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Michael J. Baltzley^a, Christopher J. Paradise^a & William A. Dunson^a

^a Department of Biology, The Pennsylvania State University, University Park, PA, 16802, USA

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Interactive Effects of Density and Water Sodium Concentration on Growth of Insect Larvae Inhabiting Treeholes

Michael J. Baltzley^a, Christopher J. Paradise^b, and William A. Dunson^c

Department of Biology
The Pennsylvania State University
University Park, PA 16802 USA

ABSTRACT

There is an inverse correlation between sodium concentration ([Na]) and larval densities of insects in water-filled treeholes, suggesting that sodium might be a limiting resource or that it is controlled by biota. The effects of water [Na] and population density on the growth of the culicid *Aedes triseriatus* and the scirtid beetles *Helodes pulchella* and *Prionocyphon discoideus* were studied in a laboratory experiment. At low densities, microcosms high in [Na] produced significantly larger adult *A. triseriatus* than those low in [Na]. Scirtids showed the opposite trend. Sodium level had no impact on size at high density for either mosquitoes or scirtids. Dry masses of adult mosquitoes and larval scirtids were significantly smaller at high than at low density. High densities were also associated with longer development and lower survival of mosquitoes. Despite the fact that mosquitoes at low density grew faster at high [Na], the negative effects of high density were not alleviated by high [Na]. High densities of scirtids decreased the [Na] of the water in the microcosms, indicating that the insects may regulate water [Na].

INTRODUCTION

Treeholes are cavities in trees filled with water, leaf litter, and sediment (Kitching 1971), with leaf litter inputs occurring once a year in temperate forests (Carpenter 1983, Walker et al. 1991), and water inputs from precipitation in the form of throughfall and stemflow (Walker et al. 1991). These detritus-based communities are home to a variety of macroinvertebrate species (Kitching 1971, Barrera 1996a). Three of the more common of these in Pennsylvania are the mosquito *Aedes triseriatus* (Say), and two species of scirtid (= helodid) beetles, *Helodes pulchella* (Guerin) and *Prionocyphon discoideus* (Say) (Barrera 1996a, Paradise 1997). *A. triseriatus* is a filter feeder and browser (Wallace and Merritt 1980, Merritt et al. 1992), removing particulate matter from suspension and the surface of leaves. Scirtids are leaf shredders (Barrera 1996a) and feed on leaf litter, while also facilitating its decay (Paradise and Dunson 1997a).

Treeholes are small but complex communities with many varying abiotic and biotic factors; interspecific interactions and water chemistry are well-studied factors (Fish and Carpenter 1982, Livdahl 1982, Bradshaw and Holzapfel 1983, Hard et al.

^a current address: 1365 York Ave., New York, NY 10021

^b author to whom correspondence should be directed at the following current address:
Department of Biology, King's College, Wilkes-Barre, PA 18711

^c current address: PO Box 3330, Immokalee, FL 34143

1989, Kitching and Beaver 1990, Walker et al. 1991, Barrera 1996a, 1996b). Water chemistry parameters in treeholes may act as resources or toxins, depending on the particular chemical and its concentration. Various chemicals, such as dissolved organic carbons (DOC) and inorganic cations and anions have been investigated in treeholes (Peterson and Chapman 1969, Sota 1993, Mercer and Anderson 1994, Walker et al. 1991, Paradise and Dunson 1997b, 1998a, 1998b), and have numerous impacts on insect populations therein. The densities of insects in treeholes are often related to water chemistry and the size of treeholes (Peterson and Chapman 1969, Bradshaw and Holzapfel 1983, Sota 1996, Paradise and Dunson 1997b). Densities of mosquitoes are higher in treeholes with high water volume and low [Na], and scirtid densities are higher in treeholes with low [Na] and larger total treehole volume (Paradise and Dunson 1998a). Concentrations of Na in stemflow and precipitation are relatively low, generally below 60 μM (Paradise and Dunson 1998a), but sodium may also come from decaying leaf litter and dead insects.

