

# Dietary sodium levels affect grasshopper growth and performance

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**Abstract.** Anthropogenic activities are increasing terrestrial sodium availability through application of both saline irrigation water and road salt. Sodium often limits herbivore abundance, but less is known about the physiological, developmental, and behavioral means by which moderate increases in sodium availability can increase herbivore fitness. Here, we raised a grasshopper species on three no-choice diets of wheatgrass watered with no sodium (control), a 1% (medium) sodium solution, and a 5% (high) sodium solution to examine the effects of sodium intake on grasshopper survival, morphology, and jumping performance. Grasshopper nymphs raised on a high sodium diet had lower weights and reduced survival compared to those raised on control or medium sodium diets. However, nymphs on a high sodium diet developed larger eye size standardized by body size and demonstrated increased jumping distance compared to nymphs on the control or medium sodium diets. As adults, grasshoppers on the medium sodium diet had the highest survival and grasshoppers on the high sodium diet had the least amount of cannibalism of the three treatments. Understanding the response of herbivore fitness to increasing diet sodium content is an important first step toward predicting how anthropogenic inputs of sodium into terrestrial systems will alter food webs.

**Key words:** Acrididae; diet; herbivory; jumping; *Melanoplus differentialis*; no-choice; opportunistic carnivory; survival.

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## INTRODUCTION

Both the need for and concentration of sodium in food varies with trophic level, yielding consequences for the structure of ecological food webs. Sodium is not required by most plants, whereas it is a crucial micronutrient for animals, necessary for osmoregulation and use in Na-K pumps (Clausen and Everts 1991). One consequence of this dynamic is that herbivores are often sodium limited—with shortfall leading to decreases in growth, survival, performance, and reproduction. This is true for both vertebrate and invertebrate herbivores (Jones and Hanson 1984, Hellgren and Pitts 1997, Welti et al. 2019b, 2020, Kaspari 2020). Invertebrate herbivores sate their

sodium needs by puddling (Molleman 2010), opportunistic carnivory (Simpson et al. 2006, Clay et al. 2017), and seeking out more sodium-rich plant tissue and exudates (Welti et al. 2019b, Kaspari et al. 2020).

Anthropogenic activity is increasing terrestrial sodium availability. Salinization affects more than 20% of the irrigated lands worldwide, saturating fields with sodium and decreasing crop yield (Ghassemi et al. 1995). Road salt additions in northern latitudes are substantial, with the application of 109 kg/lane-mile following every freezing precipitation event (Mattson and Godfrey 1994, Massachusetts Department of Transportation 2019). The negative consequences of sodium fertilization for plants (Munck et al.

2010) and freshwater systems (Ramakrishna and Viraraghavan 2005) have been well documented. In contrast, a growing literature demonstrates widespread attraction by terrestrial insects to moderate levels of sodium fertilization (Clay et al. 2017, Prather et al. 2018a, Welte et al. 2019b) with repercussions for pest management and the trophic structure of invertebrate communities.

While sodium supplementation is known to enhance the survival and growth of livestock and vertebrate wildlife (Jones and Hanson 1984, McDowell 2003), less work has explored the potential for sodium applications to nutritionally supplement invertebrate herbivores (Kaspari et al. 2020). One important counter-example that inspired this study revealed how sodium supplements to Lepidopteran caterpillars increase abdominal muscle size in males, and neural investment (eye and brain size) in females, illustrating that sodium has major consequences for both butterfly behavioral development and foraging with a potential cost, however, in higher mortality during ontogeny (Snell-Rood et al. 2014).

Here, we explore similar questions with a dominant grassland herbivore: grasshoppers (Acrididae). Choice experiments assessing preferences for sodium suggest grasshoppers regulate the amount of sodium in their diets with responses varying from stimulating to deterring consumption depending on sodium concentration (Dadd 1961, Trumper and Simpson 1994, Simpson et al. 2006, Kilpatrick and Behmer 2018). Grasshoppers demonstrate reduced growth within one day of sodium deprivation and grasshoppers on low sodium diets tend to consume more (Trumper and Simpson 1993, Kilpatrick and Behmer 2018), while excessive dietary sodium may affect gut microbiota composition and therefore limit protein uptake (Wang et al. 2017). However, we know of no tests of sodium shortfall over the duration of grasshopper ontogeny to adulthood, nor tests of its effects on behaviors associated with survival. Grasshoppers suffer from a variety of predators (Joern and Rudd 1982, Joern 1986, Schmitz 2008) and depend on their enlarged hindlimbs to escape predators. These limbs, like the abdominal muscle mass of butterflies, may benefit from a diet of enhanced sodium.

Here, we observed the effects of dietary sodium levels through ontogeny on several

aspects of fitness using *Melanoplus differentialis* grasshoppers (Acrididae). Using no-choice laboratory experiments, we subjected grasshoppers to three wheatgrass-based diets varying in sodium content, and measured grasshopper fitness in terms of growth, survival, and jumping ability. We test four hypotheses, predicting increased sodium supply would increase (1) survival and growth, (2) grasshopper eye size, (3) jumping performance (which integrates over both muscle mass and neurological control), and decrease (4) cannibalism, which provides an alternate source of dietary sodium. We use three levels of sodium to allow for non-linearities in response (e.g., excessively high sodium content in diet may be detrimental to growth, survival, and physical fitness). By documenting the physiology and survival of grasshoppers raised on three diets varying in sodium content, we take the first step toward predicting the repercussions of anthropogenic sodium fertilization for terrestrial herbivore populations.

## MATERIALS AND METHODS

### *Grasshopper collection*

The subject of our study was the differential grasshopper, *M. differentialis* (Thomas 1895). Grasshoppers were collected using sweep nets in mid-June 2019 in a roadside field in Norman, Oklahoma, USA (35°10'49" N, 97°26'33" W). Prior to experimental setup, we housed grasshoppers in cages and provided them with water, organic lettuce, and spinach.

