Oecologia (2023) 201:941–952
https://doi.org/10.1007/s00442-023-05366-1

BEHAVIORAL ECOLOGY – ORIGINAL RESEARCH

Anthropogenic sodium influences butterfly responses to nitrogen-enriched resources: implications for the nitrogen limitation hypothesis

Alexander M. Shephard[1] · **Kyle Knudsen**[1] · **Emilie C. Snell-Rood**[1]

Received: 18 January 2023 / Accepted: 20 March 2023 / Published online: 27 March 2023

Abstract

Humans are increasing the environmental availability of historically limited nutrients, which may significantly influence organismal performance and behavior. Beneficial or stimulatory responses to increases in nitrogen availability (i.e., nitrogen limitation) are generally observed in plants but less consistently in animals. One possible explanation is that animal responses to nitrogen enrichment depend on how nitrogen intake is balanced with sodium, a micronutrient crucial for animals but not plants. We tested this idea in the cabbage white butterfly (*Pieris rapae*), a species that frequently inhabits nutrient-enriched plants in agricultural settings and roadside verges. We asked (1) whether anthropogenic increases in sodium influence how nitrogen enrichment affects butterfly performance and (2) whether individuals can adaptively adjust their foraging behavior to such effects. Larval nitrogen enrichment enhanced growth of cabbage white larvae under conditions of low but not high sodium availability. In contrast, larval nitrogen enrichment increased egg production of adult females only when individuals developed with high sodium availability. Ovipositing females preferred nitrogen-enriched leaves regardless of sodium availability, while larvae avoided feeding on nitrogen-enriched leaves elevated in sodium. Our results show that anthropogenic increases in sodium influence whether individuals benefit from and forage on nitrogen-enriched resources. Yet, different nitrogen-to-sodium ratios are required to optimize larval and adult performance. Whether increases in sodium catalyze or inhibit benefits of nitrogen enrichment may depend on how evolved nutrient requirements vary across stages of animal development.

Keywords Anthropogenic change · Ecological stoichiometry · Foraging · Nutritional ecology · Sodium

Introduction

Human activities are drastically altering the abundance, distribution, and availability of chemical nutrients in natural ecosystems (Vitousek et al. 1997; Smith 2003), which may have major effects on organismal behavior, development, and performance (Snell-Rood et al. 2015; Espeset et al. 2019). For instance, nitrogen is a crucial macronutrient for virtually all organisms, playing a pivotal role in genetic coding, cell structure, and metabolism (Mattson 1980). Although nitrogen makes up ~ 78% of the earth's atmosphere, the amount of nitrogen available to organisms in usable forms has been historically limited (White 1978; Mattson 1980). However, human activities have increased the relative availability of usable nitrogen more than any other nutrient, largely through fossil fuel combustion and agriculture (Vitousek et al. 1997; Schlesinger 2009). Moderate increases in the amount of available nitrogen are generally thought to have beneficial or stimulatory effects on organismal performance, and this is referred to as the nitrogen limitation hypothesis (White 2012). Consistent with this hypothesis, nitrogen fertilization has repeatedly been shown to have beneficial effects in plants, including enhanced growth and biomass (LeBauer and Treseder 2008; Harpole et al. 2011; Fay et al. 2015). Herbivores have much higher nitrogen requirements than plants, suggesting that herbivores should also benefit from increases in available nitrogen due to plant fertilization (Mattson 1980; White 2012). Indeed,

---

Communicated by Konrad Fiedler.

Alexander M. Shephard
sheph095@umn.edu

[1] Department of Ecology, Evolution, and Behavior, University of Minnesota, Twin Cities, 1987 Upper Buford Circle, St. Paul, MN 55108, USA

numerous studies have shown that dietary nitrogen enrichment improves aspects of herbivore performance, such as growth rate, body size, and fecundity (Slansky and Feeny 1977; Myers and Post 1981; Schetter et al. 1998; Han et al. 2014; Klop et al. 2015; Kurze et al. 2017), and herbivorous insects often seek out nitrogen-enriched plants for feeding and oviposition (Chen et al. 2008; Jaumann and Snell-Rood 2017). However, evidence for benefits of nitrogen enrichment in herbivores (particularly invertebrates) has been weaker and less consistent than in plants (see Kaspari et al. 2017). In some cases, nitrogen enrichment can be detrimental for herbivore performance, even at characteristic levels of agricultural fertilization (Fischer and Fiedler 2000; Kurze et al. 2018). Additionally, meta-analytic evidence has shown that macronutrient enrichment negatively affects invertebrate species abundance (Nessel et al. 2021). These conflicting observations call into question the generality of the nitrogen limitation hypothesis across taxa and complicate our predictions about how animals will respond to anthropogenic increases in nutrient availability.

One potential explanation for why the nitrogen limitation hypothesis has mixed support in animals is that the benefits of increased nitrogen are dependent on the relative availability of other dietary nutrients. This idea is central to the "ecological stoichiometry" framework, which proposes that organismal performance depends on optimal ratios of all required nutrients rather than levels of a single nutrient (Sterner and Elser 2017). Under this framework, it would be predicted that the benefits of nitrogen enrichment may depend on the relative availability of micronutrients, which are required by organisms only in trace amounts but quickly become toxic at higher levels (e.g., sodium, zinc, and calcium). One micronutrient that has a crucial role in the behavior and physiology of animals (but not plants) is sodium (Kaspari 2020). Like nitrogen, sodium has been historically limited in animal diets (especially herbivores). Of all micronutrients, the discrepancy between sodium levels in animal tissue and diet tends to be greatest (e.g., insect tissue can range from being 6–90 × more concentrated in sodium than their food supply; Snell-Rood et al. 2014; Kaspari 2020). Yet, human activities are increasing the bioavailability of sodium through the overuse of groundwater, crop irrigation in arid regions, and the application of road de-icing salt in northern climates (Jackson and Jobbagy 2005; Rengasamy 2006; Daliakopoulos et al. 2016). Although plants do not require sodium, they accumulate it through leaf surfaces or root uptake from the soil (Benes et al. 1996). Sodium can build up in leaf tissue and nectar of plants (Santiago-Rosario et al. 2021), where it becomes available to feeding animals (Mitchell et al. 2020). Consistent with sodium limitation, animals display a range of sodium-seeking behaviors, including puddling for sodium in butterflies (Arms et al. 1974), salt licking in large mammals (Hebert and Cowan 1971), increased herbivory of sodium-enriched leaves by leaf cutter ants (Chavarria-Pizarro et al. 2012), higher rates of termite activity and decomposition in sodium-enriched plots (Kaspari et al. 2014), and increased visitation of sodium-enriched floral nectar by pollinators (Finkelstein et al. 2022).