Initially, the inverse relationship of high insect densities and low [Na] seems counter-intuitive. One might expect more mosquitoes and scirtids in treeholes with higher [Na], possibly due to preferential oviposition by females or lower survival in treeholes with low [Na]. However, the inverse relationship between density and [Na] could be explained by sodium absorption by a dense population of insects. Sodium is vital to these insects, and a high density might absorb a large amount of the available sodium, depressing [Na] to the low levels observed in the field. Freshwater insects are hyperosmotic to the surrounding medium, and this causes problems of ion and water balance (Sutcliffe 1962, Wharton 1985). To help maintain ion balance, mosquitoes and scirtids use anal papillae to take up sodium in freshwater (Wigglesworth 1938, Treherne 1954, Stobart 1960).

In aquatic insects, growth is also dependent on population density (Hard et al. 1989, Broadie and Bradshaw 1991, Leonard and Juliano 1995). Density dependence is partially a result of resource limitation, where increasing densities decrease the per capita availability of resources. This may be especially important to growth in temporary habitats, such as treeholes. Larvae inhabiting treeholes are restricted to the site of female egg oviposition. Since oviposition preferences may result in extremely high densities in some treeholes, this increases the potential for density-dependent growth effects. The adverse effects of density on mosquitoes are especially well documented (Hard et al. 1989, Broadie and Bradshaw 1991, Leonard and Juliano 1995, Barrera 1996b). Proposed mechanisms for decreased growth under high densities include resource limitation, interference by physical contact, waste accumulation, and chemical inhibition. Competition increases under conditions of limiting resources and high density, and decreased growth and increased mortality result (Hard et al. 1989).

To better understand the relationship between insect density and the effects of a potentially limiting resource, sodium, on growth and survival of treehole insects, we performed a laboratory experiment using simulated treehole microcosms. High and low densities of *A. triseriatus* and scirtid beetles were separately placed in high and low [Na] solutions. Considering both the need for sodium and the effects of density in insects, the following hypotheses were made: 1) both scirtids and mosquitoes will have the largest body masses and survival at

high [Na] and low densities, and the smallest masses and survival at low [Na] and high density treatments; 2) mosquitoes at low [Na] will have a longer development time, and; 3) the water [Na] will decrease by a greater proportion in microcosms with high insect densities than in those with low densities.

METHODS AND MATERIALS

Experimental Design

The experiment included five distinct insect treatments: no insects (NI), high (HM) and low (LM) mosquito densities, and high (HS) and low (LS) scirtid densities. Each insect density treatment had two sodium levels, high and low (Table 1), yielding a full factorial design. High (200 insects/L) and low (100 insects/L) densities for both mosquitoes and scirtids were chosen from natural insect density ranges in Pennsylvania treeholes, as were high and low [Na] (Paradise 1997). Low [Na] treatments had distilled water, while High [Na] treatments had 300 μ M Na water made from NaCl. There were four replicates for each treatment combination.

The microcosms were constructed from 1L plastic containers capped with lids. The center of each lid was removed and 0.2 mm mesh netting was attached to prevent insects from escaping. Fiberglass mesh was attached to the sides of the container with silicon caulk to provide the scirtid larvae with a surface on which to climb. Treehole sediment (2.5g) collected from nearby treeholes and 2.5g of red oak (*Quercus rubra*) leaf litter collected from the forest floor, both dried at 80°C for one week, were added to each microcosm to provide habitat. The appropriate solution was added to each microcosm for a total volume of 250 mL. One week later, 25 mL of natural treehole water was added to each microcosm to inoculate it with protozoans. Initial water samples were taken for determination of [Na], and the appropriate number of insects was added. Insects were collected from local treeholes, pooled and sorted by size; we used only first instar larvae. Because separation of scirtids by species is difficult at early development stages, they were not identified to species. On 5 April 1997, the microcosms were randomly placed in a controlled temperature room at 24°C with a 15:9 light:dark cycle, and were rotated between shelves at regular intervals during the course of the experiment. The experiment continued until 20 June 1997.

Data Collection and Statistics

Five mL water samples were taken from each microcosm prior to insect addition for initial [Na] analysis, and final samples were collected on the last day of the experiment (day 78 for mosquitoes and day 43 for scirtids). Samples were filtered through 0.45 mm cellulose nitrate filter paper, and [Na] was measured using atomic absorption spectrophotometry (Perkin-Elmer 2280). As expected, initial [Na] for both scirtids and mosquitoes did not show any density effects (mosquitoes, $F = 0.69$; $df = 2, 18$; $P = 0.50$, scirtids, $F = 0.72$; $df = 2, 18$; $P = 0.50$), but [Na]s were significantly different (mosquitoes, $F = 417$; $df = 1, 18$; $P < 0.001$, scirtids, $F = 661$; $df = 1, 18$; $P < 0.001$). Periodically, distilled or 300 μ M Na water was added to the appropriate microcosms to return the water volume to its initial level.