### *Grasshopper diets*

Experimental diets consisted of wheatgrass watered with three levels of a NaCl solution and then dried and ground. To grow wheatgrass, we first soaked seeds in distilled water for 24 h. Seeds were then planted in soil, watered daily with distilled water, and kept in a climate chamber set to 25°C and a 12-h light cycle. After 10 d, once grass was fully grown, three planter trays each of wheatgrass (13.5 × 17.8 cm) were allocated to one of three treatments: control, medium sodium, and high sodium. Experimental treatments consisted of 200 mL of distilled water, with the medium sodium treatment additionally containing 2 g of NaCl (a 1% NaCl solution), and the high sodium solution containing 10 g of

NaCl (a 5% NaCl solution). Each grouping of wheatgrass was misted with a spray bottle containing its corresponding treatment solution and left to sit for 6 h. We chose to spray plants rather than water soil as this method imitates overhead sprinkler irrigation such as center pivot systems, and plants directly uptake more sodium from leaves than from roots (Benes et al. 1996). Wheatgrass was then cut, dried at 60°C for 48 h, and ground into a fine powder using a coffee grinder.

To determine content of sodium and other nutrients, we analyzed two samples of each diet for elemental chemistry, one taken from wheatgrass grown at the beginning and one from the end of the nymph experiments. Additionally, we analyzed two samples of grasshopper frass (pooled to reach the 2 g needed for analysis) from each diet treatment to determine elemental excretion. Elemental chemistry was determined using combustion analysis for C, N, and H, and using hot plate digestion and inductively coupled plasma atomic emission spectroscopy for metals (including sodium) by the Cornell Nutrient Analysis Laboratory (<https://cnal.cals.cornell.edu/>).

### *Nymph experiments*

Initially, we divided grasshoppers into two experimental setups: nymph colony and solitary. Colonies consisted of 15 aluminum mesh cages (30.3 × 30.3 cm), five for each treatment. Each nymph colony initially contained 10 grasshoppers, of which five were 2nd instar and five were 3rd instar (totaling 150 grasshoppers in all colonies). Colonies were provided with two water sources (vials of distilled water capped with cotton balls for water access without spillage) and ~2.0 g of dried wheatgrass (Appendix S1: Fig. S1A, B). The second experiment consisted of isolated grasshoppers in clear plastic cylindrical cages with mesh lids (height 10 cm, diameter 11.5 cm). Each solitary cage was equipped with 0.2 g of dried and ground wheatgrass from one of the three treatments (food), a water source, and an aluminum mesh structure for grasshoppers to hang from when molting (Appendix S1: Fig. S1C). The experiment included 60 solitary cages (20 per treatment), each containing one grasshopper starting at 3rd instar. We weighed food for each colony and solitary cage

approximately twice a week. We provided food *ad libitum* and regularly added and recorded weights of added food as needed. All grasshopper cages were stored in climate chambers maintaining 25°C and a 12-h light/dark cycle.

To test how grasshopper food weight changed over time due to water absorption in the absence of grasshopper consumption, three additional solitary cages initially containing 0.2 g of the grasshopper diets from each treatment with the same setup as those in the solitary experiment were kept in a climate chamber and the food was weighed 10 times over a 21-d period. For all three treatments, the food gained weight for two days and then remained at a stable weight. Both the control and the medium sodium food increased by 7% from the initial weight, whereas the high sodium food increased by 22%, presumably because the sodium in the food led to higher water absorption. We used these values to correct calculations of food weight measurements, with new food added to cages assumed to gain 7% of its weight for control and medium sodium diets and 22% for high sodium diets.

In the nymph colony experiment, mold contaminated the provided food in two cages of grasshoppers in each treatment, corresponding to six total cages (all cages in one growth chamber), 21 d into the experiment. We replaced this food within 24 h; however, the mold event did affect the survival within those colonies. Thus, we removed these colonies from our survival analysis.

### *Grasshopper measurements*

We weighed individual grasshoppers in the solitary experiment approximately twice a week. Molt skins and instar status were recorded for grasshoppers in the solitary experiment. Within the colonies, we recorded molt skins and the number of grasshoppers present in each instar. We weighed individual grasshoppers within the nymph colonies once, 29 d into the experiment; we used these weights to calculate mean grasshopper weight for each nymph colony. Both femur and eye size were measured once, one month into the nymph experiments, for each grasshopper in both the nymph colony and solitary experiments. This was accomplished by lowering the temperature within the climate chambers by 5°C every 2 h until the climate

chamber reached 10°C and then reducing the temperature to 8°C the following hour to ensure grasshoppers were less mobile. We then recorded grasshopper eye and femur size using an electronic digital caliper by swaddling grasshoppers in pantyhose to limit movement. We returned grasshoppers to 25°C after 8 h.

### *Jumping trials*

In order to test grasshopper agility, we constructed an enclosed jumping arena and recorded the length of five jumps of each grasshopper, following 22 and 32 d after experimental setup for the solitary and nymph colony experiments, respectively. The jumping trials were conducted in an indoor lab kept at 25°C. The jumping arena consisted of a 2.44 m diameter plastic shaded pool (H2OGO!), filled with 150 lbs of fine play sand, and covered with an outdoor umbrella screen (Ideaworks; Fig. 1A). The sand functioned to mark indentations of locations grasshoppers landed in-between jumps, and the umbrella screen kept the grasshoppers contained. We individually placed each grasshopper from the nymph colony and solitary experiments into the center of the jumping arena (Fig. 1B). We did not record each grasshopper's first jump, but the next five consecutive jumps were marked and their length was measured to the nearest mm. Flags were placed in the sand on indentations left by grasshoppers only after grasshoppers had completed each jump. We leveled the sand in the jumping arena between each grasshopper trial.