There are multiple ways by which animal responses to nitrogen enrichment may interact with the relative availability of dietary sodium. For instance, animals may not benefit from nitrogen enrichment unless sodium levels are also elevated. In this regard, sodium may co-limit the effects of nitrogen enrichment on animal performance, such that increased availability of one nutrient increases demand for the other. Recent evidence suggestive of co-limitation comes from ecosystem studies, where sodium supplementation in prairies increases invertebrate abundance in nitrogen-enriched plots (Kaspari et al. 2017; Prather et al. 2018), and grassland plants enriched with both nitrogen and sodium receive more herbivory (Borer et al. 2019). Despite these ecosystem-level observations, it remains unclear how this nutrient co-limitation principle may generalize to responses at the level of animal performance and behavior (but see Adams and Pennings 2022), which is relevant for understanding the fitness consequences of anthropogenic changes in nutrient availability (Snell-Rood et al. 2015). For instance, it is also possible that increases in sodium could inhibit or disrupt the benefits of nitrogen enrichment if sodium levels become toxic or stoichiometrically imbalanced with nitrogen (Sterner and Elser 2017). Unlike nitrogen, which is incorporated as a basic building block of proteins in animal cells, sodium cannot be stably stored in the cell, and ~ 1/3 of the cell's resting metabolic rate is devoted to maintaining sodium homeostasis (da Silva and Williams 2001). If sodium levels become toxic or imbalanced with nitrogen, it is unclear whether animals can adaptively modify their behavior to avoid exposure costs, or whether once-reliable nutritional cues might act as an "ecological trap," drawing animals to forage on harmful or poor-quality resources (Schlaepfer et al. 2002). Additionally, in many animals (including herbivorous insects, such as butterflies and moths), nutrient requirements can vary across juvenile and adult stages (Altermatt and Pearse 2011), raising the possibility that responses to anthropogenic increases in nitrogen and sodium availability may reveal tradeoffs between different aspects of organismal performance, such as larval growth and adult reproduction.

In this paper, we use the cabbage white butterfly (*Pieris rapae*) as a model herbivore to study how anthropogenic increases in nitrogen and sodium availability interact to affect performance and foraging behavior. Butterflies are an ideal system to study such effects because females commonly use nutritional cues to select host plants suitable for oviposition (Nylin and Janz 1993; Chen et al. 2008; Jaumann and Snell-Rood 2017), and caterpillars can make foraging decisions based on variation in plant nutritional quality (Lee et al. 2002). Additionally, nutrient levels vary greatly both within and across species of butterfly dietary host plants (Watanabe et al. 2007; Swanson et al. 2016). The cabbage white butterfly is a particularly interesting system for studying responses to changes in nutrient availability because it is a widespread pest species across North America that consumes plants in the Brassicaceae family (e.g., cabbage and mustards; Ryan et al. 2019). The cabbage white also frequently inhabits weedy plants growing in urban areas, such as roadsides, which receive significant input of nitrogen and sodium from human sources (Cape et al. 2004; Mitchell et al. 2020; Shephard et al. 2022). In northern climates, sodium salts are applied to roads in large quantities during winter (Novotny et al. 2009) and sodium remains in the soil through the growing season (Mitchell et al. 2020). Plant surveys of roadside sites across the upper midwestern region of the United States have shown that leaf sodium content is higher in plants collected from busier roads, and caterpillars collected from saltier leaves contain higher whole-body sodium concentrations (Mitchell et al. 2020). Macronutrients, such as nitrogen and phosphorous, are also elevated along roadsides from traffic and agricultural spillover, and in the United States, vehicles are the largest contributor to anthropogenic nitrogen release (Bettez et al. 2013). Yet, we are just beginning to understand how these novel increases in nutrient availability are affecting animal performance and behavior (Finkelstein et al. 2022).

We conducted a series of experiments to test (1) how anthropogenic increases in nitrogen and sodium availability in the larval diet interact to affect performance of cabbage white larvae and adults and (2) whether larvae and adults can adaptively adjust their foraging behavior to such effects. We began by rearing cohorts of cabbage white larvae in a 2 × 2 factorial experiment to test the effects of nitrogen and sodium on larval development time, growth rate, adult body size, egg production, and survival to adulthood. We considered two possible hypotheses for how increases in nitrogen and sodium availability may interact. Both hypotheses, which are based on the ecological stoichiometry framework (Sterner and Elser 2017), can provide potential solutions to the paradox of why positive effects of nitrogen enrichment on animal performance (i.e., nitrogen limitation) are not consistently observed. First, the *micronutrient co-limitation hypothesis* is based on the idea that increased nitrogen availability increases demand for micronutrients, such as sodium. This hypothesis predicts that the performance benefits of combined increases in nitrogen and sodium availability will be greater than any benefits of increased nitrogen availability alone (Kaspari et al. 2017). Alternatively, the *micronutrient masking hypothesis* is based on the idea that increases in sodium availability will inhibit any benefits of increased nitrogen availability if dietary sodium levels are stressful or stoichiometrically imbalanced. This hypothesis predicts that any performance benefits of combined increases in nitrogen and sodium availability will be lower than the benefits of increased nitrogen availability alone. In addition to the rearing experiment, we tested the effects of leaf nitrogen and sodium variation on adult oviposition behavior and larval feeding preference. Here, we considered the preference-performance hypothesis (Jaenike 1978), which predicts that, assuming nutritional cues are reliable indicators of resource quality, individuals will prefer to oviposit and feed on nutrient treatment combinations that confer the greatest performance benefits. Alternatively, the ecological trap hypothesis (Schlaepfer et al. 2002) predicts that if historically reliable cues are no longer indicators of high resource quality, individuals may oviposit or feed on nutrient combinations that have detrimental effects on performance.

Methods

Study animals

To test how variation in N and Na availability affect cabbage white butterfly performance and behavior, we performed three experiments: performance (Experiment 1), female oviposition preference (Experiment 2), and larval feeding preference (Experiment 3). Butterflies used in all three experiments originated from wild female cabbage whites captured on the University of Minnesota Saint Paul campus over a six-week period in June–July 2021. Upon capture, we immediately transferred all butterflies to a greenhouse and housed them in Bug Dorm cages (61 × 61 × 61 cm) for egg collection. We housed no more than 10 females in a single cage. We provided butterflies with ad libitum access to sponges soaked with 10% honey water (changed daily), and we provided each cage with a moist towel to maintain humidity. Each day, we provided each cage with fresh host plant leaves for oviposition. All larvae used for Experiments 1 and 2 were derived from eggs collected on kale (*Brassica oleracea*), and larvae used for Experiment 3 were derived from eggs collected on radish (*Raphanus sativus*). Each day we removed host plant leaves from oviposition cages housed all leaves containing eggs in a climate chamber maintained at 25˚C on a 14-h photoperiod until hatched larvae were assigned to one of the three experiments. All larvae used in our experiments were derived from approximately 30 wild-caught female butterflies.

