Compensatory Feeding of Grasshopper Nymphs (*Melanoplus femurrubrum*) In Nitrogen-limited Habitats

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Abstract

We placed grasshopper nymphs (*Melanoplus femurrubrum*) from a nitrogen-sufficient environment into nitrogen-limited mesocosms (and nitrogen-sufficient mesocosms as a control) in order to test for scavenging behavior when given insect detritus; the growth of nymphs were monitored over a seven-day period. Nymphs placed into nitrogen-limited mesocosms were predicted to supplement their nitrogen-lacking diets with ant detritus. However, the difference in ant detritus weights before and after placing detritus into the mesocosms was statistically insignificant. Differences in percent weight change, and percent length change over a seven-day period were also insignificant. According to the statistical data obtained, we reject our hypothesis; however, the data may have been skewed due to flaws in experimental design. Suggestions for future studies are discussed.

Introduction

Nitrogen is often the key nutrient required for growth in various herbivorous arthropods (Mattson, 1980; Lincoln et al., 1982; Joern and Behmer, 1997; Wheeler and Halpern 1999; Cruz-Rivera and Hay, 2000). When nitrogen resources are low, growth rate and biomass can be reduced (Wheeler and Halpern, 1999); this has led to the nitrogen limitation concept which holds that limitation of nitrogen resources from plant foods will have a negative impact on the growth and performance of the consumer. The alternative to this concept has been presented in the past, focusing on a combination of nitrogen and carbohydrate resources rather than just nitrogen (Joern and Behmer, 1997); this study found that, though carbohydrate resources are essential for

grasshopper growth, sufficient energy is only obtained if enough nitrogen is ingested. For this reason we limited our nutrient focus to nitrogen levels only.

Sturgeon Bay (Lake Michigan) contains a number of sand dunes with vegetation and nitrogen levels that vary with distance from the shore. Beach grass, shrub-bunchgrass, conifers, and hardwoods inhabit mostly dunes of ~25yr, ~150-250yr, ~200-500yr, and ~400-500yr respectively (Lichter, 2008). Wind velocities and sand movement decrease with increasing distance from the lake as moisture holding-capacity and nitrogen levels also increase.

The Lake Huron locust resides in the fore-dune front of the ~25yr dunes with soils having low nitrogen and moisture, normally feeding on beach grass (e.g. *Ammophila breviligulata*, the grass used in our study) at ~31˚C (Scholtens, personal communication). These grasshoppers were observed ingesting insect detritus (composed mostly of mayfly carcasses) on the shore of Sturgeon Bay, suggesting a nitrogen flow from the lake to the dunes. We believe that these grasshoppers are supplementing their diet with insect detritus due to a lack of nitrogen in the Sturgeon Bay vegetation (Scholtens, personal communication).

Studies have shown that nitrogen flow from aquatic environments can have a significant impact on the terrestrial shores. For example, studies involving nitrogen flow from aquatic to terrestrial habitats showed that salmon carcasses in riparian habitats dramatically increase nitrogen composition in terrestrial soils, which can enhance plant growth near the region of detritus (Gende et al., 2007; Hocking and Reimchen, 2002). Though the region in Sturgeon Bay containing insect detritus does not show growth in nearby vegetation, there may still be an influx of nitrogen into the dunes; we believe there are other effects, such as the unusual behavior of the Lake Huron locust in ingesting insect detritus. Given a nitrogen-limiting environment, we believe that grasshoppers will consume insect detritus to supplement their nitrogen-deficient

diets. Since the Lake Huron locust is endangered, we tested a similar species, *Melanoplus femurrubrum*, predicting that it would resort to ingesting insect detritus when subjected to nitrogen-limited conditions.

Materials and Methods

The experiment was held at the University of Michigan's Biological Station, located near Douglas Lake in Pellston, MI. Mesocosms were kept inside the Biological Station's greenhouse for the entire seven-day period.

In making mesocosms, twenty, two-gallon Ziploc bags were filled with soil (sandy soil) and vegetation (*Ammophila breviligulata*) from Sturgeon Bay and twenty bags were filled with soil (clay loam) and vegetation (*Poa pratensis*) from the UV Field of the Biological Station. We considered samples from Sturgeon Bay and the UV Field to be nitrogen-poor and nitrogen-rich respectively according to recent speculations (Scholtens, personal communication). Eighty grasshopper nymphs (*Melanoplus femurrubrum*) were obtained from the UV Field and kept in small glass vials. Before placing the nymphs into the bags, lengths of each nymph were measured with an electronic caliper from the head to the end of the abdomen; the nymphs were measured while inside the vials. Their weights were recorded afterwards on an analytical scale. After weighing, two nymphs were placed in each bag.