### *Adult colonies*

The completion of the jumping trials concluded the two nymphal (solitary and colony) experiments. We then moved the 20 largest grasshoppers from each treatment in the colony experiment (average femur lengths, control 11.12 mm  $\pm$  0.26 standard error [SE], medium sodium 11.57 mm  $\pm$  0.42 SE, high sodium 9.74 mm  $\pm$  0.32 SE) into three new aluminum mesh cages, one for each diet treatment. We continued to feed the 20 individuals in each of these three cages the previously described diets containing three levels of NaCl. In addition to food and two water sources, these new cages each included two containers of sand, to provide a location for females to lay eggs. We recorded

when these individuals became adults, the sex of the adults, and deaths. Additionally, we scored deceased grasshoppers for cannibalism using an index ranging from 0 to 3: 0 (no cannibalism), 1 (slightly chewed), 2 (missing entire appendage up to half the body missing), and 3 (more than half the body missing). We did not score the last grasshopper remaining alive in each cage, as they could not be cannibalized. Descriptive information on the three experiments (solitary, nymph colony, and adult colony) is included in Table 1.

### *Statistical methods*

In order to determine whether grasshoppers in the solitary and nymph colony experiments differed in body measurements and jumping ability, we conducted Welch's *t* tests. Grasshoppers in the solitary cages compared to the colonies did not differ in femur size ( $t = -1.17$ ,  $df = 82$ ,  $P = 0.24$ ) or eye size ( $t = 0.14$ ,  $df = 67.4$ ,  $P = 0.89$ ); thus, one analysis was conducted combining the solitary and nymph colony experiments to determine the effects of diet treatment on these measurements and eye to femur ratio. We tested whether slopes of the relationship between eye and femur length differed with diet using an ANCOVA, and included experiment (solitary or nymph colony) as a covariate. Additionally, whether grasshoppers were in the solitary cages or the colonies did not affect jumping ability ( $t = 0.38$ ,  $df = 65.1$ ,  $P = 0.71$ ). Therefore, we combined jumping trial data from the solitary and nymph colony experiments to examine the effect of diet treatment on jumping ability. Grasshoppers in the solitary experiment were weighed ~2 times/week, whereas grasshoppers in the nymph colony experiment were weighed once; thus, grasshopper weights from the solitary and nymph colony experiments were analyzed separately. In order to evaluate the effects of diet on eye size, femur size, and jumping ability for both experiments, and weights of grasshoppers in the nymph colony experiment, we used Tukey's honestly significant difference (HSD) tests. We conducted a principle component analysis (PCA) of the elemental chemistry of the frass and wheatgrass diet samples to examine differences between intake and output and variation due to sodium treatment. A broken stick model was used to evaluate the number of significant

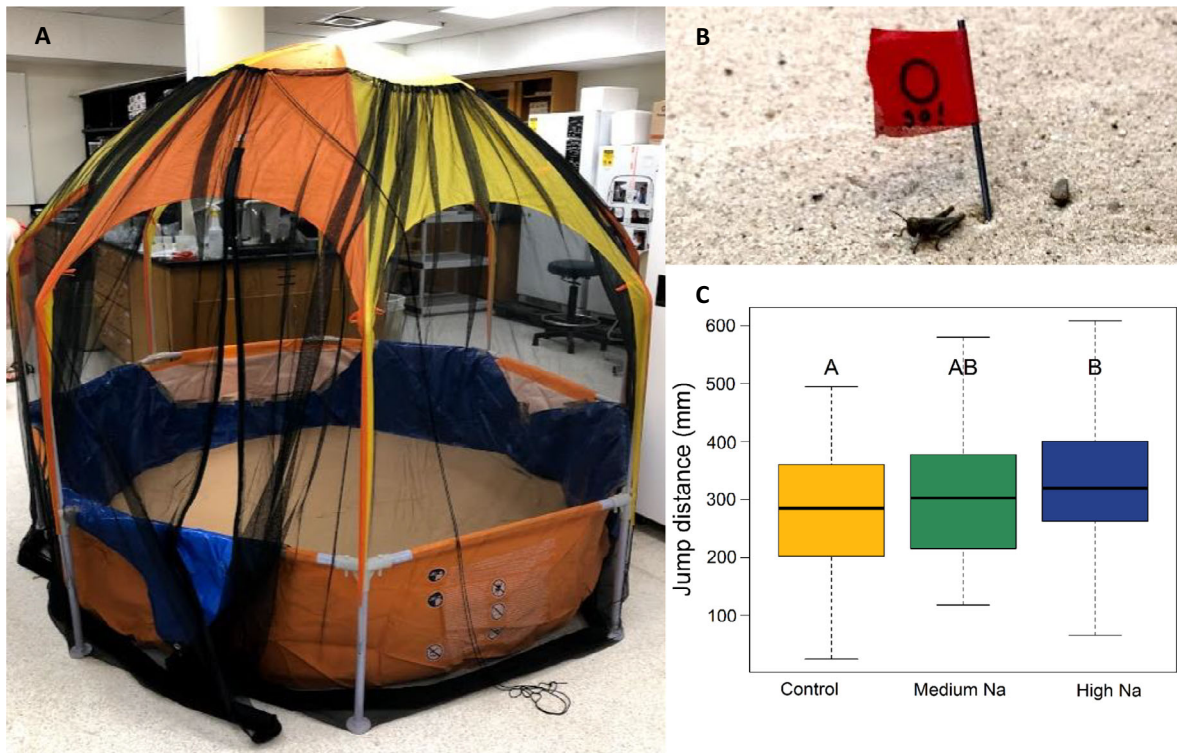


Fig. 1. Photographs of the grasshopper jumping arena setup (A) and grasshopper at the start of a jumping trial (B). Grasshoppers from both the solitary and nymph colony experiment jumped furthest on high sodium diets (C).

Table 1. Description of the three experiments.