Experiment 1: performance

We tested the effects of larval exposure to dietary N and Na variation on multiple aspects of cabbage white performance: survival from larva to adulthood, larval development time, adult body size, larval growth rate, and female egg size and number. For this experiment, we reared all larvae on an agar-based artificial diet designed for cabbage white butterflies (modified from Troetschler et al. 1985) in a 2 × 2 factorial design (treatments included low N/low Na, high N/low Na, low N/high Na, and high N/high Na). This artificial diet approach allowed us to carefully manipulate N and Na levels. For the low Na treatment, we followed Snell-Rood et al. (2014) in creating a base salt mix without NaCl that supports butterfly development. This base salt mix included 43% potassium monobasic, 17% potassium chloride, 14% calcium carbonate, 11% tricalcium phosphate, 13% magnesium sulfate, 1.5% ferric phosphate, and trace levels (< 0.5%) of copper sulfate, potassium aluminum sulfate, potassium iodide, sodium fluoride, and magnesium sulfate. Accordingly, our low N/low Na artificial diet contained the following ingredients: 6.4 g of the base salt mix, 20 g casein, 8 g Torula yeast, 50 g wheat germ, 24 g sucrose, 3.6 g cholesterol, 10.5 g Vanderzant Vitamin mix, 0.75 g methyl paraben, 1.5 g sorbic acid, 3 g ascorbic acid, 0,175 g streptomycin, 5 mL of flaxseed oil, and 23.6 g of cellulose. For the high N treatment, we removed 11 g of cellulose and replaced it with 7 g of casein and 4 g of Torula yeast. For the high Na treatment, we removed 1 g of cellulose and replaced it with 1 g of NaCl. To prepare the diets, 15 g of fine mesh agar was boiled in 400 mL of water and cooled by adding an additional 400 mL of distilled water before mixing all ingredients in a food blender. On average, the Na concentration of the high Na diet was ~ 7 × greater than the Na concentration of the low Na diet (low Na diet mean = 520.17 mg/kg, high Na diet mean = 3666.3 mg/kg; Table S1). For reference, the low and high Na dietary treatments fall within the range of Na in Brassicaceae leaves (min = ~ 400 mg/kg, max = ~ 14,600 mg/kg, median = ~ 3800 mg/kg) collected from the field (Watanabe et al. 2007). The high N diet contained ~ 20% more N than the low N diet, on average (low N diet mean = 3.564% N, high N diet mean = 4.374% N; Table S1). For reference, the low and high N dietary treatments fall within the range of N in Brassicaceae leaves (min = ~ 3.3%, max = ~ 6.5%, median = ~ 4.8%) collected from the field in Minnesota (Espeset et al. 2019). Element concentrations were measured by ICP-AES at the University of Minnesota Research Analytical Lab.

Seven days after egg collection, we assigned cabbage white larvae to one of the four artificial diet treatments (N = 51 larvae assigned to each treatment). The artificial diet was provided in 118-mL plastic cups (3 larvae per cup). We housed all diet cups in a climate chamber (24 °C with 14-h photoperiod) for the duration of larval development and the pupal period.

We measured survival as whether an individual successfully transitioned from larva to adult eclosion with wings fully intact. We measured development time as the number of days from egg collection until eclosion. On the day of eclosion, we labeled each butterfly with a number on its hindwing. A random subset of female butterflies was transferred to a greenhouse for 48 h to allow for egg development without access to mates or host plants. After this egg development period, females were stored at − 20 °C in airtight containers. All other butterflies emerging from the artificial diet were stored at − 20 °C immediately upon eclosion. We measured forewing length only in butterflies surviving to adulthood with intact forewings by carefully removing and photographing one forewing from each butterfly. Using ImageJ (NIH), we measured forewing length as the distance between the wing's apex and the articulation of the wing with the thorax. Finally, we measured growth rate for each butterfly as forewing length divided by development time.

We measured egg number as the total number of mature eggs in the ovaries of each female surviving the 48-h egg development period. Egg size was measured as the average length of 5 eggs randomly selected from each female. Egg length measurements were performed using ImageJ. We dissected all female butterfly ovaries in 1 × PBS buffer and visualization occurred under a Leica M165C microscope (10 × magnification).

Experiment 2: female oviposition preference

To test the effects of leaf N and Na variation on female oviposition preference, we provided mated female cabbage white butterflies with a choice of leaves belonging to each of the four treatments: low N/low Na, high N/low Na, low N/high Na, and high N/high Na. All individuals used for our oviposition preference experiment were reared as larvae on cabbage white artificial diet (3 larvae per 118-mL plastic diet cup). Upon eclosion, all male and female butterflies were labeled on the hindwing with an individual number and housed in a mating cage (61 × 61x 61 cm Bug Dorm) in a greenhouse with ad libitum access to sponges soaked with 10% honey water. Females were housed in the cage for 48 h to allow egg development and mating. We never housed more than 30 butterflies in the mating cage at once, and we always maintained an equal sex ratio. All oviposition trials took place during late June–early July 2021, and no supplemental light was provided in the greenhouse during this time.

After the mating period, we transferred female butterflies from the mating cage to small mesh cages (30 × 30 × 30 cm) for oviposition preference tests. To increase the likelihood of successful oviposition, we housed 3 females in each test cage, as we have previously observed that only 20–30% of cabbage white females will successfully oviposit in the greenhouse when housed individually (personal observations). In each preference test cage, we placed 4 size-matched radish leaves, each belonging to one of the four treatment combinations of N and Na levels. All leaves were derived from 21- to 28-day-old radish plants grown from seed in a greenhouse in individual (15 × 15 × 15 cm) pots containing Professional Growing Mix soil (Sunglow Horticulture). To produce high N leaves, we treated a subset of radish plants with 3 g of NPK fertilizer at 7 and 14 days of age. Plants in the low N treatment received no added fertilization, since our goal was to generate conditions of N limitation and N enrichment rather than two different levels of N enrichment. Throughout the growth period, all plants were watered with deionized water to minimize Na exposure before experimental Na treatments were applied. On the morning of each oviposition preference test, we removed high- and low-N leaves from their respective plants and immediately sprayed the front and back side of each leaf with a ~ 1.6 mL spray of either a high Na solution (2.1 g of NaCl dissolved in 0.74 L of deionized water) or a low Na solution (deionized water with no added NaCl) from a 0.74-L Rubbermaid spray bottle. Each leaf was then placed upright in a separate water-filled tube to provide hydration. Leaf spray treatments were allowed to dry before being introduced to butterflies for oviposition preference tests. We ensured that the four leaves assigned to each preference test cage were approximately equal in size. In each cage, we spatially assorted each leaf randomly, and leaves were never in contact with one another. For each preference test, butterflies were left to oviposit for 24 h starting at 12 PM on the set-up day. Immediately following each preference test, we removed the leaves from each cage and counted the number of eggs laid on each leaf. Each female butterfly was used in a preference test only once.

Experiment 3: larval feeding preference

We tested the effects of leaf N and Na variation on larval feeding preference by providing early instar cabbage white caterpillars with a choice of radish leaves belonging to each of the four treatments: low N/low Na, high N/low Na, low N/high Na, and high N/high Na. We applied all N and Na treatments to the leaves in the same way as described above for Experiment 2 (Na solution or deionized water spray treatments were also allowed to completely dry before starting the experiments). For this experiment, we tested young larvae at the 2nd instar stage of development (7–8 d past the date of egg laying) because larger, later instar caterpillars eat a lot more and tend to be less choosy with respect to leaf quality (Moreau and Bauce 2003). For each preference test, we removed four size-matched leaves (each belonging to one of the four nutrient treatments) and submerged their stems in a water wick. The water wick was placed upright in a 946-mL plastic cup, and we placed a single caterpillar on the surface of the water wick adjacent to the intersection of the four stems. We chose this set-up for the feeding preference test because it allows the four leaves to be in close enough proximity to each other to enhance the ability of the caterpillar to explore each leaf. Before the start of each preference test, we covered each cup with a mesh cloth to prevent the caterpillar from escaping. We ran each preference test for 48 h starting at 3 PM on the set-up day. We chose this test duration because larval feeding in Lepidoptera occurs in bouts that are distributed randomly throughout the day (Reynolds et al. 1986), making it difficult to perform short-term feeding assays. Additionally, because our experimental larvae were quite small, we wanted to ensure that larvae had sufficient time to explore feeding options without completely consuming any of the leaves (larvae never completely consumed a single leaf in any of the feeding preference trials). To assess larval feeding preference, we individually photographed each leaf after the preference test and quantified the area of each leaf that was consumed (see details below).

