δ^{13}C and δ^{15}N Isotopic Signatures of Carabid spp. in Seasonal Pools of the Northern Woods

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Abstract:

Vernal or seasonal pools are a type of wetland in which low lying areas experience seasonal drying and inundation. These ecosystems are found in all climates, however, characteristics of these ecosystems vary greatly based on geographic range. The support of unique flora and geographic specificity of these pools makes local studies of these ecosystems important. Insects and other invertebrates often play important roles within such ecosystems. Ground beetles are common organisms with a wide geographic range. They have a large potential for acting as bioindicators in many different ecosystems. Functional roles played by organisms with in ecosystems can act as indicators for environmental variability and ecosystem function. Examining the diet of organisms can provide information on the functional role of the species. In this study, the trophic role and diet of ground beetles in seasonal pools was examined using δ^{13}C and δ^{15}N. Limitations in data and methods of data analysis made results rather unambiguous. There was a difference between isotopic values of different dates indicating there may be some influence of seasonality on ground beetle diet.

Introduction

Wetlands are unique ecosystems which bridge terrestrial and aquatic habitats. They serve a variety of important functions such as absorbing pollutants and protecting shorelines from wave impacts (Richardson 1994). Restoration and conservation of these endangered ecosystems are of particular interest because they support endangered and threatened fauna and flora disproportionate to their own abundance (Brook and Croonquist 1990). Vernal or seasonal pools are wetlands which exhibit distinct dry and inundated stages correlated with seasonality (Ciccotelli et al. 2011). While vernal pools can be found in a variety of climates, characteristics of these pools and the functions they serve are unique to their location().

Invertebrates play prominent roles in these wetlands as their evolution and diversity allows them to fill a variety of roles at different trophic levels. Due to the variability within insect communities, many insects are useful bioindicators. Variance in the traits of and the functional diversity of insects makes them useful indicators for abiotic variability, intensity and frequency of disturbances, and trophic hierarchies. Members of the Carabidae family, also known as Ground beetles, are common in many areas. They feed on a wide range of resources capable of acting as generalist predators, omnivores, or herbivores. Their wide range and ability to respond to changes in the environment makes them promising candidates as bioindicators in ecosystems. Ribera et al. used morphological characters of ground beetles to demonstrate that changes in the abiotic environmental factors impact morphology (2014). However, structural characteristics are not the sole determinant of the functional or trophic role played by an individual. Differentiation in the rates of evolution between morphological structures and physiological mechanisms makes it possible for a species to structurally remain a generalist but physiologically become a specialist (Lovei et al 1996).

Dietary studies offer an alternative way to study the active functional role played by an individual. The use of stable carbon and nitrogen isotopes has proven to be a promising method for dietary studies of organisms (Tillberg et al 2006). Stable carbon isotopes can be used to determine the basal carbon sources of a specie's diet this includes differentiating between C3, aquatic, and C4 plant sources (Rasmussen 2009). Nitrogen isotope values are used primarily to study the trophic level of a species. The more N^{15} enriched a species, the higher their trophic level (Elshayeb et al. 2009, Hyodo et al 2015, Tillberg et al. 2006).

Based on the cyclical drying and flooding of vernal pools I predict that ground beetles will exhibit a varied diet. The diet of ground beetle will be influenced by food resource availability and will therefore change with season. Since vernal pools support aquatic insects but lack fish, common predators, ground beetles will occupy a predacious role in the food web. Predation would be indicated by a more enriched N^{15} level and similarity in C^{13} signatures.

Materials and Methods

Field Sampling

Pitfall traps (figure 1) were set up in three different locations across the property of the University of Notre Dame Environmental Research. These were composed of buckets dug into the ground with the tops left ajar to prevent water from getting in, but still collect samples. There was netting placed between each bucket that helped draw the insects to the collected buckets. Sites were characterized by assessing vegetation, soil moisture, and pH. The areas chosen were set in early successional forests nearby seasonally wet areas or areas where the water table was located close to the surface. Samples were collected from traps during the periods of 17/05/29-17/06/02, 17/06/12-17/06/16, and 17/07/10-17/07/14. Samples were collected in glass jars or vials which had been rinsed with water and ethanol and allowed to dry in a fume hood or in paper bags and labeled. Plant samples were collected at each site on 17/06/13. Plants were selected based on their commonality between all three sites, as well as visible evidence of insect interactions (bite marks, larvae on leaves, etc.).

