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Growth Rates of Grasshopper Nymphs in Nitrogen Deficient Versus Nitrogen Rich Habitats

Abstract

We observed changes in length and weight of grasshopper nymphs in controlled environments of nitrogen rich and nitrogen deficient soil until they reached adulthood. Dead ants were added to the environments to see if the grasshoppers would deviate from their herbivorous nature in order to supplement their nitrogen intake. There was no significant evidence that the grasshoppers ate any of the dead ants. We found no significant difference in growth rates for nitrogen rich versus nitrogen deficient environments. Initial size and length of grasshopper nymphs had no effect on survivorship. The insignificance of the data may be attributed to the extreme temperatures to which the grasshopper nymphs were exposed.

Introduction

Nitrogen is an important element to life on earth because it is a necessary component of ribonucleic acids, deoxyribonucleic acids, and amino acids. Amino acids polymerize to form proteins, which are essential to growth and numerous functions in organisms, ranging from catalysis of chemical reactions to communication between and within cells to structural support for cells. Thus, there is an innate behavior present in organisms to efficiently gather sufficient levels of nitrogen necessary for development and reproduction.

Higher levels of nitrogen correlated with better survivorship and reproductive performance in grasshoppers (Joern and Behmer, 1997). Grasshoppers can distinguish between food sources of different quality and base their decision on nitrogen levels (Joern and Behmer, 1997). In grasses, nitrogen levels may vary from between 0.5%-7%, with

optimal levels for grasshopper growth and reproduction around 4% (Joern and Behmer, 1997). In the Sturgeon Bay sand dunes, the soil nearer the water was unproductive due to the effects of strong winds, high evaporation rates, and leaching of nutrients through the fragmented sand, correlating to lower levels of nitrogen (Lichter, 1998). Thus, the grass, *Ammophila breviligulata,* collected from this area was nitrogen deficient with nitrogen levels between 1–1.5% (Lichter, 1998). In order to supplement their nitrogen intake, these grasshopper nymphs, like the Lake Huron Locust, may scavenge for dead insects.

As grasshopper nymphs continue to feed on nitrogen deficient plants they may also increase consumption in order to meet their nitrogen needs. According to Berner et al. (2005), an increase of 82% in mean food consumption of the grasshopper *Omocestus viridulus* was observed when subjected to nitrogen deficient grass.

Conversely, *Poa pratensis* (Kentucky bluegrass), which is presumed to be nitrogen rich, was collected from the UV field at the University of Michigan Biological Station. The cohesive soil of the UV field allowed for better water retention and increased nitrogen availability. As such, grasshoppers feeding on this grass should exhibit normal growth and survivorship.

Thus, grasshoppers exposed to nitrogen deficient plants should have slower growth rates compared to grasshoppers feeding on nitrogen rich plants, since lower levels of nitrogen are available to form essential proteins required for growth and function. By feeding grasshopper nymphs grasses from either the Sturgeon Bay sand dune ecosystem or the Douglas Lake ecosystem differences in growth rates due to levels of nitrogen present should become apparent.

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<u>Materials & Methods</u>

  We measured the growth rates of 80 grasshopper nymphs until they molted into adult grasshoppers. The nymphs were collected in the Douglas Lake ecosystem, specifically the UV field of the University of Michigan Biological Station, with nets and then individually placed into small glass vials. We took initial length and weight measurements for each nymph using an electronic scale and electronic calipers. To ensure accurate data, the nymphs were first transferred into a pre-tared vial and then weighed. We froze the grasshoppers and then measured them with electronic calipers in order to accurately measure the initial lengths. Nitrogen rich soil and the grass *Poa pratensis,* which was common in the UV field, were collected with garden trowels and placed in buckets. The nitrogen deficient dune grass, *Ammophila breviligulata,* and sand were collected from Sturgeon Bay, again using garden trowels and buckets.