Experiment	Life stage	Housing type	Treatment	Replicates ( <i>n</i> )	Grasshoppers/replicate	Grasshoppers in jumping trials	Duration (days)
Solitary	Nymph	Solitary	Control	20	1	15	36
			Medium	20	1	19	
			High	20	1	6	
Nymph colony	Nymph	Colony	Control	5	10	37	37
			Medium	5	10	34	
			High	5	10	34	
Adult colony	Adult	Colony	Control	1	20	NA	135
			Medium	1	20	NA	
			High	1	20	NA	

Notes: All experiments contained grasshoppers subjected to three no-choice diet treatments (control, medium sodium, and high sodium). Replicate (*n*) refers to the number of cages corresponding to each treatment within each experiment. All grasshoppers still alive in the solitary and nymph colony experiments were included in the jumping trials, with the number alive recorded here as grasshoppers in jumping trials.

axes (Legendre and Legendre 2012). The PCA was run using the R package *vegan* (Oksanen et al. 2018).

Survival rates were calculated separately for each experiment and diet treatment using Kaplan–Meier models and the R package

survival (Therneau 2019). To test for differences in survival between treatments, we used Cox proportional hazard models (Murray 2009). Survival data for all experiments met the assumption of proportional hazards (nymph colony experiment  $P = 0.99$ ; solitary experiment  $P = 0.41$ ; adult colony experiment  $P = 0.18$ ) based on scaled Schoenfeld residuals (Fox and Weisberg 2002). To examine when grasshoppers on the three diet treatments were likely to perish over the course of the experiments, we examined temporal risk of mortality using hazard functions. Hazard functions report treatment effects as a hazard ratio where a value of 1 indicates no treatment effect on survival rates. Differences in survival for each treatment and across the duration of the experiments were examined using smoothing splines (with the smoothing parameter set at the default of  $\alpha = 1.2$  to avoid overfitting) of hazard functions in the R package *gss* (Gu 2014). For the nymph colony experiment, the two cages from each treatment in which food was contaminated with mold were not included in the survival analysis. We included the categorical variable of colony as a random variable in the colony survival analysis to account for pseudoreplication of grasshoppers within colonies. While we did not have true replication (multiple cages per treatment) for the adult colony experiment, we maintained experimental treatments until the death of all grasshoppers, and calculated survival of individual grasshoppers from the three treatments in an exploratory analysis of long-term responses to fixed sodium intake.

All analyses were conducted using Program R ver. 3.6.1 (R Core Team 2020).

## RESULTS

### *Diet and frass elemental chemistry*

Sodium concentration in wheatgrass in the three diet treatments varied two orders of magnitude (control 249 ppm  $\pm$  58 SE, medium sodium 5352 ppm  $\pm$  4627 SE, high sodium 27,070 ppm  $\pm$  2081 SE). Grasshopper frass also increased with sodium content in diets (control 406 ppm  $\pm$  3 SE, medium sodium 12,826 ppm  $\pm$  1337 SE, high sodium 29,440 ppm  $\pm$  1771 SE).

Wheatgrass had decreasing carbon content with increasing sodium (control 38.7%  $\pm$  0.7 SE, medium sodium 37.4%  $\pm$  0.5 SE, high sodium

34.2%  $\pm$  0.5 SE) and lower nitrogen in the high sodium treatment (control 5.82%  $\pm$  0.07 SE, medium sodium 5.94%  $\pm$  0.16 SE, high sodium 5.29%  $\pm$  0.09 SE). Frass carbon reflected changes in wheatgrass carbon in diet treatments (control 35.4%  $\pm$  0.1 SE, medium sodium 32.6%  $\pm$  0.03 SE, high sodium 29.4%  $\pm$  0.2 SE), whereas frass nitrogen was highest from grasshoppers consuming the high sodium treated wheatgrass (control 5.84%  $\pm$  0.05 SE, medium sodium 5.74%  $\pm$  0.1 SE, high sodium 6.13  $\pm$  0.09 SE). In a PCA of wheatgrass and frass chemistry, the second PCA axis captured the variation in sodium content (Appendix S1: Table S1, Fig. S2). Additional elemental chemistry differences between wheatgrass and frass were captured by the first axis of the PCA with wheatgrass having higher concentrations of H, Cu, and Mo in addition to C, and frass having higher concentrations of many metals (Al, B, Ba, Ca, Cd, Cr, Fe, K, Mg, Mn, P, Pb, S, and Zn; Appendix S1: Table S1, Fig. S2).

### *Body measurements, consumption, and growth*

Combining the solitary and nymph colony experiments, grasshoppers on the high sodium diet had larger eye to femur ratios than grasshoppers on the control diet (Tukey's HSD,  $P = 0.001$ ) or the medium sodium diet (Tukey's HSD,  $P < 0.001$ ; Fig. 2). For example, for a femur length of 10 mm, high sodium diets yielded eyes that were 8% larger than control or medium sodium diets. The same result of larger eye to femur ratios was found when analyzing the grasshoppers from the solitary and nymph colony experiments separately (Appendix S1: Fig. S3). An ANCOVA conducted to test differences in slopes of eye to femur length relationship found no effect of experiment (solitary or nymph colony), but did find a significant interaction between diet treatment and femur length on eye length (Appendix S1: Table S2). Grasshopper eye to femur ratio increased less slowly with body size for grasshoppers on the high sodium diet with a 1 mm increase in leg size accompanied by a 0.787 mm increase in eye size in the control treatment, a 0.686 mm increase in medium treatment and a 0.438 mm increase for grasshoppers on the high sodium diet. While only three *M. differentialis* fed the high sodium diet reached adulthood, they maintained

the higher eye to femur ratio found in *M. differentialis* nymphs on the high sodium diet (control  $0.1735 \text{ mm} \pm 0.0047 \text{ SE}$ , medium sodium  $0.1784 \text{ mm} \pm 0.0038 \text{ SE}$ , high sodium  $0.1967 \text{ mm} \pm 0.0062 \text{ SE}$ ).