Sample Processing

Once collected samples were left in the lab for between 11-13 hours to excrete any recently consumed resources. Samples were then moved to a -20^{o}C freezer. After remaining in the freezer for at least 8 hours, samples were placed in a drying oven at 50^{o}C for 12 hours. Once dry, the samples were ground using an ethanol cleaned mortar and pestle then packaged in labelled penny envelopes. Insects were identified down to order or family level and preserved photographically using Leica Application Suite software and a dissecting light microscope with a camera.

Samples were mailed sent to the University of Notre Dame's Center for Environmental Science and Technology (CEST). Each sample was weighed to around 0.8 mg on a microbalance and placed into 4 x 6 mm pressed tin capsules. The stable carbon and nitrogen isotope ratios were found using an elemental analyzer coupled to a Delta V Isotope ratio mass spectrometer (EA-IRMS). Stable C and N isotope ratios are expressed as: δ^{13}C or δ^{15}N =[(Rsample/Rstandard) - 1] X 1000 where R is 13C/12C or 15N/14N for δ^{13}C or δ^{15}N, respectively. The standard for C is the Peedee Belemnite (PDB) and for N the standard is atmospheric N2.

Data Analysis

Data collected from EA-IRMS was exported into an excel document. δ^{13}C and δ^{15}N values were corrected for using sorghum flour, protein powder, and peach leaf standards provided by CEST facilities. An acetanilide standard was used to calculate the %C and %N using the following equations: %C = (71.09 x std.amount (mg) / std. peak area 44) x (sample peak area 44 / sample amount (mg)) and %N = (10.36 x std.amount (mg) / std. peak area 28) x (sample peak area 28 / sample amount (mg)). $\delta^{13}C$ values were used as an indicator of different food resources used by insects. $\delta^{15}N$ were used as an indication of the trophic level of insects. The impact of different sampling sites on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$ / $\delta^{15}N$ on ground beetles was assessed by using 3 individuals Kruskal Wallis tests. Kruskal Wallis tests were also used to compare $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$ / $\delta^{15}N$ between different families. Seasonal changes were assessed by using $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$ / $\delta^{15}N$ of ground beetles collected during different weeks of the summer. Date was analyzed as a categorical variable due to proximity in sampling dates and limitations in ground beetle data obtained from different days. $\delta^{15}N$ values for date were not normally distributed; to achieve a normal distribution values were transformed using an arcsine square root function. An analysis of variance was used to compare the transformed values of different collection dates. A Shapiro Wilkes test for $\delta^{13}C$ and $\delta^{13}C$ / $\delta^{15}N$ showed non-normal distribution of data so Kruskal Wallis tests were used.

Results

Table 1 shows the mean values of $\delta^{13}C$ and $\delta^{15}N$ of different Linnaean groups collected. The table shows that insects collected exhibited a much larger C signature than plants. C signature of plants ranged from 7.90-8.57 while insects ranged from 32.70-50.64 (Table1). There was no statistical significance between $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$ / $\delta^{15}N$ observed based on different sampling sites (Table 2). Ground beetles and other invertebrate families and orders (Table 2, Figures1&2) did not show statistically significant difference in on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$

/ $\delta^{15}N$ values. A significant difference may not have been observed between different orders and families due limited sample sizes. Seasonality did have a statistically significant impact on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$ / $\delta^{15}N$ (Figures 3,4,5). Unfortunately, while the Kruskal Wallis test reveals a significant difference between the groups it does not allow for a post hoc test therefor, it is not possible to determine which groups varied statistically from each other.

Discussion

It is unsurprising that there was no statistical difference observed between samples from different sites. The sites were all early successional forests with low lying areas that showed seasonal pooling. Since ground beetles were sampled from similar microenvironment, it is probable that they played similar functional roles and occupied similar trophic levels. It probable that $\delta^{13}C$ and $\delta^{15}N$ signatures are similar between organisms belonging to the same family and found in similar microenvironments in the same geographical region.

