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The Flow of Aquatic Nitrogen from Ants to Antlions on the Sturgeon Bay Dunes

Abstract

In order to determine the effect of distance from the shoreline on nitrogen levels in terrestrial organisms living in a dune habitat, we quantified the nitrogen content of both ant and antlion species looking at the $\delta^{15}N/^{14}N$ *ratio. We collected ants and antlions at Sturgeon Bay of Lake Michigan in Wilderness State Park, taking samples from ten different locations at varying distances between the tree line and the shoreline and measured the nitrogen levels found in all of the samples. Additional samples were collected from a terrestrial environment as well as algae samples collected from an aquatic environment which served as a base comparison standard for Nitrogen values. When looking at nitrogen values and distances from the shoreline we found no significant statistical correlation suggesting that further research may be needed in order to find a more significant correlation.*

Introduction

The Sturgeon Bay Dunes of Lake Michigan which are located in the northern part of the state in the Lower Peninsula are a desired habitat for a wide range of species and are an ideal site to easily observe the study of ecological succession and nutrient cycling. Like most sand dunes the Sturgeon Bay Dunes are the ideal site to examine predictable changes in the composition or structure of an ecological community. The movement of materials and organisms between ecosystems is a common occurrence in nature. These exchanges in the ecosystem can be categorized into three major groups (1) transport of nutrients and materials by physical agents such as wind or water; (2) transport of nutrients and materials by biotic agents such as vertebrates; and (3) movement of prey and consumers between habitats (Polis, Anderson and Holt 1997) and is prominent in both terrestrial and aquatic ecosystems.

The Sturgeon Bay dunes were formed when sand from Lake Michigan was carried from the bay by currents from the westerly winds. The sand gradually accumulated on the shoreline and was blown into dunes during extreme stages of drought (Litcher, 1998). While the dunes are typically a nutrient poor environment, plants and other vegetation inhabit the dunes by adapting to the challenges of winds, abrasion and burial from sand. Each organism functions best within a certain range of environmental factors such as temperature, soil acidity and nutritional quality of food items. A significant effect on the survival, growth and reproduction of many species can be observed within the ecosystem by examining the changing environmental conditions of the Sturgeon Bay dunes.

In addition to changes in vegetation and relative location of the dunes with respect to the lake, the soil content changes as well. One factor that contributes to this process is the decomposition of plant materials, which gradually enriches the otherwise nutrient poor environment. Looking at dune succession, the overall nitrate production increases with succession age, while nitrate production in the secondary sere shows no consistent pattern except that soils from the oldest stage produced the most nitrate (Robertson and Vitousek 1981). Therefore looking at these data, we would expect to see overall higher nitrogen levels in greater distances from the shoreline.

Examining patterns of nutrient content between animals in different trophic levels is important to understand the significance of this study. Recently, the Lake Huron locust is an endangered species endemic to the northern Great Lake dune area that was previously known as a strictly herbivorous organism, but has been discovered to have acquired scavenger behaviors (Scholtens, personal communication). The nitrogen-poor plants on the northern Great Lake dunes suggest that this newly discovered behavior may be an indication that the flow of nitrogen from the terrestrial community is not sufficient for survival of the Lake Huron locust and may be an observable adaptation that is used to supplement the low nitrogen levels that are fixed on the dunes. The goal of this study is to examine the amount of aquatic nitrogen flowing from Lake Michigan into two terrestrial trophic levels. Like the Lake

Huron locusts, ants have been observed utilizing aquatic insects as a supplement to their food supply and can used to measure the flow of aquatic nitrogen through trophic levels of the food chain.

Because the Lake Huron locust is an endangered species we looked at the nitrogen ratio of aquatic and terrestrial nitrogen in ant and antlion species in order to find an accurate measurement of the flow of aquatic to terrestrial nitrogen through the different trophic levels of the food chain. Ants are opportunistic in nature and like the Lake Huron locust have been observed consuming dead, aquatic insects to supplement their food supply on the dunes of Sturgeon Bay. Antlions are also opportunistic organisms and consume other insects that may happen to fall into their pit traps.