One month into the nymph colony experiment, body mass of grasshoppers on the high sodium diet averaged 62% of those on the control diet (Tukey's HSD,  $P = 0.006$ ) and 58% of those on the medium sodium diet (Tukey's HSD,  $P = 0.001$ ; Fig. 3A). Grasshoppers in the nymph colony experiment on high sodium diets consumed an average of 30% less food over time compared to the other treatments (Appendix S1: Fig. S4A).

Sodium levels shaped development time. In the solitary experiment, where we could monitor individuals over time, grasshoppers in the high sodium diet averaged 68% and 83% of the body mass of grasshoppers on the control and medium sodium diets, respectively (Fig. 3B), and tended to consume less food over time, although food consumption was variable (Appendix S1:

Fig. S4B). Grasshoppers in the solitary experiment on the control diet spent an average of  $18.5 \pm 1.1 \text{ SE}$  days in the 4th instar ( $n = 12$  grasshoppers reaching 5th instar). Grasshoppers on the medium sodium diet spent an average of  $18.6 \pm 0.9 \text{ SE}$  days in the 4th instar ( $n = 14$  grasshoppers reaching 5th instar). One grasshopper on the control diet and two on the medium sodium diet reached 6th instar with all three spending 13 d in 5th instar. Only one grasshopper on the high sodium diet reached 5th instar during the experiment and spent 33 d in 4th instar.

#### Jumping ability

Mean grasshopper jumping distance did not vary with grasshopper weight (solitary  $F_{1,38} = 1$ ,  $R^2 = 0.03$ ,  $P = 0.31$ ; colonies  $F_{1,103} = 1.1$ ,  $R^2 = 0.01$ ,  $P = 0.3$ ) nor grasshopper femur length (solitary  $F_{1,38} = 0.002$ ,  $R^2 < 0.001$ ,  $P = 0.97$ ; colonies  $F_{1,103} = 21$ ,  $R^2 = 0.02$ ,  $P = 0.15$ ). Consequently, we used Tukey's HSD test to examine the effects of diet treatment on mean jumping

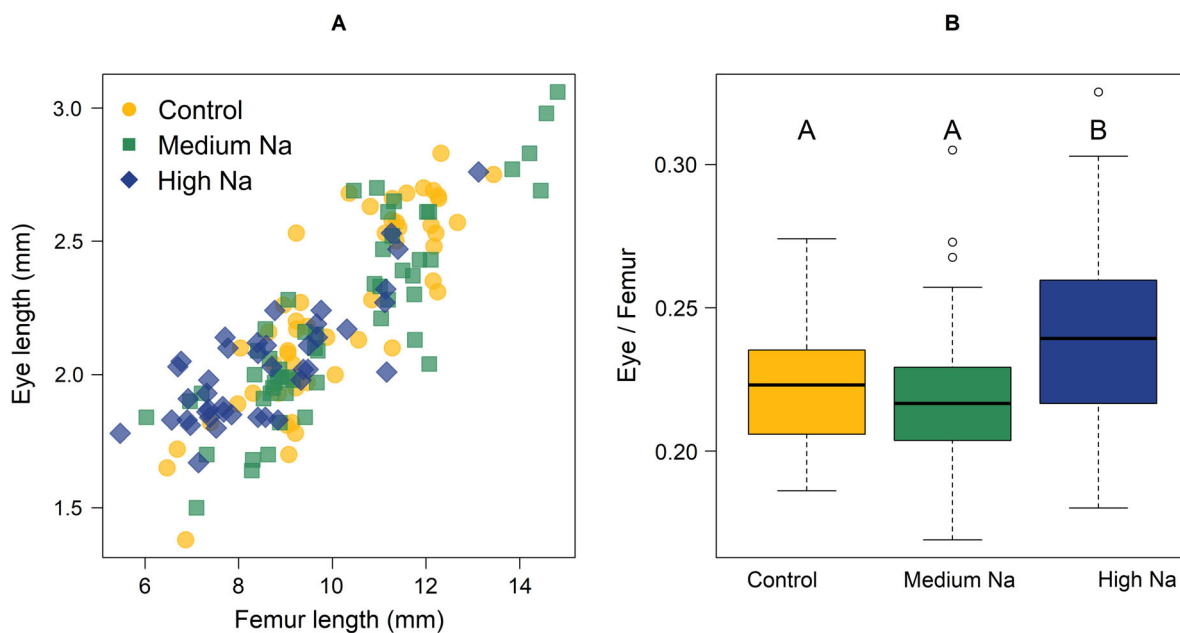


Fig. 2. Eye to femur ratio of grasshoppers on three diets varying in sodium content. Eye length increased with femur length (A). Grasshoppers on the high sodium diet had larger eye to femur ratios than those on the lower sodium diets (B). However, as the slope of eye to femur length was less steep for grasshoppers on the high sodium diet, the increased eye to femur length ratio occurred only when grasshoppers on the high sodium diet were small.