Families also failed to show significance between on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$ / $\delta^{15}N$ signatures. The result is contradictory to that of dietary studies which used stable isotopes(). Many report finding difference in signatures between species and in some instances within species(). One possible explanation for this may be small sample size. For instance, only three individuals belonging to the order Diptera were collected. Other samples were equally underrepresented or the size of the individuals made it difficult to obtain $\delta^{13}C$ and $\delta^{15}N$ values. A second explanation may be related to the role of ground beetles as generalist predators. $\delta^{13}C$ are used in determining the primary food resource of organisms because they change very little from consumption of the original resource. If ground beetles consume other insects, it is not surprising that they would exhibit similar $\delta^{13}C$ signatures. Such an assumption does not explain similarities in $\delta^{15}N$ and $\delta^{13}C$ / $\delta^{15}N$ between families which play different ecological roles and are known to differ in primary resource consumption. It is expected that predaceous organisms would have a more enriched $\delta^{15}N$ value and occupy a lower $\delta^{13}C$ / $\delta^{15}N$. Lastly, lack of difference between families could be due to the limitations of statistical tests used. Many individuals rely on a variety of food resources which creates a unique signature; differences in food resources are frequently distinguished through use of mixing models which were not used in this study.

As predicted the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{13}C$ / $\delta^{15}N$ differed significantly with the date sampling occurred. Differences in $\delta^{13}C$ indicates a change in the primary resource consumption of ground beetles based on seasonality. Changes in $\delta^{15}N$ and $\delta^{13}C$ / $\delta^{15}N$ indicate that the trophic level also shifted based on some sort of seasonal impacts. The two changes are not mutually exclusive but rather may support each other. Change in primary food resource may result in occupation of different trophic levels if ground beetles consume a greater proportion of secondary consumers. As demonstrated by Ikeda et al., ground beetles may shift diet from primarily herbivorous to omnivorous based on resource availability (2010). Unfortunately, while the Kruskal wallis test showed a statistically significant difference between $\delta^{13}C$ and $\delta^{13}C$ / $\delta^{15}N$ it is not possible to distinguish the area in which this difference occurs. Similarly, while an ANOVA revealed a statistical difference between date and the arcsine square root of $\delta^{15}N$ the transformed data was not able to be analyzed using a post hoc Tukey test and therefor it is not possible to state where such a difference occurs.

In conclusion, it appears that seasonal factors impact the diet and trophic level occupied by ground beetles near seasonal pools. The result is consistent with the results of other studies which looked at the diet of ground beetles using isotopic data. Questions remain as to how such seasonal factors impact the diet of ground beetles and the trophic and functional roles played by ground beetles in seasonal pools. A future study may use stable isotope methodology to perform a more comprehensive study of insect food web interaction of seasonal pools. Greater sampling of other invertebrate families may aid in clarification of the role played by ground beetles in relation to other invertebrates. In addition, use of more sophisticated models may help to distinguish between primary food resources and changes which occur in these sources.

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	Mean %C	Standard Error	Mean %N	Standard Error
Scarabidae	45.57991	±0.52926	5.803639	±0.278959
Buprestidae	49.64462	±0.619869	11.1818	±0.016562
Pentatamide	47.90404	±0.083331	5.803639	±0.025325
Elateridae	47.16254	±2.247601	5.52468	±0.278959
Carabidae	38.74964	±3.550139	10.97849	±2.658177
Isoptera	47.3548	±0.091331	4.676851	±0.008756
Diptera	32.69859	±15.61732	19.39646	±14.73474
Formicidae	47.30406	±0.339502	4.844512	±0.213875
Fraxinus pennsylvanicus	8.374661	±0.043648	3.105548	±0.253805
Betulae papryfa	8.306088	±0.056012	3.067111	±0.029761
Acer saccharum	8.488849	±0.034241	2.030211	±0.010762
Fraxinus americana	8.56858	±0.012036	2.308996	±0.012064
Convalleri majalis	7.897827	±0.047119	1.863534	±0.009822
Dryopteris intermedias	8.073505	±0.063802	2.415666	±0.025761