Unlike the locust, ants have an insect predator, the antlion that lives in the same location as the ant species. Ants are the typical prey for the antlion, *Myrmelean immaculatus*, thus an accurate measurement of aquatic nitrogen can be taken as it flows up through the food chain. Antlions are insects that dwell in concave pits in the sand which make them easy to find. The antlion sits at the bottom of the pit with its mandibles protruding, waiting for any potential prey items to stumble into its pit. As soon as the ant falls to the bottom, the antlion uses its mandibles to seize and kill its prey (Marshall 2006). By examining nitrogen content of both ants and antlions, we can look for a possible correlation between the effect of distance from the shore and nitrogen levels as well as the flow of nitrogen from aquatic systems. Differences in nutrient content, specifically nitrogen and phosphorus between herbivores and their plant resources have proven to have major consequences on herbivore success, nutrient cycling, and the fate of primary production in ecosystems (Fagan et al 2002). They showed that predators exhibit, on average, 15% more nitrogen content than herbivores. Therefore we would expect to see higher nitrogen content in antlions compared to ants.

Stable isotope ratios are used to assign individual organisms to a particular trophic level. Analysis of trophic structure has found that N^{15} values increase in a consistent manner as trophic levels increases (Adams and Sterner 2000). The aquatic and terrestrial nitrogen can be measured by looking at the percentage of specific isotopes found in insects. Nitrogen has two stable isotopes, N^{14} and N^{15}. Aquatic nitrogen has a signature isotope ratio (N^{15}/N^{14}) that differs from the ratio of terrestrial nitrogen in that it is typically higher. This ratio allows researchers to calculate the amount of aquatic verses terrestrial nitrogen that is fixed in an organism. Looking at the value of stable isotopes can reveal a relationship between an organism's diet and its corresponding isotopic composition. Comparison of consumers and their resources indicate that N^{15} values increase approximately 3.4% with each trophic level. Therefore by analyzing the N^{15} values of various organisms within an ecosystem it may be possible to explain part of the trophic structure within the ecosystem (Kling et al. 1992; Hecky and Hesslein 1995). Experiments that trace N^{15} either naturally occurring concentration gradients or those that monitor additions of N^{15} enriched or depleted substrates can be used to examine biogeochemical processes such as nutrient cycling.

The objective of this experiment was to quantify the amount of aquatic nitrogen in two trophic levels, looking at ant and antlion species living various distances from the shoreline of the Sturgeon Bay Dunes. The combination of aquatic and terrestrial nitrogen in ants should indicate the amount of aquatic versus terrestrial nitrogen present in the food they are scavenging. If ants in one location have a combination of both aquatic and terrestrial nitrogen fixed in their system, then their predator, the antlion in that same location will contain the same ratio of aquatic to terrestrial nitrogen magnified by the trophic level change.

Materials and Methods

Before gathering specimens for analysis, we first measured the distance of the tree line to the shore at Sturgeon Bay of Lake Michigan. We collected samples from ten different locations at various distances in meters from the tree line to the shoreline in order to find an overall average correlation of nitrogen flow within the dune habitat. In this case measurements were taken at 12, 33.5, 40, 58, 72.7, 81, 90, 101, 112.5 and 129.8 meters from the shore to the tree line. At each site location, a minimum of 10-20 individual ants from the same colony were collected using an aspirator. Because this was a rather time consuming process, we often would first kill the ants with a hand trowel and then use forceps to transfer them into vials. The ants that were collected using an aspirator were also transferred to a vial and labeled with the site number and distance from the shoreline.

Antlions were much easier to retrieve because they are pit-dwelling insects. Antlion pits can be easily located and then retrieved using a hand trowel to scoop up the sand which was then transferred to a sifter in order to remove the sand and easily locate the antlion. Using forceps the antlion was removed and placed in a vial, again labeled with the site number, name of the species and distance from the shoreline. A total of five antlions were collected from each site. Since antlions are predatory organisms and may potentially eat each other, only one antlion was placed in each vial.

In order to have a base sample to compare the nitrogen levels of ants and antlions that do not scavenge for aquatic insects, we collected additional samples from the UV field at the University of Michigan Biological Station using the same methods. These samples served as a baseline for the amount of N^{15} that would be expected in ants and antlions that are foraging strictly on terrestrial sources. Other researchers from the University of Michigan Biological Station collected algae samples from the shore of Lake Michigan at Sturgeon Bay where the algae samples served as the base amount for aquatic N^{15} values.