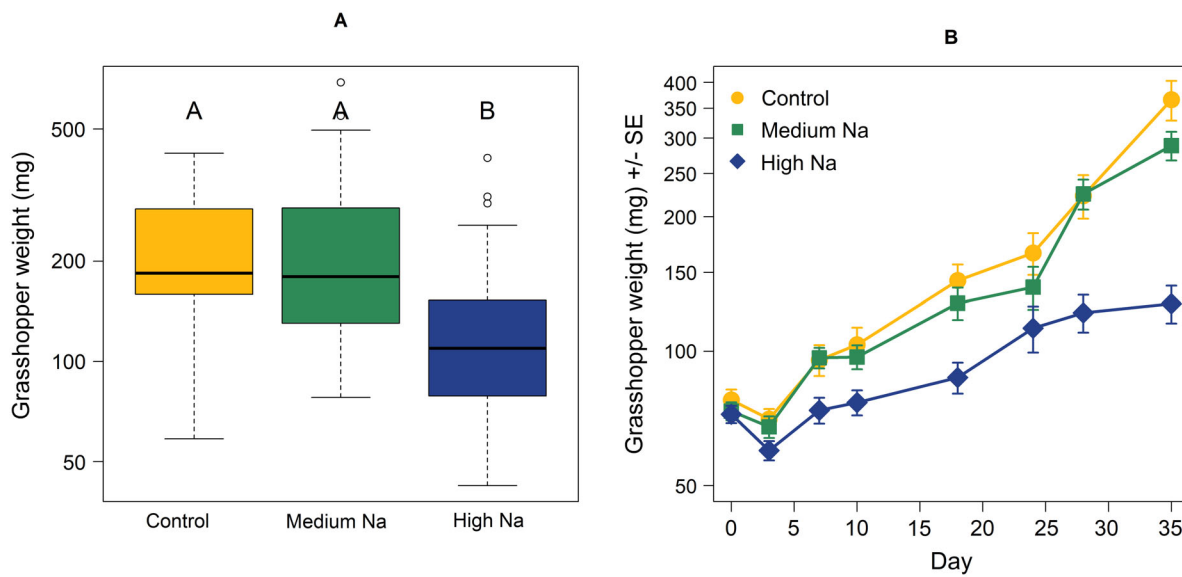


Fig. 3. Grasshopper weights were lower for grasshoppers on the high sodium diets. In the nymph colony experiment, grasshoppers were weighed 30 d after the start of the experiment (A). In the solitary experiment, grasshoppers were weighed ~twice weekly (B).

distance without including grasshopper size as a covariate.

Grasshopper jumping length increased with the sodium concentration in their diet (average jumping lengths, control 273 mm  $\pm$  14.4 SE, medium sodium 301 mm  $\pm$  14.8, and high sodium 333.6 mm  $\pm$  19.5 SE). Grasshoppers on the high sodium diet jumped and average of 22% further than grasshoppers on the control diet (Tukey's HSD,  $P = 0.03$ , Fig. 1C). When analyzing the solitary and nymph colony experiments separately, grasshoppers in the solitary experiment did not display differences in jumping distance with diet treatment (Appendix S1: Fig. S5A), but this test included a small sample size ( $n = 6$ ) for grasshoppers on the high sodium diet (Table 1). Grasshoppers from the nymph colony experiment were found to jump further on the high sodium diet compared to control (Appendix S1: Fig. S5B).

### Survival

Grasshoppers had consistently low survival on high sodium diets, consistently high survival on medium sodium diets, and on control diets switched from high survival as nymphs to low survival as adults.

In the nymph colony experiment, grasshopper survival on the control diet ( $90\% \pm <1\%$  SE) and the medium sodium diet ( $87\% \pm 5\%$  SE) was higher than grasshopper survival in the high sodium diet ( $80\% \pm 5\%$  SE;  $z = 2.82$ ,  $P = 0.005$ ; Fig. 4A). Grasshopper survival in all treatments was lower in the solitary experiment ( $57\% \pm 6\%$  SE) than the nymph colony experiment ( $86\% \pm 4\%$  SE). However, similar to the nymph colony experiment, grasshopper survival in the solitary experiment on the control diet ( $70\% \pm 10\%$  SE) and the medium sodium diet ( $80\% \pm 9\%$  SE) was higher than grasshopper survival in the high sodium diet ( $23\% \pm 9\%$  SE;  $z = 2.73$ ,  $P = 0.006$ ; Fig. 4B).

In the adult colony experiment and out of the original 20 stocked grasshoppers in each treatment, the number of grasshoppers that reached the adult stage where 10 (4 females, 6 males) on the control diet, 15 (2 females, 13 males) on the medium sodium diet, and 3 (1 female, 2 males) on the high sodium diet. On average, grasshoppers in the adult colony experiment lived much longer on the medium sodium diet ( $72.1 \pm 7.4$  SE days;  $z = 2.25$ ,  $P = 0.025$ ) than on the control diet ( $45.1 \pm 6.8$  SE days) or the high sodium diet ( $47.6 \pm 6.6$  SE days; Fig. 4C).