Table 1: Mean $\delta^{13}C$ and $\delta^{15}N$ with standard error by family

	Chi squared values	Degrees of freedom	p-value
$\delta^{13}C$ ~Site	0.77	2	0.67
$\delta^{15}N$~Site	2.30	2	0.32
$\delta^{13}C$ / $\delta^{15}N$~Site	3.02	2	0.22
$\delta^{13}C$ ~Family	4.31	6	0.64
$\delta^{15}N$~Family	5.80	6	0.45
$\delta^{13}C$ / $\delta^{15}N$~Family	7.54	6	0.27

Table 2: Results of Kruskal Wallis statistics

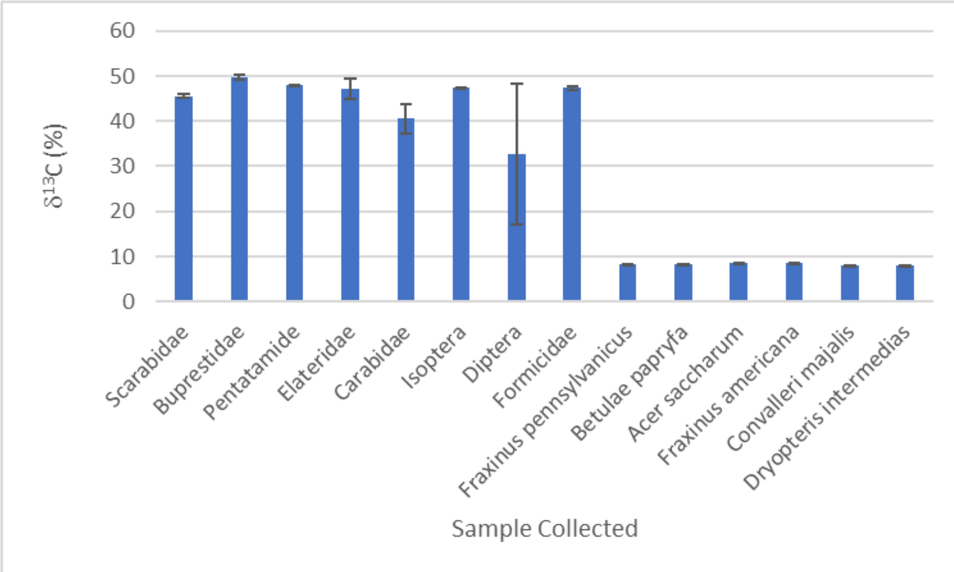