Statistical analysis

All statistical analyses were performed in R Studio version 3.6.3. We measured nutrient effects on development time, forewing length, growth rate, egg number, and survival to eclosion using a linear mixed effect modeling approach. For each model, we initially included nitrogen treatment, sodium treatment, and their interaction as fixed effects. In models where the interaction between nitrogen and sodium was not significant ($p > 0.05$), we dropped the interaction from the model and considered the simpler additive model containing only additive effects of nitrogen and sodium. In all models, we included the random effect of egg collection date (i.e., the date on which eggs of experimental individuals were collected from breeding females in the greenhouse), to serve as a proxy for variation in maternal identity and condition across the experiment. In the egg number model, we used a Poisson error distribution and included the fixed effect of forewing length (a proxy for adult body size in butterflies), given that egg number was positively correlated with forewing length across all individuals (Pearson's $r = 0.35$, $p = 0.021$). In the survival model, we used a binomial distribution with a logit link function. Significant effects for each model were determined by Type III Wald X^2 tests using the Anova function in the "car" package (Fox and Weisberg 2011). For each model in which we detected a significant nitrogen-by-sodium interaction, we used the *emmeans* package to compute planned pairwise contrasts of the estimated marginalized means for sodium treatment conditioned on nitrogen treatment to test our hypotheses about how responses to nitrogen enrichment may vary across levels sodium availability.

In the oviposition preference experiment (Experiment 2), we counted the number of eggs laid by female butterflies on each of the four leaves corresponding to each nutrient treatment after a 24-h oviposition period. For oviposition preference analysis, we used a generalized linear mixed effects model with a Poisson distribution. We included egg count as the response variable and nitrogen treatment, sodium treatment, and their interaction as fixed effects. We included cage number (i.e., experimental replicate) as a random effect nested within the random effect of assay date. To account for overdispersion, we also included observation as a random effect (Bolker et al. 2015). In total, we ran 21 cage replicates of the oviposition preference test with 3 females per cage in each test (tests were carried out over 12 separate days). To qualify for inclusion in our analysis, females must have laid at least 5 eggs per test. This was achieved in 19 of our replicate cages. In two of the cage replicates, zero eggs were laid, so we dropped these cages from the analysis.

For the larval feeding preference tests (Experiment 3), we used ImageJ to quantify the area consumed of each of the four leaves corresponding to each nutrient treatment. Each leaf was given a score from 1 to 5 corresponding to percent herbivory damage, where 1 = no visible damage, 2 = > 0 and < 1% damage, 3 = > 1 and < 3% damage, 4 = > 3 and < 5% damage, and 5 = > 5% damage. We modeled herbivory score using a cumulative link model for ordinal regression in the *clm* package in R. This model included nitrogen treatment, sodium treatment, and their interaction as fixed effects. We set up a total of 26 cup replicates for this experiment. To qualify for inclusion in our analysis, herbivory must have been detected on at least one of the four leaves in each cup, and the caterpillar had to survive the experiment. These inclusion criteria were not met in only 3 of our cup replicates, which we dropped from the analysis.

Results

Experiment 1: performance

We found significant interactions between larval sodium and nitrogen availability on performance measures. Under conditions of low sodium availability, cabbage white larvae with higher access to nitrogen developed earlier ($t = 2.99$, $p = 0.0065$), but nitrogen did not affect development time under high sodium availability ($t = -0.61$, $p = 1.00$), indicating a significant interactive effect of dietary nitrogen and sodium availability on development time (Table 1; Fig. 1A). Similarly, under conditions of low sodium availability, larvae with higher access to nitrogen grew faster ($t = -3.09$; $p = 0.0047$), but nitrogen enrichment did not affect growth rate under conditions of high sodium availability ($t = 0.25$, $p = 1.00$), representing a significant interactive effect of nitrogen and sodium availability on growth rate (Table 1; Fig. 1C). Variation in larval nitrogen availability did not affect adult forewing length, yet high larval sodium availability reduced adult forewing length ($t = 2.37$, $p = 0.019$; Table 1, Fig. 1B).

Table 1 Linear mixed effect model results for the effects of larval dietary nitrogen and sodium availability on development time, forewing length, and growth rate in the cabbage white butterfly (*Pieris rapae*). Sex is included as a fixed effect in each model

	X^2	*df*	*p*
Development time ($N = 181$ observations)			
Nitrogen	9.13	1	0.0025
Sodium	0.25	1	0.62
Sex	3.55	1	0.059
Nitrogen x Sodium	6.45	1	0.011
Forewing length ($N = 168$ observations)			
Nitrogen	0.84	1	0.36
Sodium	5.67	1	0.017
Sex	26.14	1	< 0.001
Growth rate ($N = 168$ observations)			
Nitrogen	9.82	1	0.0017
Sodium	0.0001	1	0.99
Sex	1.44	1	0.23
Nitrogen x Sodium	5.28	1	0.021

There were no effects of larval nitrogen or sodium availability on cabbage white survival from larva to adult eclosion (Table 2). Under conditions of low sodium availability, larval nitrogen enrichment did not affect adult egg number ($z = 0.68$, $p = 0.99$); yet under conditions of high sodium availability, individuals with higher access to nitrogen developed more eggs ($z = -2.51$, $p = 0.024$), representing a significant interactive effect of nitrogen and sodium availability on female egg production (Table 2; Fig. 2).

Experiment 2: female oviposition preference

We detected a significant main effect of leaf nitrogen availability on female cabbage white oviposition preference (Table 3; Fig. 3A). Females laid more eggs on leaves that were high in nitrogen relative to leaves that were low in nitrogen ($z = -3.33$. $p = 0.0009$). However, there was no effect of leaf sodium availability on oviposition preference ($z = -0.43$, $p = 0.66$).

Experiment 3: larval feeding preference

We detected a significant interactive effect of leaf nitrogen and sodium availability on feeding preference (i.e., herbivory score) of cabbage white larvae (Table 3; Fig. 3B). Under conditions of high leaf nitrogen availability, herbivory of high sodium leaves was lower than herbivory of low sodium leaves ($z = 2.37$, $p = 0.018$). Yet, under conditions of low nitrogen availability, herbivory score did not differ between low and high sodium leaves ($z = -1.61$, $p = 0.11$).