Insect detritus was prepared manually by crushing various species of ants (primarily field ants) between two sturdy planes (e.g. between ground and a person's thumb). Remains were then transferred into paper dishes (made from Dixie cups: diameter of ~5.08cm, height of ~2.54cm) - each plate containing an average of two whole ants. Weights of ant detritus in each plate were recorded using the analytical scale (Note: ant detritus was used since ants were easily obtainable given our limited resources). We assumed ant detritus to be similar enough in

nitrogen content to the mayfly detritus of Sturgeon Bay. Also, if condensed water filled the plates, they were placed under sunlight inside the greenhouse to dry before weighing).

Half of the bags containing the soil and grass samples from the UV Field received ant detritus dishes, and the other half did not (to serve as a control). This process was repeated for the bags containing Sturgeon Bay samples.

Bags were kept in the Biological Station's greenhouse for a period of seven days. The bags were opened enough for ventilation, but not enough for nymphs to escape. Ant detritus dishes where changed every other day; the contents of every dish were weighed before and after placement inside the bags. Final lengths and final weights of the surviving nymphs were taken at the end of the seventh day. The length and weight measurements of dead nymphs were not used in our data set.

SPSS 15.0 was used in statistical analysis of our data. An independent samples t-test was done with the initial and final ant detritus weights for Sturgeon Bay and UV Field; another independent t-test was done with ant detritus weight changes between the two environments after calculating the weight changes beforehand; one-way ANOVA tests were used in determining if there were significant differences in the four environments (two with ants, and the two controls) in terms of percent change in nymph lengths and percent change in nymph weights; means and standard deviations were also calculated for each environment.

Results

*Note: the following statistics use a 95% confidence level

Initial and final ant detritus weights for both environments (Sturgeon Bay and UV Field) showed no significant difference ($F=0.016$, $p=0.9$). The two environments did not differ significantly in terms of detritus weights ($F=0.007$, $p=0.993$).

**Habitat Key: 1=UV Field mesocosm, no ants 2=UV Field mesocosm, with ants
3=Sturgeon Bay mesocosm, no ants 4=Sturgeon Bay mesocosm, with ants

Descriptive Statistics for Percent Increase in Nymph Length

Habitat	N	Mean (%)	Std. Deviation(%)
1	4	43.0750	49.77894
2	5	12.0818	13.50272
3	5	5.9356	8.68940
4	8	7.1268	14.64316
Total	22	14.5182	25.87802

Figure 1. Means and standard deviations for % length increase in nymphs of each mesocosm in a seven-day period.

Habitat 1 had the highest mean percent increase in nymph length, but it also had the highest standard deviation (Figure 1). Standard deviations of all four habitats were greater than their respective means. One-way ANOVA showed no significant difference among the four different environments in terms of percent change in length of the (twenty-two) surviving grasshopper nymphs (F=2.467, p=0.095).

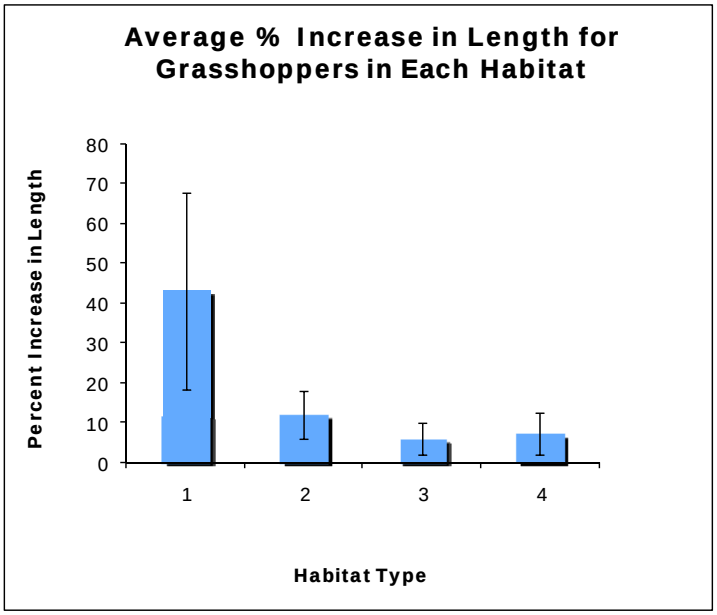

Figure 2. Means and standard deviations (error bars) for % length increase in nymphs of each mesocosm in a seven-day period.