Once all the specimens were collected from the both aquatic and terrestrial locations the samples were then placed in a freezer of -80°C for an approximate time of two hours. The ant specimens were then transferred to a lyophilizer to be freeze-dried overnight. Because the antlion specimens would not completely dry using the lyophilizer, the frozen antlion samples had to first be punctured and flattened with a small spatula and then placed in a 50 degree oven in order to dry overnight. To finally prepare the samples for nitrogen analysis the samples were ground up using a SPEX

8000D Mixer/Mill machine. Once all the samples were ground they were transferred to scintillation vials and labeled with an ID number. To analyze the samples, they were ran through the Thermo Finnigan Delta XP, then later were run through the Costech Elemental Combustion System where ISODAT 2.1 computer software was used to obtain the carbon and nitrogen content of the samples and the stable isotope ratio.

The data were graphed into scatter plots using Microsoft Excel software and were also analyzed using regression analysis on SPSS software. A regression analysis determines the strength of a dependent relationship between variables. In this case the dependent variable was the nitrogen content of the ants and the independent variable was the distance from the shore.

Results

The $\delta^{15}N$ data for ants and antlions are shown in Figures 1 and 2. The mean value for the ant samples was 4.370 with a standard deviation of 0.4398. The summary for linear regression of N^{15} values for ants and the distance found from the shore gave an R-squared value of 0.005. (F=, P= 0.852).

The mean value for antlions was 3.543 with a standard deviation of .8586. The summary of the linear regression for nitrogen values for antlions and the distance from the shore gave an R-squared value of .070. (P= .461).

Discussion

Generally, since aquatic habitats are more enriched with nitrogen in comparison to terrestrial habitats (Adams and Sterner 2000), we expected a lower $\delta^{15}N$ value in ants and antlions as their distance from the shore increased. Our results do not show any significance because both P-values for ants and antlions are greater than .05. There is no significant difference in nitrogen levels as ants and antlions were sampled farther from the shore.

The R-squared value for nitrogen in ants was .005 and reveals no significant trend between the nitrogen levels in ants and their distance found from the shore. The R-squared value for antlions showed no significant trend as well with an R-squared value of 0.070. Figure 1 (Ants) and Figure 2 (Antlions) show no significance since the trend line for both ants and antlions are noticeably flat instead of showing a downward slope which may imply the same amount of nitrogen levels are found throughout the dunes.

From the observed data, antlions displayed more of a variation in δN^{15}. This variation may be due to the fact that each time you go up a trophic level you may see more variation because you can not control what they are eating. Antlions, like ants and the Lake Huron locust, may also be opportunistic in nature and may possibly feed on various prey items, not strictly ant species, that just so happen to fall into their pit traps. The results may also suggest that the highest amount of nitrogen in ants is fixated in their exoskeleton. When antlions seize their prey they puncture the abdomen and only eat the innards and discard the exoskeleton (Marshall 2006).

Since the study did not show a significant affect on nitrogen levels in ants and antlions when looking at distance from the shoreline further experiments and research may be needed. This could have something to do with varying food supplies as well as the time frame in the abundance of aquatic organisms, presuming that at the time of research the amount of aquatic insects on the shoreline may have been low.