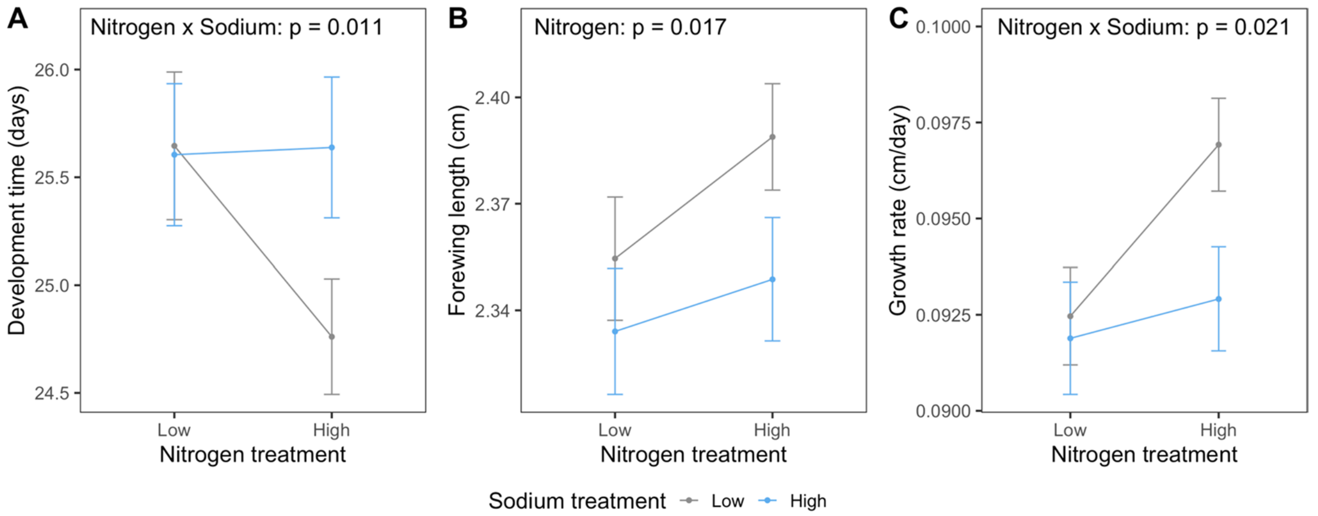

Fig. 1 Effects of larval exposure to dietary nitrogen and sodium on performance traits in the cabbage white butterfly (*Pieris rapae*). Development time **A** was quantified as the number of days between the date larvae were transferred to each diet treatment and the date of adult emergence. Forewing length **B** was measured as the distance between them forewing apex and the forewing articulation with the thorax. Growth rate **C** was quantified as forewing length divided by development time. Points on each graph indicate mean ± standard error

Table 2 Linear mixed effects model results for the effects of nitrogen and sodium availability on survival from larva to adult eclosion and egg number of female cabbage white butterflies (*Pieris rapae*)

	X^2	*df*	*p*
Survival (*N* = 204 observations)			
Nitrogen	0.18	1	0.67
Sodium	0.93	1	0.33
Female egg number (*N* = 41 observations)			
Nitrogen	0.46	1	0.50
Sodium	1.66	1	0.2
Forewing length	21.42	1	< 0.001
Nitrogen x sodium	5.27	1	0.022

Discussion

Our study shows that increases in sodium availability characteristic of anthropogenic inputs can influence whether butterflies seek out and benefit from nitrogen-enriched resources, providing one potential solution to the problem of why nitrogen limitation is inconsistently observed in animals. We found that in cabbage white larvae, individuals developing on a nitrogen-enriched diet grew faster and matured earlier in the presence of low but not high sodium availability. Consistent with these performance effects, larvae avoided feeding on nitrogen-enriched leaves that were high in sodium. However, in contrast to larval performance effects, we found that dietary nitrogen enrichment increased egg production of adult female butterflies only in the presence of high sodium availability. These results show that anthropogenic increases in sodium catalyze or inhibit the performance benefits of nitrogen enrichment in a manner that may be consistent with how specific nutrient requirements vary across stages of animal development.

In terms of growth and development, cabbage white larvae benefitted from dietary nitrogen enrichment in the presence of low but not high sodium availability (Fig. 1). This observation is consistent with the micronutrient masking hypothesis, which predicts that high levels of dietary sodium will inhibit performance benefits of nitrogen enrichment, potentially due to sodium levels being stressful or stoichiometrically imbalanced with nitrogen (Sterner and Elser 2017). In further support of this hypothesis, larvae avoided feeding on nitrogen-enriched leaves that were high in sodium (Fig. 3B). These results are consistent with previous observations that herbivores can self-select diets that benefit performance (e.g., Waldbauer and Friedman 1991; Telang et al. 2001). While previous evidence suggests that variation in dietary sodium alone does not influence larval foraging decisions (Mitchell et al. 2019), our results extend these previous observations by showing that sodium levels may have an important influence on foraging decisions when variation in nitrogen availability is also considered. An additional factor that could influence why caterpillars in our study were choosier with respect to leaf nutritional variation could be that while we focused on relatively young second instar caterpillars, the Mitchell et al. (2019) study focused on fifth instar caterpillars. Previous evidence suggests that caterpillars are less choosy

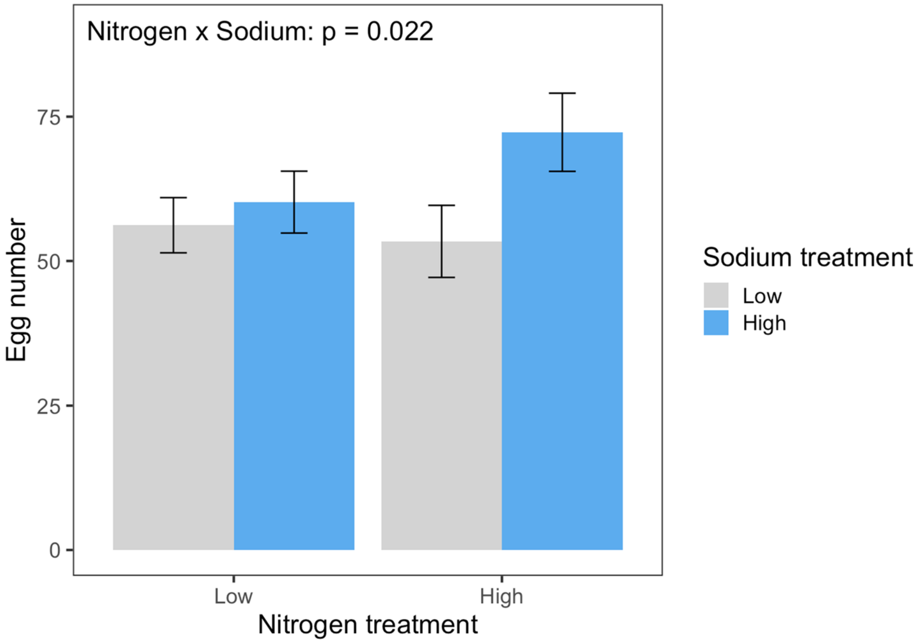

Fig. 2 Effects of larval exposure to dietary nitrogen and sodium on egg number of adult female cabbage white butterflies (*Pieris rapae*). Egg number was measured by counting the total number of mature eggs in ovaries of females dissected 48 h after emergence from pupae. Bars indicate least square means from a model controlling for body size (forewing length) ± standard error

Table 3 Model results for the effects of nitrogen and sodium availability on female oviposition preference and larval feeding preference of cabbage white butterflies (*Pieris rapae*)

	X^2	*df*	*p*
Female oviposition preference (*N* = 76 observations)			
Nitrogen	11.12	1	< 0.001
Sodium	0.19	1	0.66
Larval feeding preference (*N* = 92 observations)			
Nitrogen	7.06	1	0.0079
Sodium	8.16	1	0.0043
Nitrogen x Sodium	19.11	1	< 0.001

with respect to leaf nutritional quality in later stages of development (Moreau and Bauce 2003).