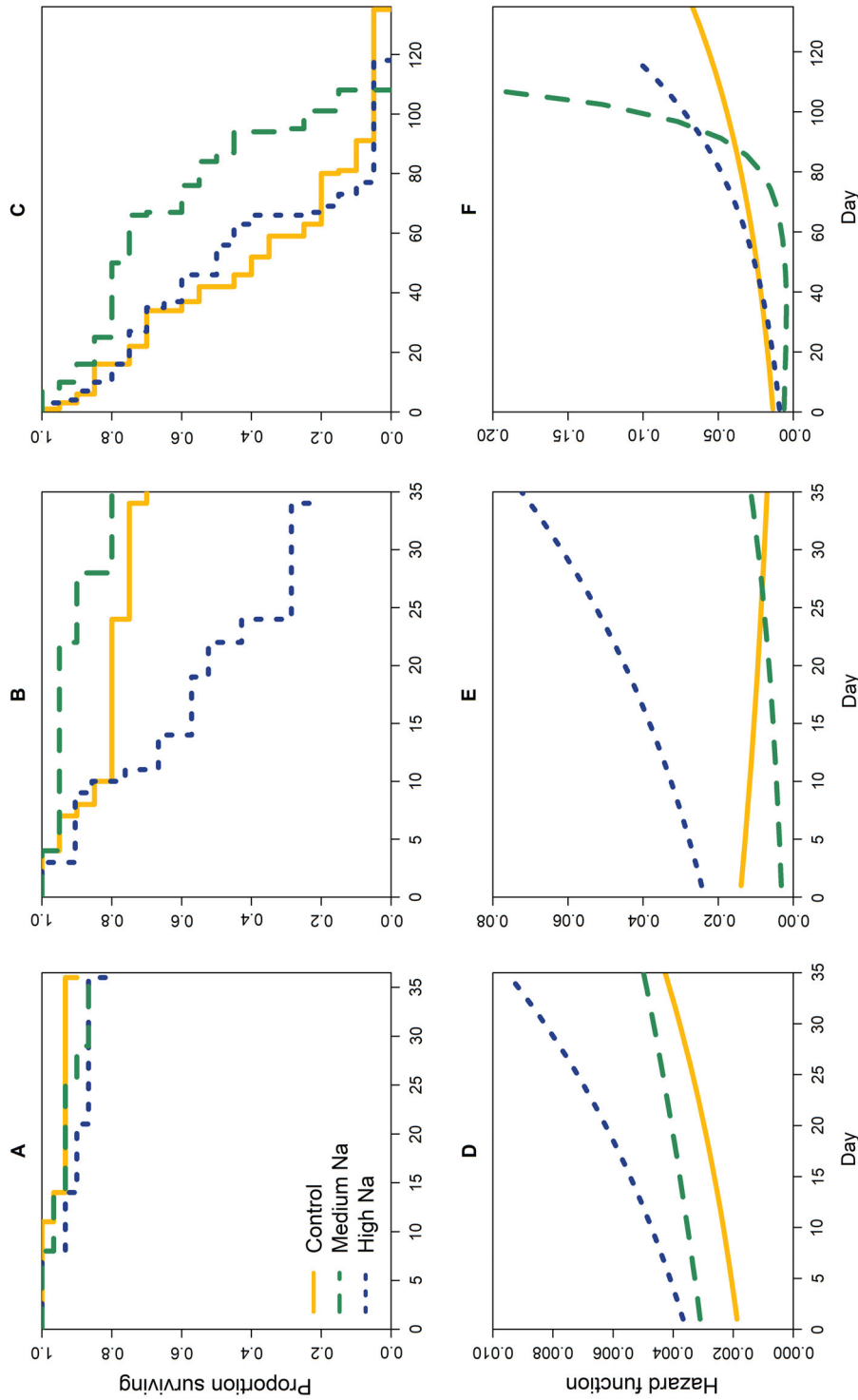


Fig. 4. Kaplan–Meier curves show grasshopper survival on the three sodium diet treatments for grasshoppers in the nymph colony (A), solitary (B), and adult colony (C) experiments. Hazard functions depict survival rates for grasshoppers in the nymph colony (D), solitary (E), and adult colony (F) experiments. Note that hazard function plots have different  $y$ -axis constraints.

Grasshoppers in the nymph colony experiment had increased risk of mortality with grasshoppers in the high sodium treatment showing the steepest rise in mortality over time (Fig. 4D). In the solitary experiment, grasshoppers on the control and medium sodium diets had little change in mortality risk over the course of the experiment, whereas grasshoppers on the high sodium diet had increased mortality risk over time (Fig. 4E). In the adult colony experiment, grasshoppers on medium sodium diets had the lowest risk of mortality for the first 90 d of the experiment. Grasshoppers on the control and high sodium diets had higher overall mortality but a few individuals were long-lived (Fig. 4F).

### **Cannibalism**

The severity of cannibalism, scored on 17 dead grasshoppers recovered from each of the three treatments (51 total), was highest in grasshoppers not supplemented with salt. Cannibalism was highest on grasshoppers in the control treatment ( $1.35 \pm 0.08$  SE cannibalism index), followed by grasshoppers on the medium sodium diet ( $1.12 \pm 0.07$  SE cannibalism index), and least common on grasshoppers on the high sodium diet ( $0.24 \pm 0.03$  SE cannibalism index). Grasshoppers on the high sodium diet had lower cannibalism index scores than those on the control diet (Tukey's HSD,  $P = 0.007$ ) and medium sodium diet (Tukey's HSD,  $P = 0.041$ ), but there was no difference between grasshoppers on the control and medium sodium diet (Tukey's HSD,  $P = 0.78$ ; Appendix S1: Fig. S4). The cannibalism index did not vary with grasshopper sex ( $t = 0.6$ ,  $df = 9.6$ ,  $P = 0.55$ ) nor between dead nymph and adult grasshoppers ( $t = 1.5$ ,  $df = 47.2$ ,  $P = 0.14$ ).

## **DISCUSSION**

As the Anthropocene becomes saltier via road salt application and the mining of sodium-rich irrigation water (Qadir et al. 2014), documenting the sodium's effects on the growth, reproduction, and activity of the often sodium-limited herbivores is the first step toward predicting repercussions for ecological food webs. Here, changing the concentration of sodium content in a common grasshoppers' diet had physiological and survival ramifications with mixed effects on grasshopper fitness. High sodium diets

generated grasshoppers with reduced food consumption, prolonged development times, frass with higher nitrogen content, smaller adult body mass, and reduced survival. At the same time, consistent with studies on Lepidoptera (Snell-Rood et al. 2014), grasshoppers on the high sodium diet had relatively larger eyes and evidence for increased muscle development via jumping. Additionally, grasshoppers on diets with intermediate levels of sodium addition had the highest survival rates as adults, demonstrating fitness benefits of sodium consumption. Thus while in the relatively benign environment of a laboratory cage with ad libitum food, high levels of sodium consumption can decrease average survival and protein assimilation (see also Wang et al. 2017), it also enhances neural and muscle investment that can increase a grasshopper's ability to escape predation. Given this tradeoff, we predict that grasshoppers in the wild may enhance their sodium intake when subject to high predation pressure.