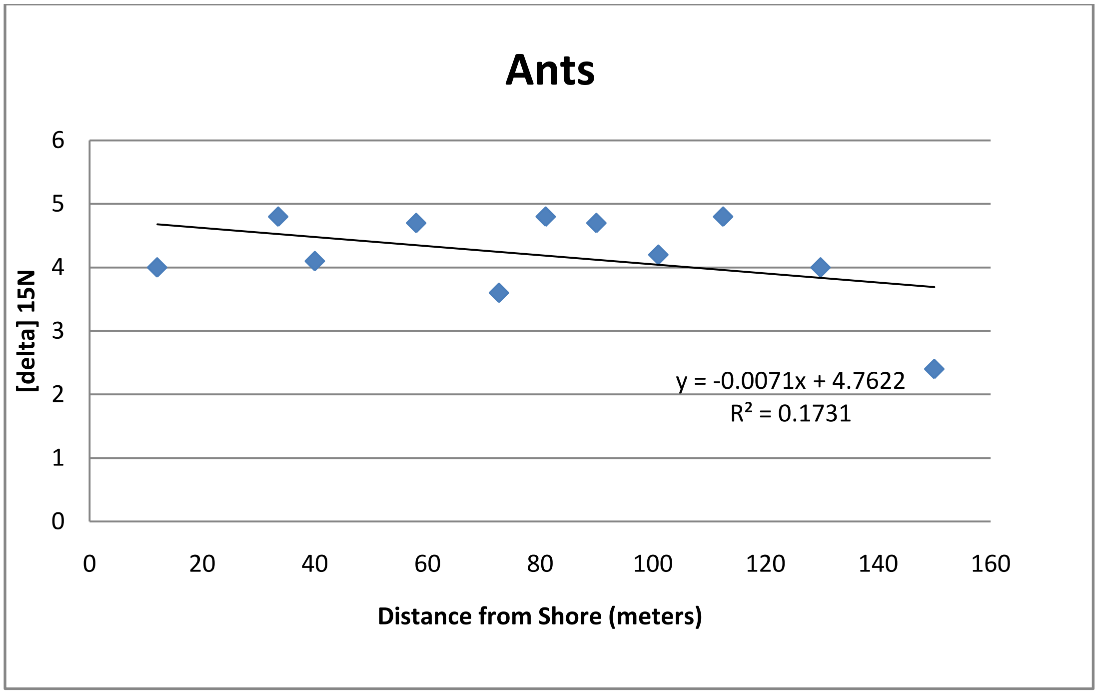


Figure 1. N^{15} values of ants as distance from the shore increases.

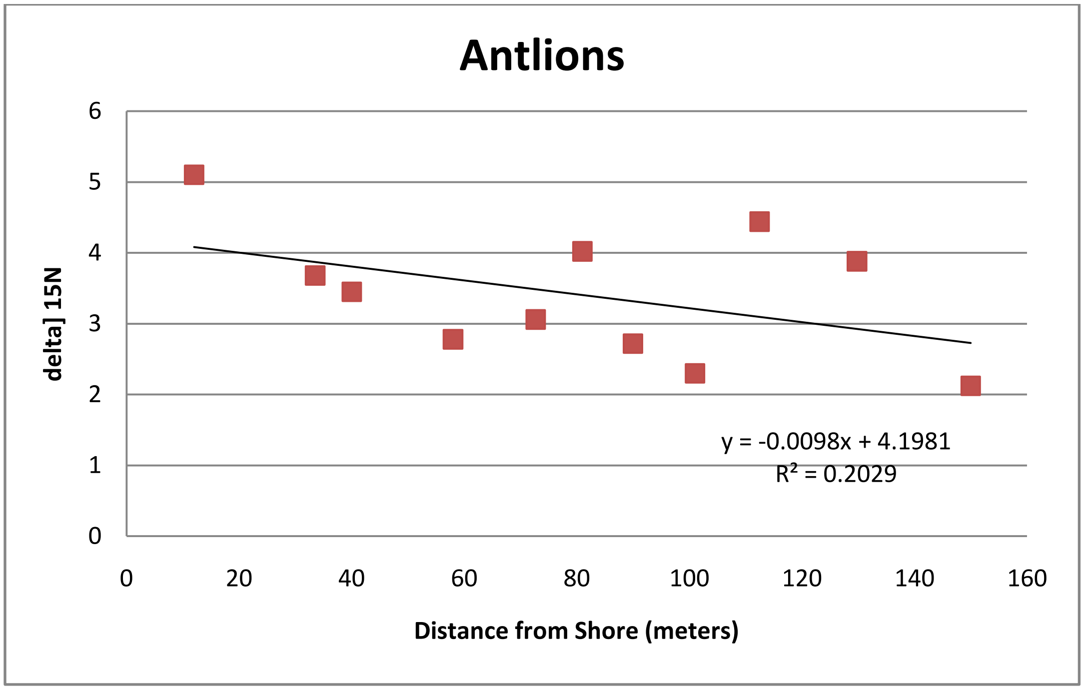
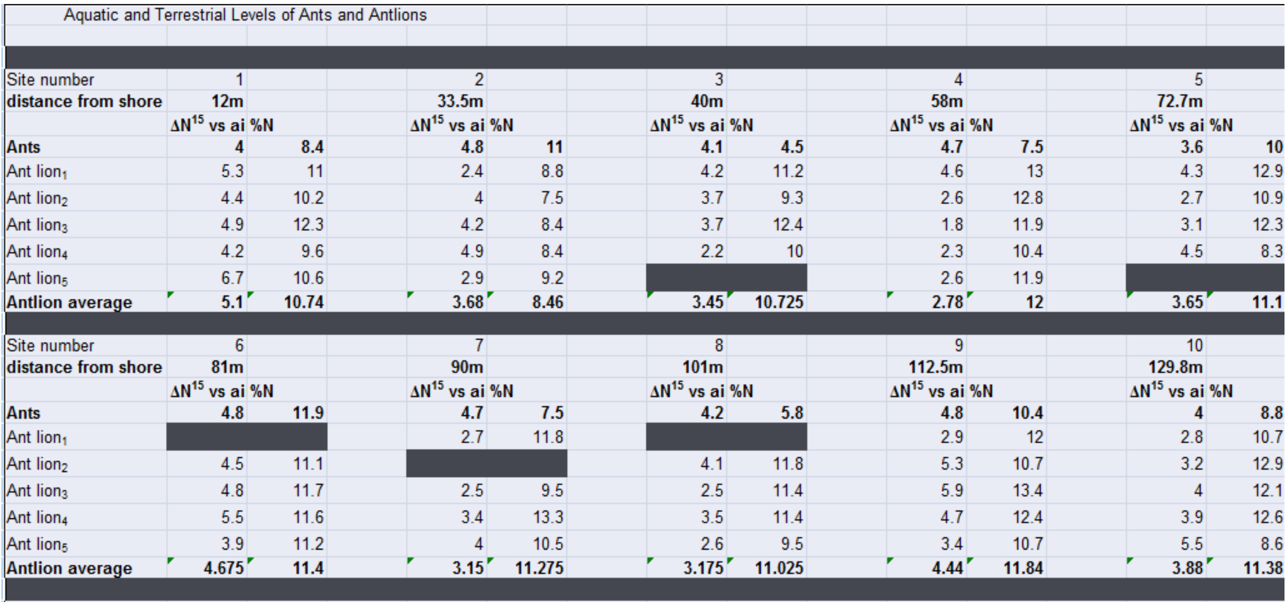


