial ecosystems by directly serving as resources for predators. Surprisingly though, where most studies found terrestrial consumers to be birds, lizards, spiders, and beetles (Murakami and Nakano 2002, Sabo and Power 2002, Sanzone et al. 2003, and Paetzold et al.

2005 in Gratton et al. 2008), our experiment found ants to be the primary terrestrial consumer of aquatic herbivore insects.

The gradient of δ^{15}N and abundance/density of aquatic herbivore insects from Lake Michigan into terrestrial dune ecosystems was as expected, decreasing with distance from shore. The aquatic herbivore insects had enriched δ^{15}N of 5.40, 3.51, and 5.77 for midges, mayflies, and caddisflies respectively. These values were expected because these insects develop in the aquatic environment and take up aquatic nitrogen N^{15} into their tissues when developing. The δ^{15}N values as found along the *Ammophila breviligulata* transects show a decreasing trend as samples increased in distance from the shoreline (Figure 4). The most enriched δ^{15}N values being at the vegetation start along the shoreline, and decreasing with distance. This trend was also expected and accepted since the percentage of aquatic herbivore insects followed the same trend/pattern (Figure 5). This pattern for the percentage of aquatic herbivore insects, however, is only seen on the standard emergence night, thereby supporting our earlier claim in that these nights tend to be most frequent. Although the large emergence nights happen occasionally, we also theorize that since they are not that common and that aquatic herbivore insects blanket the terrestrial dune ecosystems with no decreasing or increasing trend, that the δ^{15}N values along the dunes are not affected because theoretically all parts of the dunes are receiving the same amount/inputs of aquatic herbivore insects. Vander Zanden and Sanzone (2004) found that once these aquatic insects have emerged in their adult stages from the aquatic habitats that their densities decrease rapidly with distance from their aquatic beginnings; and where they are deposited results in a significant fertilization effect on terrestrial inhabitants with effects on community structure and plant quality (Gratton et al. 2008). As found in our experiment, midges in Iceland as studied along the shores of the Mývatn lake were also found to decline in abundance with distance from shore but were still present in fairly significant quantities up to 150 meters inland (Gratton et al. 2008). Although the previous study was done on extreme cases of massive midge hatchings, it was found that study lakes with low to moderate aquatic herbivore insect productivity followed similar patterns of aquatic insect distribution and the distribution of essential elements such as nitrogen (Dreyer et al. 2012).

Since aquatic herbivore insects were shown to be more abundant near shore than away from shore (if we assume the standard emergence night is most common) and the δ^{15}N values of *Ammophila breviligulata* were found to become less and less enriched with distance from shore, it was not surprising that the calculated percent aquatic nitrogen in *Ammophila breviligulata* tissue also decreased with distance from shore. We used the midge δ^{15}N for one of the two source mixing model points, as well as the lowest δ^{15}N value along the *Ammophila breviligulata* transect as to get conservative estimates of percent aquatic nitrogen along the transects. It was found that little fractionation of nitrogen isotopes occurs during the uptake by plants and all of the available nitrogen is used up (Owens 1987, Peterson and Fry 1987 in Schindler and Lubetkin 2004). As such, the δ^{15}N of plants reflect that of their nitrogen sources (Peterson and Fry 1987 in Schindler and Lubetkin 2004). With nitrogen being such a limiting nutrient in dune ecosystems, ranging between 1.02-2.02% in our samples *Ammophila breviligulata* will make use of whatever nitrogen is available. Because *Ammophila breviligulata* has access to precious aquatic nitrogen in the terrestrial dune ecosystems, made available primarily via midges, we feel justified in saying that whatever the percent of aquatic nitrogen is within *Ammophila breviligulata* tissue also represents the equivalent amount of extra biomass they are able to acquire by being able to sequester more carbon through photosynthesis. In other words, it is expected that the biomass of *Ammophila breviligulata* would be larger the closer the individuals are to the aquatic habitat, assuming that the terrestrial nitrogen supplies are the same all along the transects. Other studies found similar results or conclusions. In a study done by Hoekman et al. (2011) it was found that plant growth is stimulated by midge additions. This was confirmed by Dreyer et al. (2012) when they found that as midge decomposition occurs, elemental nutrients such as nitrogen are recycled through the soil with benefits to plant growth and quality. Even UK sand dunes that were along a nitrogen deposition gradient showed that the cover and biomass of *Ammophila arenaria* increased (UKREATE). This was also the case in the Jones et al. (2004) paper when they discovered a positive relationship between plant biomass and nitrogen inputs.

As our results show, the movement of nutrients, prey, energy, and consumers across habitat boundaries can be dynamically and energetically important to the receiving ecosystem; and consequences of such movement can range in its effects from population dynamics to influences on broader ecosystem processes (Vander Zander and Sanzone 2004). Being able to understand the various factors that end up influencing the dispersal of specific organisms across ecotone boundaries is particularly essential and necessary for predicting the extent of linkages between this water to land ecotone, and how much material, nutrients, etc. is delivered inland; and what their effects may be (Gratton et al. 2008). Mobile links, such as midges, and their correlated habitats (aquatic, and terrestrial) need to be considered more extensively in approaches to biodiversity conservation and ecosystem management. They play a very significant role in the dynamics of ecosystems and help to maintain the capacity of ecosystems to supply the ecological services that are essential for economic development and social welfare (Lundberg and Moberg 2003).

Our results raise many more questions and provide ideas for future experiments, and additional information would be valuable to extend our findings. Because sticky trap transects were done only over two nights, we were only able to collect snapshots of the types of emergences there may be over the course of the summer, so not enough data were collected to paint a comprehensive picture about the phenology of aquatic herbivore insects over the entire summer. We believe that sticky traps and flying insect traps would and should be used over the entire course of the summer to be able to know the relevant rates of these insects as to create a contour map on the dunes of the aquatic herbivore insect ‘rain’. Also, better data could be gathered and interpreted if the *Ammophila breviligulata* and sticky trap transects were 150+ meters in length. This would give a better picture of where aquatic herbivore insects stopped supplying aquatic nitrogen. Sampling biomass of *Ammophila breviligulata* and removal rates of aquatic herbivore insects would also help to compliment the above data. Additional similar experiments on the other Great Lakes examining similarities and/or differences, as well as finding and testing what and where the inputs are of nitrogen into the Great Lakes would be great complimentary experiments. Many new questions have arisen with this experiment, and we are sure many more will follow. With the continuation of studies done on the same study system, a better and more comprehensive story can be constructed, which will ultimately help our understanding and conservation of coastal environments.

Acknowledgements

I am very grateful and would like to thank my REU mentor, Brian Scholtens, for all of the valuable guidance during all phases of this project, the University of Michigan Biological Station staff for logistical support, Mary Anne Carroll and Dave Karowe for constructive suggestions about experimental design, and of course my REU peers for constructive suggestions in the beginning phases of this project. This work was supported by NSF REU grant # AGS-0851421 to M.A. Carroll and D. N. Karowe.

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