Descriptive Statistics for Percent Increase in Nymph Weight

Habitat	N	Mean (%)	Std. Deviation(%)
1.00	4	178.8800	210.70910
2.00	5	37.7380	52.71301
3.00	5	20.8420	28.95077
4.00	8	23.7500	58.02529
Total	22	54.4736	108.61595

Figure 3. Means and standard deviations for % length increase in nymphs of each mesocosm in a seven-day period.

Habitat 1 had the highest mean percent increase in nymph weight, but it also had the highest standard deviation (Figure 3). Standard deviations of all four habitats were greater than their respective means. One-way ANOVA showed no significant difference among the four different environments in terms of percent change in length of the (twenty-two) surviving grasshopper nymphs (F=2.681, p=0.078).

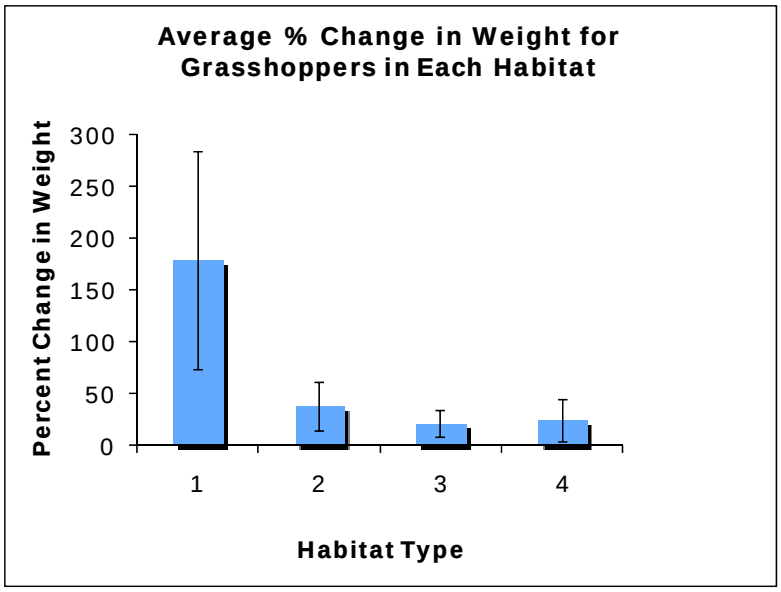

Figure 4. Means and standard deviations (error bars) for % weight increase in nymphs of each mesocosm in a seven-day period.

Many of the grasshoppers died within the seven-day period (Figure 5).

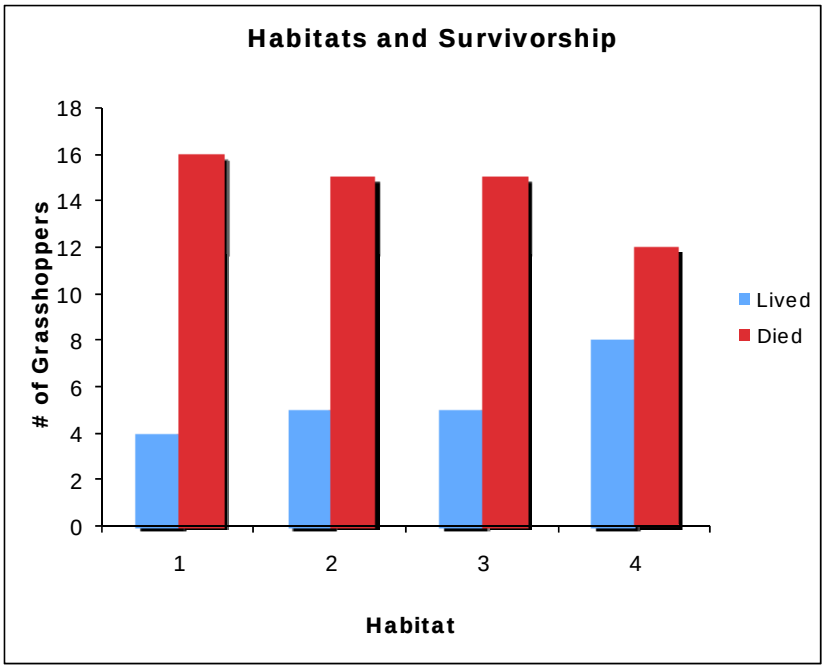

Figure 5. Number of nymphs surviving for each habitat at the end of the seventh day.