Figure 2. N^{15} values of antlions as distance from the shore increases.

Table 1. The data chart of N^{15} values from collected of ants and antlions. The black boxes indicate outliers of N^{15} values.



Aquatic and Terrestrial Levels of Ants and Antlions

Site number	1		2		3		4		5	
distance from shore	**12m**		**33.5m**		**40m**		**58m**		**72.7m**	
	ΔN^{15} vs ai	**%N**	**ΔN^{15} vs ai**	**%N**	**ΔN^{15} vs ai**	**%N**	**ΔN^{15} vs ai**	**%N**	**ΔN^{15} vs ai**	**%N**
Ants	**4**	**8.4**	**4.8**	**11**	**4.1**	**4.5**	**4.7**	**7.5**	**3.6**	**10**
Ant lion$_1$	5.3	11	2.4	8.8	4.2	11.2	4.6	13	4.3	12.9
Ant lion$_2$	4.4	10.2	4	7.5	3.7	9.3	2.6	12.8	2.7	10.9
Ant lion$_3$	4.9	12.3	4.2	8.4	3.7	12.4	1.8	11.9	3.1	12.3
Ant lion$_4$	4.2	9.6	4.9	8.4	2.2	10	2.3	10.4	4.5	8.3
Ant lion$_5$	6.7	10.6	2.9	9.2			2.6	11.9		
Antlion average	**5.1**	**10.74**	**3.68**	**8.46**	**3.45**	**10.725**	**2.78**	**12**	**3.65**	**11.1**
Site number	6		7		8		9		10	
distance from shore	**81m**		**90m**		**101m**		**112.5m**		**129.8m**	
	ΔN^{15} vs ai	**%N**	**ΔN^{15} vs ai**	**%N**	**ΔN^{15} vs ai**	**%N**	**ΔN^{15} vs ai**	**%N**	**ΔN^{15} vs ai**	**%N**
Ants	**4.8**	**11.9**	**4.7**	**7.5**	**4.2**	**5.8**	**4.8**	**10.4**	**4**	**8.8**
Ant lion$_1$			2.7	11.8			2.9	12	2.8	10.7
Ant lion$_2$	4.5	11.1			4.1	11.8	5.3	10.7	3.2	12.9
Ant lion$_3$	4.8	11.7	2.5	9.5	2.5	11.4	5.9	13.4	4	12.1
Ant lion$_4$	5.5	11.6	3.4	13.3	3.5	11.4	4.7	12.4	3.9	12.6
Ant lion$_5$	3.9	11.2	4	10.5	2.6	9.5	3.4	10.7	5.5	8.6
Antlion average	**4.675**	**11.4**	**3.15**	**11.275**	**3.175**	**11.025**	**4.44**	**11.84**	**3.88**	**11.38**

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