Table 1. Insect densities and the actual range (μM) of [Na] over the length of the experiment. Nominal [Na] in low and high Na treatments was 0, and 300 μM , respectively. For insect densities, the first number is the actual number of insects in each microcosm, while the number in parenthesis is the resulting density ($\#/L$). NI = no insects, LM = low mosquito density, HM = high mosquito density, LS = low scirtid density, and HS = high scirtid density.

[Na]		NI	LM	HM	LS	HS
Low	Range	36 - 110	26 - 122	21 - 113	36 - 95	32 - 109
	Density	0 (0/L)	25 (100/L)	50 (200/L)	25 (100/L)	50 (200/L)
High	Range	143 - 616	197 - 637	145 - 698	145 - 465	159 - 425
	Density	0 (0/L)	25 (100/L)	50 (200/L)	25 (100/L)	50 (200/L)

Adult mosquitoes were collected daily as they emerged within the microcosms. The insects were collected in individual vials, frozen, dried at 100°C for 48 hours, and weighed to the nearest 0.01 mg on a Mettler M5 microbalance. The experiment was terminated after 11 weeks when mosquitoes ceased to emerge. The microcosms were then disassembled and all remaining larvae and pupae were counted in order to determine mosquito survival. After six weeks, all scirtids were removed from their microcosms and counted to calculate survival. The scirtids were frozen, separated into individual vials, then dried and weighed as above.

For the mosquitoes, adult dry mass, length of time to emergence, and percentage survival and emergence were all used to measure the effects of density and [Na] on the insects. Analyses on individual dry mass and days to emergence (an indicator of development time) were performed separately on the two sexes. A two-way analysis of variance (ANOVA) was used for males. A one-way ANOVA was used for the females, since none emerged from high Na/high density microcosms, using the three [Na]/density combinations that produced females as the factor levels. Microcosm, or replicate, was nested within treatment combination to account for variation among mosquitoes emerging from the same microcosm (Neter et al. 1990). Percentage survival and percentage emergence were also analyzed with a two-way ANOVA. Masses were log-transformed and percentages were arc-sine transformed to approximate normality.

As a final measure of mosquito performance, we used a composite index of population growth rate, which incorporated the number of females, their individual dry mass and development time, and larval survivorship from each treatment (Livdahl 1982). This index converted female biomass into the expected per capita number of female offspring for each treatment. We then transformed the estimate of population growth rate, r' into λ' ($= e^{r'}$), to avoid an r' value of $-\infty$ when no females emerged from certain treatment combinations. We analyzed these data using a nonparametric Kruskal-Wallis test (Neter et al. 1990).

For scirtids, two-way ANOVA models were used for final larval dry mass and percentage survival, with microcosm nested within treatment as above. A square-root transformation was used on scirtid dry mass to obtain normality, and percentage survival was arc-sine transformed. Two-way ANOVAs were also used

for analyzing initial and final [Na]. Log transformations were used on initial and final [Na] for the *A. triseriatus* microcosms to approximate normality, while the [Na] from the scirtid microcosms were not transformed. Since the mosquito experiment ran five weeks longer than the scirtid experiment, there was greater variation in the [Na], making the transformation necessary.

We used an α level of 0.05 for all tests. When any test with more than two factor levels was deemed significant, we used a Tukey test to determine differences among means. All residuals were tested for normality and homogeneity of variance.

RESULTS

Aedes triseriatus

For male size, there was a significant interaction among [Na] and larval densities (Table 2). In the low density (LM) treatments, larger adults were associated with high sodium treatments (Fig. 1). In the high density (HM) treatments, there was no effect of sodium on size. No females emerged from any high Na/HM treatment replicates. Comparisons of the remaining three treatment combinations showed a significant difference among adult females from all three treatments (Table 2). The largest females emerged from the high Na/LM treatment while the smallest females emerged from the low Na/HM treatment (Fig. 1), following the trend seen in males. Females at low density were approximately 85% larger than the males from the same treatments. However, at high density, females were only about 45% larger than males.