Discussion

During the first few days, we had cases when the ants would disappear from the dishes; however we did not include the (0.000g) ant detritus weights of these dishes in our statistical analysis since we did not believe the ants were ingested by the nymphs. Rather, we believed that the ants were washed away by the condensation of water that occurred in the bags (but, if nymphs did ingest the ants, weight and length increase was not significant). The moisture inside the sand/soil was observed to evaporate and condense at the sides of the bags. Although the bags were sealed tightly enough to prevent the nymphs from escaping, the seals were also tight enough to contain most of the water content inside the bags. The observed water vapor that condensed on the bag sides would either travel down the sides of the bag or fall down in the form of droplets from the top of the bag – we believe some of these droplets flooded the ant dishes and washed out the ant detritus into the nearby soil, making the ant detritus difficult to detect with the naked eye, hence difficult to weigh.

We believe the flooding could have been prevented if we had constructed an environment allowing water vapor to escape the test environment in a controlled manner. In our case, the

nymphs would have escaped if we held the bags open too wide for too long. One solution for future studies would be to perforate the bags with holes that are small enough so that nymphs cannot escape.

Also, only 22 out of 80 nymphs survived up to day seven. We believe this was primarily due to extreme temperatures and humidity inside the bags. The greenhouse which housed the bags was not well ventilated, so cool air could not flow inside the bags during peak hours of sun exposure and high temperatures. We did not properly take into consideration the humidity and temperature at which grasshopper nymphs of *Melanoplus femurrubrum* survive. For future studies, we recommend engineering a microcosm that allows full control over these variables in order to increase survival rates of nymphs. Designing a microcosm that accounts for environmental chances would be ideal – for instance, predation. Since we did not account for some of the natural enemies of the grasshopper nymphs, they had a better chance of survival. Though, since we did have two nymphs in each bag, it is possible that competition may have been a factor.

Since ant detritus weights did not change significantly, it is possible that the nymphs did not consume the ant detritus. However, it is also possible that the nymphs consumed all of the ants provided in their dishes. One way we could have confirmed whether or not ants were ingested by nymphs would be to run fecal analyses of nymph feces and look for traces of ant detritus. Presence of ant detritus in fecal matter would suggest ingestion of ants by nymphs.

One reason nymphs may not have ingested ant detritus could be due to the fact that ant detritus was observably tougher and drier in texture when compared to the moist mayfly carcasses. Grasshopper diet selection is affected by water content, shape, species, and many

other factors in the food item (Mulkern, 1967). This concept also applies to Sturgeon Bay vegetation. Initially, we believed the Sturgeon Bay vegetation was nitrogen-deficient to all grasshoppers since it was deficient to the Lake Huron locust; however, grasshoppers differ from species to species in what they choose to eat and in what plants have the greatest positive effect on growth and survival (Mulkern, 1967). Perhaps nymphs of *Melanoplus femurrubrum* prefer mayfly carcasses over ant detritus – this is something that can be tested in future experiments. Maybe if we had provided mayfly carcasses instead ant detritus, we might have detected more instances of insect carcasses being ingested by the nymphs.

Results from ANOVA tests for comparing all four environments in terms of percent change in weight and percent change imply that the growth rates of the nymphs were relatively the same despite the different environments they grew in. Since dune grass *Ammophila breviligulata* was nitrogen-limited and grass from the UV Field was not, it is important to note that the nymphs maintained similar growth rates in different environments even when one habitat was more nitrogen-limited than the other, but perhaps this correlation is due to certain feeding patterns in grasshoppers. Joern (1979) discovered that grasshoppers retain their original niche in various different communities. So perhaps the *Melanoplus femurrubrum* nymphs kept their niche in consuming grass and refrained from ingesting insect detritus since this would be altering their niche.

Then again, it is possible that growth was not affected over a one week period. We believe that if we had extended the time frame for our experiment (which we were unable to do since nymphs had low rates of survival in our mesocosms), we might have observed a significant difference in growth rates in the different environments.

Although our statistical data disproves our initial hypothesis, we believe our experimental parameters were not sufficient enough to properly test our hypothesis due to technical flaws in our experimental design. For future experiments, we recommend controlled experimental measures primarily focusing on environmental temperature, moisture, and proper ventilation in order to increase chances of nymph survival and longevity. For other studies, we recommend comparing the scavenging preference in *Melanoplus femurrubrum* for ant detritus and mayfly carcasses.

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