Figure 1-barplot of $\delta^{13}C$ by family

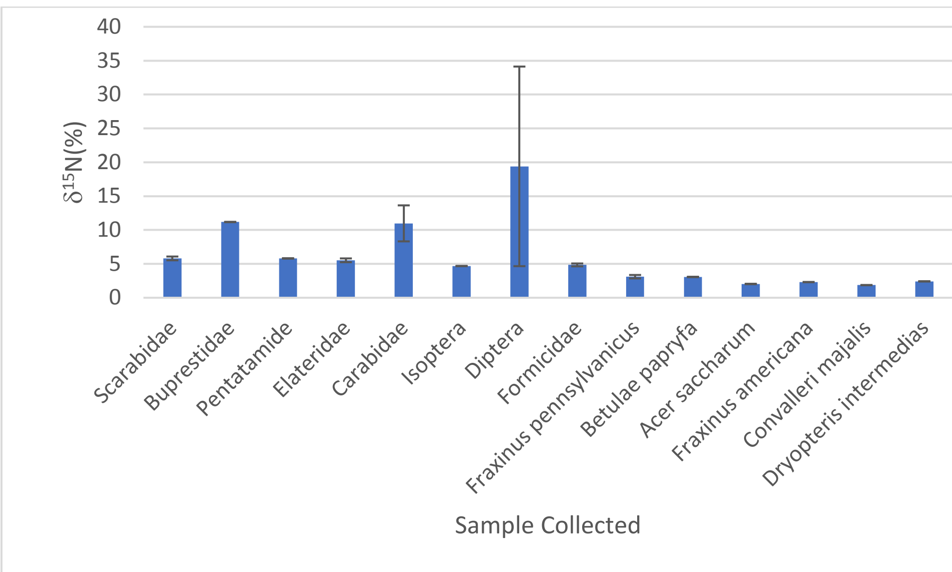

Figure 2-barplot depicting $\delta^{15}N$ by family

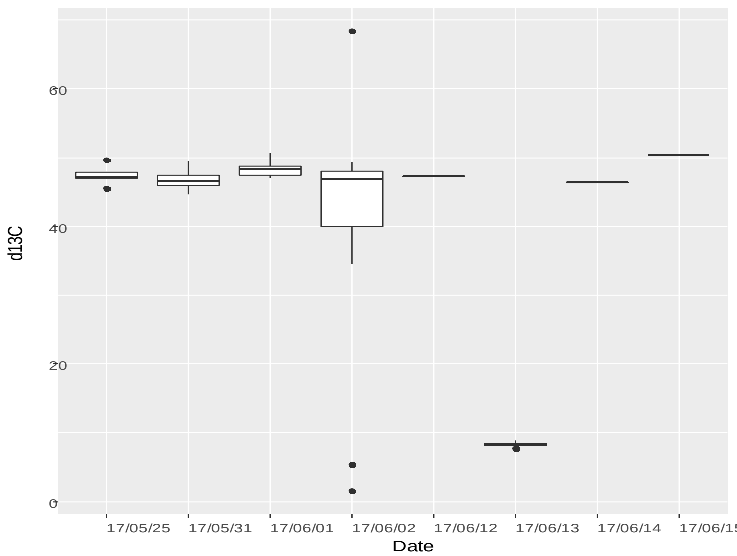

Figure 3:box whisker plot of d13C by Date

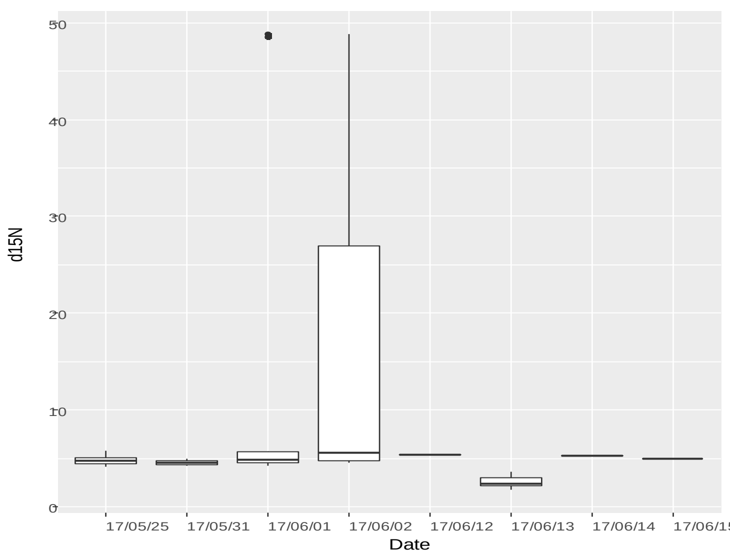

Figure 4: box whisker plot of d15N by Date

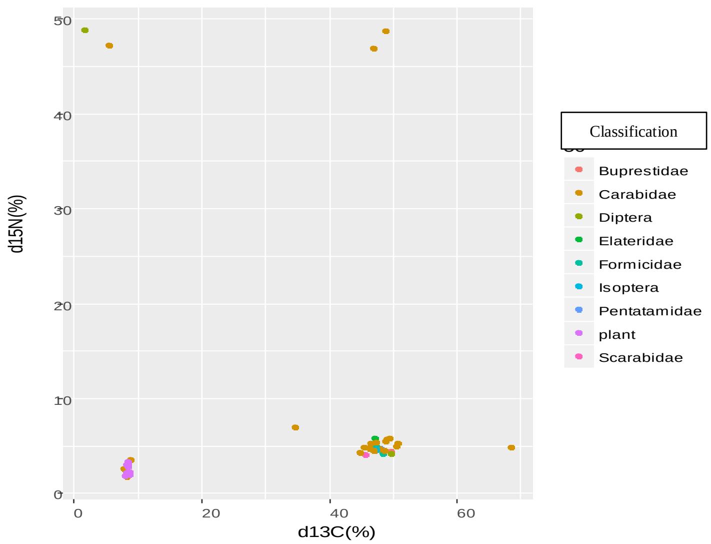

Figure 5: scatter plot of $\delta^{15}N$ by $\delta^{13}C$