Our finding that increases in sodium inhibited the benefits of nitrogen enrichment on larval growth conflicts with the micronutrient co-limitation hypothesis, which predicts that the performance benefits of combined increases in nitrogen and sodium will be greater than any benefits of nitrogen enrichment alone. Micronutrient co-limitation could be expected based on prior results showing that micronutrient additions can amplify the positive effects of macronutrient fertilization on arthropod abundance and consumption in grassland ecosystems (Kaspari et al. 2017; Prather et al. 2018; Borer et al. 2019). One possible reason for why our results differ from these prior observations could be that the nutritional requirements of cabbage white larvae differ from those of typical grassland arthropods. As an agricultural crop pest (Ryan et al. 2019), the cabbage white likely has a history of selection with consistently high levels of plant nitrogen, but not necessarily sodium, which is likely more variable in its availability. In this regard, a nitrogen-enriched diet could represent the evolved optimal diet of a crop pest, such as the cabbage white, while combined increases in nitrogen and sodium may represent a deviation from this optimal diet. Unlike crop pests, most native grassland arthropods have likely not experienced such strong selection with consistently high nitrogen levels, implying that the macronutrient additions typical of grassland fertilization experiments would be more novel for arthropods inhabiting these relatively nitrogen-limited environments. It is therefore possible that to immediately benefit from such novel increases in macronutrient availability, these species must achieve the right nutritional balance through supplemental increases in micronutrient levels. Consistent with this hypothesis, previous studies suggest that nitrogen limitation may indeed be more common in agricultural pest insects than non-pest species (see Kurze et al. 2018). For example, in Lepidoptera, benefits of nitrogen enrichment have been observed mostly in agricultural pest species, while increased nitrogen has been shown to harm non-pest species (Fischer and Fiedler 2000; Kurze et al. 2018). Altogether, these findings suggest that variation in organismal nutritional requirements shaped by evolutionary history may have an important influence on why species differ in their responses to modern changes in nutrient availability.

In contrast to larval growth, we found that nitrogen enrichment increased egg production of adult female butterflies only in the presence of high sodium availability (Fig. 2), consistent with the micronutrient co-limitation hypothesis (Kaspari et al. 2017). Why would increases in sodium catalyze the benefits of nitrogen enrichment

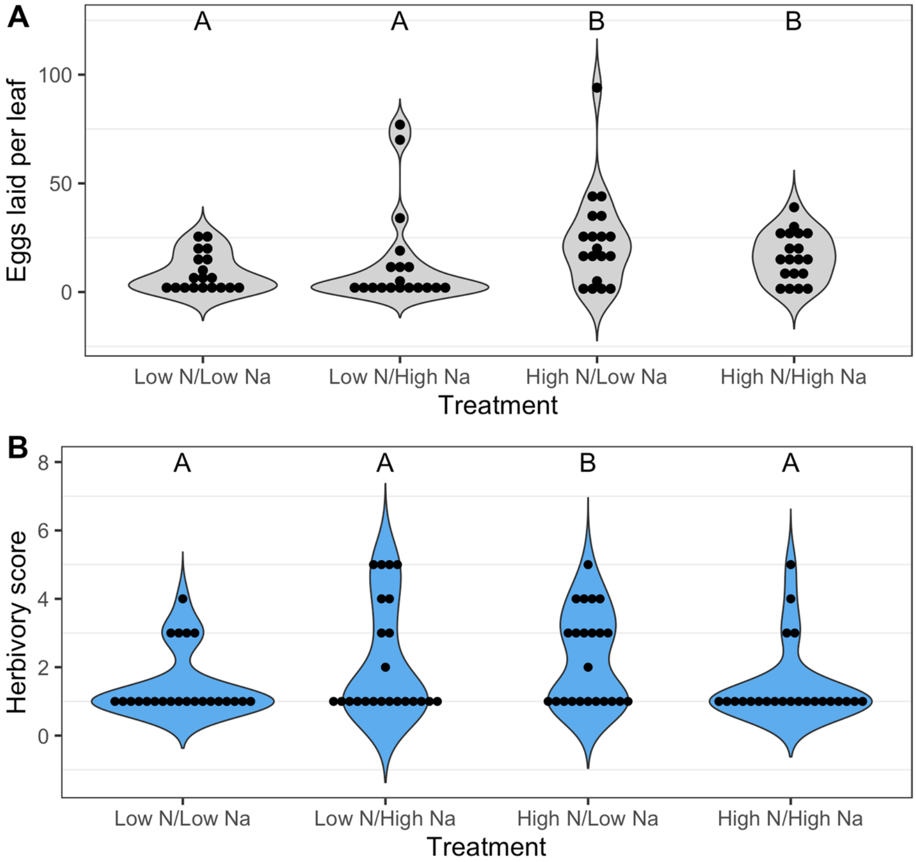

Fig. 3 Violin plots showing individual and combined effects of elevated leaf nitrogen (N) and sodium (Na) content on female oviposition preference **A** and larval feeding preference **B** in the cabbage white butterfly (*Pieris rapae*). In each preference experiment replicate, butterflies or larvae were given access to four leaves, each belonging to a different treatment: low N/low Na ("Control"), high N/low Na ("High N"), low N/high Na ("High Na"), and high N/high Na ("High N/High Na"). Oviposition preference **A** is displayed as average number of eggs laid on leaves of each treatment across $N = 19$ experimental replicates (bars represent means ± standard error). Larval feeding preference **B** is displayed as the average herbivory score of leaves in each treatment across $N = 23$ experimental replicates. Statistical results for each preference experiment are summarized in Table 2

in adult butterflies but inhibit the benefits in developing larvae? This may reflect differences in larval and adult nutritional requirements. For instance, in many butterfly species (including cabbage whites), adult males exhibit a puddling behavior, in which they seek out nutrients by feeding on fluid from resources, such as mud, urine, and carrion (Beck and Fiedler 2009). Sodium is often one of the primary nutrients that male butterflies seek out while puddling (Plotkin and Goddard 2013), and males transfer this sodium to females during mating as a nuptial gift that can enhance female fecundity and reproductive success (Pivnick and McNeil 1987; Smedley and Eisner 1996; Mitra et al. 2016). Our results show that larval nitrogen enrichment can enhance egg production of adult females only if higher levels of sodium are also available, possibly due to the particular importance of sodium in reproductive functioning. If anthropogenic increases in nutrient availability can provide organisms with resources at the larval stage that would historically have only been obtained in adulthood, it is unclear whether this might affect selection on other adult traits, such as mating behavior. Future studies should further explore the potential for such carryover effects, where changes in the availability of key nutrients at earlier developmental stages may have important behavioral or fitness consequences at later stages.

Although feeding larvae tended to avoid nitrogen-enriched leaves that were high in sodium (Fig. 3B), adult female butterflies preferred ovipositing on nitrogen-enriched leaves regardless of sodium availability (Fig. 3A). Thus, while larval foraging decisions prioritize larval growth over adult reproduction, female oviposition decisions do not favor larval or adult performance in the context of our study. Although previous research indicates that female butterflies typically prefer to oviposit on nitrogen-enriched leaves (Chen et al. 2008; Jaumann and Snell-Rood 2017), other findings suggest that butterflies do not alter oviposition decisions in response to leaf sodium variation (Mitchell et al. 2019). It is possible that ovipositing butterflies have not undergone selection to detect novel variation in host plant sodium levels in anthropogenic environments (Mitchell et al. 2019) Alternatively, it is possible that female cabbage whites may prioritize only a narrow set of cues when making oviposition decisions, as previous research has demonstrated that females prefer to spread their eggs across multiple plants, a behavior that has likely been driven by the ephemeral nature of their host plant species (Root and Kareiva 1984). Even though our results suggest that increased larval access to dietary sodium may confer adult reproductive benefits, butterflies may not be behaviorally drawn to this novel source of sodium if they are primarily adapted to acquiring sodium through puddling at the adult stage. Additionally, the inability for ovipositing butterflies to detect differences in host plant sodium availability may result in failure to avoid laying eggs on leaves with toxic sodium levels, resulting in an ecological trap (Schlaepfer et al. 2002; Mitchell et al. 2019).