Sodium is essential for neural and muscle development (Denton 1982); on a high sodium diet, grasshoppers showed increased investment in these tissue types. Increasing the amount of sodium grasshoppers consumed yielded an increase in their eye size relative to femur length (our proxy for relative brain size), albeit only when grasshoppers were small. This result is in accordance with work on Lepidoptera showing elevated sodium concentrations in host plants led to increased brain size (Snell-Rood et al. 2014). Moreover, butterflies that fed on salty plants as caterpillars have larger mass-corrected brains than those that feed on less salty species (Swanson et al. 2016). The only predictor of grasshopper jumping distance in this study was a positive effect of diet sodium content. Femur length and grasshopper weight had no effect on jumping distance. Both relatively larger eye sizes and increased grasshopper jumping distance may increase grasshoppers' ability to evade predation through improved visual detection and escape capability (Hawlena et al. 2011).

The effect of diet sodium content on grasshopper survival varied with life stage and between grasshopper nymphs in the laboratory colonies vs. those isolated in the solitary experiment. As nymphs, grasshoppers had the highest survival on both the control and medium sodium diets.

This result is consistent with previous work on the caterpillars of two butterfly species. Monarchs (*Danaus plexippus*) had lower survival when raised on milkweed leaves collected near a roadside (2065 ppm sodium) compared to milkweed leaves collected far from a road (62 ppm; Snell-Rood et al. 2014). Cabbage white butterflies (*Pieris rapae*) had higher survival on artificial diets containing lower levels of sodium (400 ppm) compared to medium (3000 ppm) and high levels (6000 ppm; Snell-Rood et al. 2014). Our preliminary analysis of adult grasshoppers suggests that the mid-level sodium diet resulted in the highest adult colony survival rates. Lower survival rates in adult controls may be due to sodium deficiency and/or compensatory cannibalism.

Likewise, grasshopper nymphs in colony had better survival rates than isolated grasshoppers in the solitary experiment. Again, one hypothesis is that grasshoppers in the colonies had increased survival because they could supplement their provided plant diet with the meat of live or dead fellow colony members. Grasshoppers may have been deficient in other nutrients as evidenced by the common occurrence of consuming their own molt skins (Mira 2000). However, we suspect cannibalism was related to sodium deficiency because all diets had sufficient nitrogen content (>5%) and cannibalism increased with decreasing sodium content of diet. Sodium deprived grasshoppers have been shown to exhibit more cannibalism than those provided with sodium (Simpson et al. 2006).

### Caveats

This study has several limitations that restrict our ability to fully quantify the effects of diet sodium content on grasshopper fitness. First, interpretations of the adult survival results are provisional, as the adult experiment did not have true treatment replication, and should be considered an exploratory study. Second, in order to control sodium responses, we provided grasshoppers with a no-choice diet. On the high sodium diet (>25,000 ppm sodium, 10 times the sodium concentration found in wheatgrass alone), grasshoppers ate less, grew slower, and remained smaller as adults. Even our medium sodium diet contained levels higher than in all but 6% of common grassland plant species

(surveyed by Borer et al. 2019), and potentially higher than levels preferred by grasshoppers. In a choice experiment, the grasshopper species *Locusta migratoria* preferred artificial diets with sodium addition up to 1522 ppm; a diet containing 3044 ppm of sodium was not preferred, albeit the result of this study is confounded by the addition of other minerals to grasshopper diets (Trumper and Simpson 1993). Third, while femur length is a common proxy for grasshopper body size (Akman and Whitman 2008, Branson 2008) it is possible that our ratio of eye to femur length may reflect slower leg growth rather than larger eye growth.

Finally, our results need replication across more species of herbivore to achieve generality. For example, our focal taxa, *M. differentialis*, generally exhibits a preference for weedy and urban areas. This species may have a higher nutritional need for sodium than other species, as suggested by the high sodium content in sunflowers, one of their common host plants (Miller 1995). However, our results are broadly consistent with the responses of two butterfly species (Snell-Rood et al. 2014).

### CONCLUSIONS AND FUTURE DIRECTIONS

Our work confirms the pervasive role of sodium nutrition on development, survival, and performance for a common grassland herbivore. Further extending this work to fecundity and the health of the F1 generation—potentially through the provisioning of sodium in eggs—would yield further insights. In that vein, mammal milk, and butterfly spermatophores are two ways that parents ensure that their offspring are supplied with adequate quantities of sodium (Hawlena et al. 2011, Kaspari 2020). Only one female each on the control and medium sodium diets of the adult colony experiment laid eggs; thus, we were not able to assess the effects of variation in sodium content of diet on grasshopper fecundity here due to the low number of egg-laying females.

A second fruitful avenue of study includes sodium demand and body size. Smaller herbivores are frequently more selective, preferring foliage with higher nutrient concentration (Ovadia and Schmitz 2002, Kleynhans et al. 2011). Moreover, the concentration of sodium in two grasshopper species—*Schistocerca americana*

(Boswell et al. 2008) and *Zonocerus variegatus* (Ademolu et al. 2010)—decreases with dry mass, a result consistent with the metabolic ecology of sodium (Prather et al. 2018b). Combined, this suggests that smaller grasshoppers may require food with higher concentrations of sodium.

Finally, the grasshopper species used in this study is a diet generalist, as are many grasshopper species (Welti et al. 2019a), so they have many choices when it comes to deciding what to consume in the wild. However, other herbivores, like many Lepidopteran larvae, are specialists and may not be able to switch host plants regardless of plant sodium content varying from limiting to toxic levels. Longer-term choice experiments could mimic conditions in the field where grasshoppers have more options to select host plants.

The availability of terrestrial sodium is increasing with continued applications of road salt (Mattson and Godfrey 1994) and increasing salinity of irrigation water (Ghassemi et al. 1995, Qadir et al. 2014). Our results suggest that understanding the relationship between sodium intake and herbivore fitness will enhance our ability to predict insect herbivore outbreaks and the abundance and structure of future ecological communities.

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