  In order to test our hypotheses we set up 4 different treatments: nitrogen deficient soil and plants from Sturgeon Bay, nitrogen deficient soil and plants from Sturgeon Bay with dead ants, nitrogen rich soil and plants from the UV field, and nitrogen rich soil and plants from the UV field with dead ants. Each treatment was placed into 2-gallon Ziploc bags, with 10 replicates of each treatment. Two grasshopper nymphs were placed arbitrarily into each bag and the bags were kept inside a greenhouse. Placed within the appropriate bags were two dead ants on paper petri dishes, which were changed every two days. The ants were dried before entry and after removal from the bags to ensure there were no discrepancies based on water weight. After being removed from the Ziploc bags, the ants were weighed to determine if there were any changes in weight due to consumption by grasshoppers. The nymphs were checked and observed every two days for approximately 15 to 30 minutes. Paper towels were used to wipe off condensation on the inside of the Ziploc bags, and the

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status of the grass and ants were checked as well. The surviving grasshopper nymphs were taken out of the Ziploc bags after 7 days and were placed into small vials to be measured and weighed following the procedure above. This process was repeated every 7 days until the surviving nymphs molted into adult grasshoppers. Once molted the adult grasshoppers were taken out of the bags and identified as *Melanoplus femurrubrum* (Red-legged grasshopper). The data collected were compared using ANOVA and t-tests.

Results

From the beginning of the study a low survivorship of grasshoppers was observed. After the first week only 22 grasshoppers survived, slightly more than 25% of the starting number. Many of the bodies of the dead grasshoppers were hardened and black due to the heat and sunlight. Water had condensed on the inside of the bags and both species of grasses appeared dead. All of the ants were wet from the condensed water, though some of the ants were missing. ANOVA showed that as the grasshoppers got larger they weighed more ($F = 6.940$, df $= 1$, $P = .016$). A t-test comparing the surviving grasshoppers feeding on *P. pratensis* versus *A. breviligulata* found that change in length was not significant ($t = 1.663$, df $= 20$, $P = .112$). Based on a similar t-test we found that change in weight was also not significant ($t = 1.470$, df $= 20$, $P = .157$). ANOVA among all 80 grasshoppers found nothing significant for change in length ($F = .982$, df $= 3$, $P = .423$) or change in weight ($F = 1.702$, df $= 3$, $P = .202$). Separate t-tests comparing initial length and initial weight of grasshoppers to survivorship were also not significant (Tables 1, 2, 3, and 4). Survivorship in this instance referred to the grasshopper nymphs living for at least 7 days.

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Group Statistics

	Survivorship	N	Mean	Std. Deviation	Std. Error Mean
Length	.00	58	8.5972	1.75990	.23109
	1.00	22	8.6624	1.62352	.34614

Table 1. Lengths of surviving grasshoppers varied from approximately 7 to 10.2 mm.

Initial Length versus Survivorship

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Length	Equal variances assumed	.307	.581	-.151	78	.880	-.06512	.43174	-.92464	.79440
	Equal variances not assumed			-.156	40.898	.876	-.06512	.41619	-.90569	.77545

Table 2. T-test is insignificant (t = -.151, df = 78, P = .880).

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Group Statistics

	Survivorship	N	Mean	Std. Deviation	Std. Error Mean
Weight	.00	58	.0263	.01415	.00186
	1.00	22	.0242	.01135	.00242

Table 3. Weights of surviving grasshoppers varied from approximately .0130 to .0355 g.

Initial Weight versus Survivorship

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Weight	Equal variances assumed	.571	.452	.623	78	.535	.00210	.00337	-.00461	.00881
	Equal variances not assumed			.688	47.037	.495	.00210	.00305	-.00404	.00824

Table 4. T-test is insignificant (t = .623, df = 78, P = .535)

A t-test of change in length after one week compared to treatment (UV field or sand dunes) was not significant (t = 1.270, df = 20, P = .219). Also insignificant was a t-test of change in weight after one week (t = 1.341, df = 20, P = .195) compared to treatment (UV field or sand dunes).

Concerning the ants, there was no significance between weight before and after placement in treatments (t = -.045, df = 8, P = .965). A t-test of change in length of

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grasshoppers feeding on *P. pratensis* with and without ants after 1 week was insignificant (t = 1.455, df = 10, P = .176). Substituting weight for length also yielded insignificant results (t = .122, df = 10, P = .906). A t-test of change in length (t = .515, df = 8, P = .621) and change in weight (t = .279, df = 8, P = .788) of grasshoppers feeding on *A. breviligulata* with and without ants also proved insignificant.

From the means of the grasshoppers surviving to week one, there was a 17.78% change in length of grasshoppers feeding on *P. pratensis* versus a 4.88% change in length of grasshoppers feeding on *A. breviligulata*. In terms of weight, a 30.5% change versus a 24.82% was observed.