Although we found evidence for nitrogen limitation in cabbage white larvae, we found no evidence in our study for basic sodium limitation. A potential explanation for this could be that sodium may be a more crucial limiting nutrient for traits not measured in our study. For instance, while we found no evidence of sodium limitation of performance traits related to growth, survival, or reproduction, previous work has shown that anthropogenic increases in sodium can positively affect the development of morphological traits, such as brain size (in butterflies, including the cabbage white; Snell-Rood et al. 2014; Shephard et al. 2021a, b) and muscle size (in butterflies and grasshoppers; Snell-Rood et al. 2014; Peterson et al. 2021). It is therefore possible that the fitness benefits of increased sodium availability could be realized under ecological contexts that rely more on brain or muscle function, such as learning or dispersal. For instance, increases in dietary sodium availability improved jumping performance in grasshoppers (Peterson et al. 2021), which could potentially aid in predator avoidance. A possible alternative reason for why we did not find evidence of sodium limitation in our study could be that our high sodium treatment was within a range that was somewhat stressful, as individuals developing under conditions of high sodium availability had a smaller adult body size (Fig. 1B).

In summary, our study of the cabbage white butterfly shows that the performance benefits of nitrogen enrichment (nitrogen limitation) can be influenced by the relative availability of dietary sodium. Yet, we find that increases in sodium inhibit the benefits of nitrogen enrichment for larval growth (micronutrient masking) but catalyze the benefits of nitrogen enrichment for adult reproduction (micronutrient co-limitation). These results indicate that different sodium-to-nitrogen ratios are required to optimize larval and adult performance. In this manner, anthropogenic changes in nutrient availability may generate novel tradeoffs between components of organismal fitness (Snell-Rood et al. 2015). We hypothesize that these stage-specific interactions between nitrogen and sodium availability might be explained by differences in how evolution has shaped nutritional requirements across stages of animal development, as nutritional preferences or requirements can vary markedly through ontogeny (Browne 1995). Continued attention to how nutritional requirements vary across development may yield novel insights into how organisms will respond to anthropogenic changes in nutrient availability.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-023-05366-1.

Author contribution statement AMS, KK, and ECS-R conceived and designed the experiments. AMS and KK performed the experiments and analyzed the data. AMS wrote the manuscript; other authors provided editorial advice.

Funding This study was funded by a grant awarded to KK by the Undergraduate Research Opportunities Program at the University of Minnesota.

Data availability Data will be deposited on Mendeley upon acceptance of the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval

Ethical approval is not required for non-native insects, such as *Pieris rapae*.

References

Adams T, Pennings SC (2022) Dietary protein and sodium co-limit cockroach growth and reproduction. Ecolog Entomol 47(5):831–841

Altermatt F, Pearse IS (2011) Similarity and specialization of the larval versus adult diet of European butterflies and moths. Am Nat 178(3):372–382

Arms K, Feeny P, Lederhouse RC (1974) Sodium: stimulus for puddling behavior by tiger swallowtail butterflies. Papilio Glaucus Science 185(4148):372–374

Beck J, Fiedler K (2009) Adult life spans of butterflies (Lepidoptera: Papilionoidea+ Hesperioidea): broadscale contingencies with adult and larval traits in multi-species comparisons. Biol J Lin Soc 96(1):166–184

Benes SE, Aragüés R, Grattan SR, Austin RB (1996) Foliar and root absorption of Na+ and Cl− in maize and barley: Implications for salt tolerance screening and the use of saline sprinkler irrigation. Plant Soil 180(1):75–86

Bettez ND, Marino R, Howarth RW, Davidson EA (2013) Roads as nitrogen deposition hot spots. Biogeochemistry 114(1):149–163

Bolker BM (2015) Linear and generalized linear mixed models. Contemporary theory and application, Ecological Statistics, pp 309–333

Borer ET, Lind EM, Firn J, Seabloom EW, Anderson TM, Bakker ES et al (2019) More salt, please: global patterns, responses and impacts of foliar sodium in grasslands. Ecol Lett 22(7):1136–1144

Browne LB (1995) Ontogenic changes in feeding behavior. Regulatory mechanisms in insect feeding. Springer, Boston, MA, pp 307–342

Cape JN, Tang YS, Van Dijk N, Love L, Sutton MA, Palmer SCF (2004) Concentrations of ammonia and nitrogen dioxide at roadside verges, and their contribution to nitrogen deposition. Environ Pollut 132(3):469–478

Chavarria-Pizzaro L, Mccreery HF, Lawson SP, Winston ME, O'Donnell SEAN (2012) Sodium-specific foraging by leafcutter ant workers (Atta cephalotes, Hymenoptera: Formicidae). Ecolog Entomol 37(5):435–438

Chen Y, Ruberson JR, Olson DM (2008) Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, Spodoptera exigua, on cotton. Entomol Exp Appl 126(3):244–255

Da Silva JF, Williams RJP (2001) The biological chemistry of the elements: the inorganic chemistry of life. Oxford University Press

Daliakopoulos IN, Tsanis IK, Koutroulis A, Kourgialas NN, Varouchakis AE, Karatzas GP, Ritsema CJ (2016) The threat of soil salinity: a European scale review. Sci Total Environ 573:727–739

Espeset A, Kobiela ME, Sikkink KL, Pan T, Roy C, Snell-Rood EC (2019) Anthropogenic increases in nutrients alter sexual selection dynamics: a case study in butterflies. Behav Ecol 30(3):598–608

Fay PA, Prober SM, Harpole WS, Knops JM, Bakker JD, Borer ET et al (2015) Grassland productivity limited by multiple nutrients. Nature Plants 1(7):1–5

Finkelstein CJ, CaraDonna PJ, Gruver A, Welti EA, Kaspari M, Sanders NJ (2022) Sodium-enriched floral nectar increases pollinator visitation rate and diversity. Biol Let 18(3):20220016

Fischer K, Fiedler K (2000) Response of the copper butterfly Lycaena tityrus to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. Oecologia 124(2):235–241

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*. Sage Publications.

Han P, Lavoir AV, Le Bot J, Amiens-Desneux E, Desneux N (2014) Nitrogen and water availability to tomato plants triggers bottom-up effects on the leafminer Tuta absoluta. Sci Rep 4(1):1–8

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken ME et al (2011) Nutrient co-limitation of primary producer communities. Ecol Lett 14(9):852–862

Hebert D, Cowan IM (1971) Natural salt licks as a part of the ecology of the mountain goat. Can J Zool 49(5):605–610

Jackson RB, Jobbagy EG (2005) From icy roads to salty streams. Proc Natl Acad Sci 102(41):14487–14488

Jaenike J (1978) On optimal oviposition behavior in phytophagous insects. Theor Popul Biol 14(3):350–356

Jaumann S, Snell-Rood EC (2017) Trade-offs between fecundity and choosiness in ovipositing butterflies. Anim Behav 123:433–440

Kaspari M (2020) The seventh macronutrient: How sodium shortfall ramifies through populations, food webs and ecosystems. Ecol Lett 23(7):1153–1168

Kaspari M, Clay NA, Donoso DA, Yanoviak SP (2014) Sodium fertilization increases termites and enhances decomposition in an Amazonian forest. Ecology 95(4):795–800

Kaspari M, Roeder KA, Benson B, Weiser MD, Sanders NJ (2017) Sodium co-limits and catalyzes macronutrients in a prairie food web. Ecology 98(2):315–320

Klop E, Omon B, WallisDeVries MF (2015) Impact of nitrogen deposition on larval habitats: the case of the Wall Brown butterfly Lasiommata megera. J Insect Conserv 19(2):393–402

Kurze S, Heinken T, Fartmann T (2017) Nitrogen enrichment of host plants has mostly beneficial effects on the life-history traits of nettle-feeding butterflies. Acta Oecologica 85:157–164

Kurze S, Heinken T, Fartmann T (2018) Nitrogen enrichment in host plants increases the mortality of common Lepidoptera species. Oecologia 188(4):1227–1237

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89(2):371–379

Lee KP, Behmer ST, Simpson SJ, Raubenheimer D (2002) A geometric analysis of nutrient regulation in the generalist caterpillar Spodoptera littoralis (Boisduval). J Insect Physiol 48(6):655–665

Mattson WJ Jr (1980) Herbivory in relation to plant nitrogen content. Annu Rev Ecol Syst 11(1):119–161

Mitchell TS, Shephard AM, Kalinowski CR, Kobiela ME, Snell-Rood EC (2019) Butterflies do not alter oviposition or larval foraging in response to anthropogenic increases in sodium. Anim Behav 154:121–129

Mitchell TS, Agnew L, Meyer R, Sikkink KL, Oberhauser KS, Borer ET, Snell-Rood EC (2020) Traffic influences nutritional quality of roadside plants for monarch caterpillars. Sci Total Environ 724:138045

Mitra C, Reynoso E, Davidowitz G, Papaj D (2016) Effects of sodium puddling on male mating success, courtship and flight in a swallowtail butterfly. Anim Behav 114:203–210

Moreau G, Bauce É (2003) Feeding behavior of spruce budworm (Lepidoptera: Tortricidae) larvae subjected to multiple exposures of Bacillus thuringiensis variety kurstaki. Ann Entomol Soc Am 96(3):231–236

Myers JH, Post BJ (1981) Plant nitrogen and fluctuations of insect populations: a test with the cinnabar moth—tansy ragwort system. Oecologia 48(2):151–156

Nessel MP, Konnovitch T, Romero GQ, González AL (2021) Nitrogen and phosphorus enrichment cause declines in invertebrate populations: a global meta-analysis. Biol Rev 96(6):2617–2637

Novotny EV, Sander AR, Mohseni O, Stefan HG (2009) Chloride ion transport and mass balance in a metropolitan area using road salt. Water Resour Res. https://doi.org/10.1029/2009WR008141

Nylin S, Janz N (1993) Ovi position preference and larval performance in Polygonia c-album (Lepidoptera: Nymphalidae): the choice between bad and worse. Ecolog Entomo 18(4):394–398

Peterson TN, Welti EA, Kaspari M (2021) Dietary sodium levels affect grasshopper growth and performance. Ecosphere 12(3):e03392

Pivnick KA, McNeil JN (1987) Puddling in butterflies: sodium affects reproductive success in Thymelicus lineola. Physiol Entomol 12(4):461–472

Plotkin D, Goddard J (2013) Blood, sweat, and tears: a review of the hematophagous, sudophagous, and lachryphagous Lepidoptera. J Vector Ecol 38(2):289–294

Prather CM, Laws AN, Cuellar JF, Reihart RW, Gawkins KM, Pennings SC (2018) Seeking salt: herbivorous prairie insects can be co-limited by macronutrients and sodium. Ecol Lett 21(10):1467–1476

Rengasamy P (2006) World salinization with emphasis on Australia. J Exp Bot 57(5):1017–1023

Reynolds SE, Yeomans MR, Timmins WA (1986) The feeding behaviour of caterpillars (Manduca sexta) on tobacco and on artificial diet. Physiol Entomol 11(1):39–51

Root RB, Kareiva PM (1984) The search for resources by cabbage butterflies (Pieris rapae): ecological consequences and adaptive significance of Markovian movements in a patchy environment. Ecology 65(1):147–165

Ryan SF, Lombaert E, Espeset A, Vila R, Talavera G, Dincă V et al (2019) Global invasion history of the agricultural pest butterfly Pieris rapae revealed with genomics and citizen science. Proc Natl Acad Sci 116(40):20015–20024

Santiago-Rosario LY, Harms KE, Elderd BD, Hart PB, Dassanayake M (2021) No escape: the influence of substrate sodium on plant growth and tissue sodium responses. Ecol Evol 11(20):14231–14249

Schetter TA, Lochmiller RL, Leslie DM Jr, Engle DM, Payton ME (1998) Examination of the nitrogen limitation hypothesis in non-cyclic populations of cotton rats (Sigmodon hispidus). J Anim Ecol 67(5):705–721

Schlaepfer MA, Runge MC, Sherman PW (2002) Ecological and evolutionary traps. Trends Ecol Evol 17(10):474–480

Schlesinger WH (2009) On the fate of anthropogenic nitrogen. Proc Natl Acad Sci 106(1):203–208

Shephard AM, Zambre AM, Snell-Rood EC (2021a) Evaluating costs of heavy metal tolerance in a widely distributed, invasive butterfly. Evol Appl 14(5):1390–1402

Shephard AM, Mitchell TS, Snell-Rood EC (2021b) Monarch caterpillars are robust to combined exposure to the roadside micronutrients sodium and zinc. Cons Physiol. https://doi.org/10.1093/conphys/coab061

Shephard AM, Agnew L, Herdtle A, Mitchell TS, Borer ET, Snell-Rood EC (2022) Traffic patterns, more than adjacent land use, influence element content of roadside forbs for insect pollinators. Ecolog Solut Evid 3(4):e12195

Slansky F Jr, Feeny P (1977) Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol Monogr 47(2):209–228

Smedley SR, Eisner T (1996) Sodium: a male moth's gift to its offspring. Proc Natl Acad Sci 93(2):809–813

Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems a global problem. Environ Sci Pollut Res 10(2):126–139

Snell-Rood EC, Espeset A, Boser CJ, White WA, Smykalski R (2014) Anthropogenic changes in sodium affect neural and muscle development in butterflies. Proc Natl Acad Sci 111(28):10221–10226

Snell-Rood E, Cothran R, Espeset A, Jeyasingh P, Hobbie S, Morehouse NI (2015) Life-history evolution in the anthropocene: Effects of increasing nutrients on traits and trade-offs. Evol Appl 8(7):635–649

Sterner RW, Elser JJ (2017) Ecological stoichiometry. Princeton University Press

Swanson EM, Espeset A, Mikati I, Bolduc I, Kulhanek R, White WA et al (2016) Nutrition shapes life-history evolution across species. Proc R Soc B 283(1834):20152764

Telang A, Booton V, Chapman RF, Wheeler DE (2001) How female caterpillars accumulate their nutrient reserves. J Insect Physiol 47(9):1055–1064

Troetschler RG, Malone CM, Bucago ER, Johnston MR (1985) System for rearing Pieris rapae (Lepidoptera: Pieridae) on a noncruciferous artificial diet developed for Manduca sexta (Lepidoptera: Sphingidae). J Econ Entomol 78(6):1521–1523

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW et al (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7(3):737–750

Waldbauer GP, Friedman S (1991) Self-selection of optimal diets by insects. Annu Rev Entomol 36(1):43–63

Watanabe T, Broadley MR, Jansen S, White PJ, Takada J, Satake K et al (2007) Evolutionary control of leaf element composition in plants. New Phytol 174(3):516–523

White TC (1978) The importance of a relative shortage of food in animal ecology. Oecologia 33(1):71–86

White, T. C. (2012). *The inadequate environment: nitrogen and the abundance of animals*. Springer Science & Business Media