Discussion

Based on the statistical comparisons none of the data were significant. This is attributed to the flaws of the experiment. By placing the grasshoppers inside Ziploc bags, which were kept inside a greenhouse, we essentially overheated the grasshoppers. Grasshoppers, which are ectotherms, cannot internally regulate body temperatures. Instead they regulate body temperature by staying in or out of the sun's rays. Metabolism, movement, feeding rates, digestion, and developmental rates are all affected by a grasshopper's body temperature (Gilman et al. 2008). In a recent study of the grasshopper *Trimerotropis pallidipennis* it was observed that movement began at temperatures above 18.6 C, foraging began at temperatures between 24.2-31.7 C, mating took place at temperatures between 30-40 C, and quiescence was observed at temperatures above 45 C (Gilman et. al. 2008). Furthermore, water from the grasses condensed on the inside of the plastic Ziploc bags and could not evaporate into the atmosphere, thus increasing the humidity inside the bags. By wiping the condensed water off of the inside of the bags every two days the

humidity was presumably decreased; however, the plants also died quicker because that was

their only source of water. Thus, the dead plants, the only source of food for the grasshopper

nymphs, may have lost some of their nutrients.

Based on the study by Gilman et al. (2008) and the bodies of the dead grasshoppers,

it is likely that the temperature inside the Ziploc bags exceeded 45 C for extended periods of

time. However, calculations from Dr. Brian Scholtens' Ecology 381 class showed that the air

temperature during the day at the front of the dune nearest the water, where the *A.*

breviligulata was collected, was only an average of 31 C at ground level.

While the nymphs were still alive and feeding, the concentration of nitrogen in their

diets should have impacted their growth rates (Joern and Behmer, 1997). However,

according to Berner et al. (2005), despite probable delayed development, through

compensatory feeding, grasshoppers surviving on nitrogen poor grasses reached sizes and

survived comparably to grasshoppers surviving on nitrogen rich grasses. Thus, had our data

been significant we would have reached similar conclusions.

An alternative explanation for the similar growth rates that theoretically would have

been observed in our study may be elucidated through the grasshopper, *Melanoplus*

sanguinipes, which is found in Alaska and Idaho. Despite being the same species of

grasshopper, the one endemic to Alaska, which has to deal with a harsher climate and a

shorter growing season, is more efficient at assimilating nitrogen (Fielding and Defoliart,

2007). Thus, the *Melanoplus sanguinipes* from Alaska was able to grow and develop more

quickly than its counterpart from Idaho when reared on the same diet; however, the *M.*

sanguinipes from Alaska did weigh about 5% less at adulthood comparatively (Fielding and

Defoliart, 2007). Consequently, this poses an intriguing query as to which factors influence

grasshoppers to engage in compensatory feeding or increase the efficiency by which they

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assimilate nitrogen, as well as how the importance of these factors vary with different

geographic regions and climates.

Continuing, throughout the course of the study no consumption of ants was observed.

Some of the ants were unaccounted for, but it is more likely that the ants fell off of the petri

dishes and were lost in the sand or the soil than that the herbivorous grasshoppers consumed

them.

To improve this study we could have recorded the temperatures inside the Ziploc

bags, which would have given us data that we could have compared to the study done by

Gilman et al. (2008). Thus, we could have better understood our results through the behavior

observed by the grasshopper nymphs. Also, we could have recorded the temperature, relative

humidity, and air movement of the UV field, which would have given us standard

temperatures and figures that we could have used to thermo regulate the treatments.

Continuing, we could have placed empty petri dishes in the treatments without ants as a

control to test whether the grasshopper's behavior was affected at all. Furthermore, despite a

17.78% change in length and a 30.5% change in weight for the grasshoppers feeding on

nitrogen rich grass, the figures may not be significant due to differences in initial lengths and

weights of grasshopper nymphs. Accordingly, our data would have been more conclusive

had we caught nymphs from the same instars.

In conclusion, the low survivorship of grasshoppers affected the reproducibility, data

pool, and significance of our study. Thus, based on other studies, grasshopper nymphs

feeding on nitrogen deficient grasses should exhibit slower growth rates compared to nymphs

feeding on nitrogen rich grasses; however, through compensatory feeding and metabolic

functions allowing for increased efficiency in assimilating nitrogen, ultimate sizes and

survivorship should be similar between both treatments.

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