Table 2. Results of analyses of variance (ANOVA) for all responses. An asterisk indicates significance at the 0.05 level, df for all tests are in parentheses, except for water [Na], which are 1, 18 for sodium and 2, 18 for density and the interaction. A. Results for female mosquitoes. One-way ANOVA was used to analyze the three treatment combinations for which females emerged. B. ANOVA results for remainder of mosquito responses, scirtid responses, and water [Na].

	Sodium/Density					
	F	P				
A. Female mosq.						
Dry mass (2, 7)	9.83	0.005*				
Time to emergence (2, 7)	0.78	0.47				
	Sodium		Density		Interaction	
B. Response:	F	P	F	P	F	P
Mosquitoes						
male dry mass (1, 11)	6.95	0.02*	186.9	<0.001*	8.19	<0.013*
male time to emerg (1, 11)	1.14	0.30	44.63	<0.001*	1.34	0.26
% survival (1, 12)	1.94	0.19	23.84	<0.001*	0.48	0.50
% emergence (1, 12)	2.78	0.12	66.30	<0.001*	1.10	0.32
Scirtid dry mass (1, 12)	1.07	0.32	53.58	<0.001*	4.85	0.045*
Scirtid survival (1, 12)	3.51	0.09	0.04	0.836	1.12	0.311
Final water [Na] (mosq.)	1.098	<0.001*	1.10	0.35	3.93	0.038*
Final water [Na] (scirtids)	1.598	<0.001*	4.03	0.036*	1.31	0.29

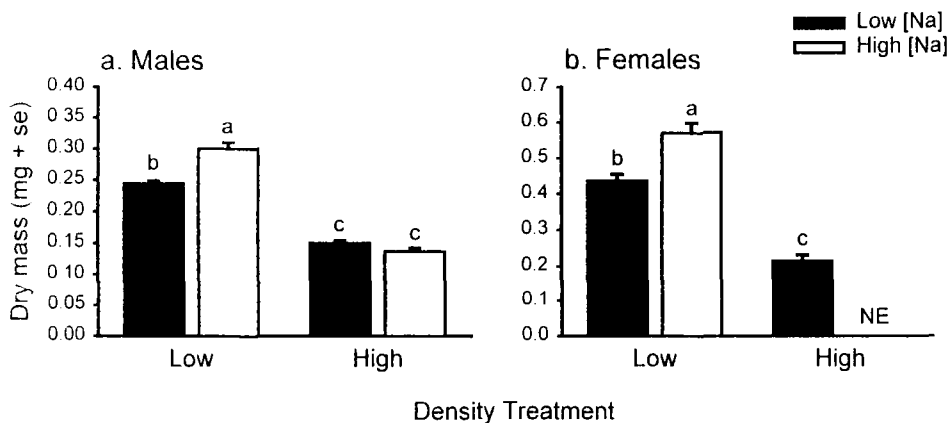


Figure 1. Mean dry mass (+ se) for (a) male and (b) female adult *A. triseriatus*. NE = no adults emerged. Bars with different letters are significantly different.

Male time to emergence, percentage survival, and percentage emergence were significantly affected only by density; time to emergence was longer, and survival and emergence were lower in the HM treatments than in the LM treatments for males (Fig. 2; Table 2). There was no effect of [Na]/density combinations on days to emergence for females. All mosquitoes that survived emerged as adults from LM treatments, while emergence was much lower than survival in HM treatments, indicating that not all survivors completed development (Fig. 2).

Since several microcosms did not have any females emerge, there were no transformations that created normality in the estimates of λ' . Nonparametric tests could not determine interactions; however, a Kruskal-Wallis test revealed a significant difference among the four treatment combinations ($H = 12.30$, $df = 3$, $P = 0.007$). In the low density treatments, λ' was > 1 ($\lambda' = 1.041 \pm 0.003$ s.e. for low [Na], and $\lambda' = 1.042 \pm 0.008$ s.e. for high [Na]), indicating the populations would increase, while in the high density treatments, λ' was < 1 ($\lambda' = 0.487 \pm 0.28$ s.e. for low [Na], and $\lambda' = 0$ for high [Na]), indicating the populations would decrease.

Scirtids

There was a significant interaction among [Na] and insect density for scirtid dry mass (Table 2). Scirtid beetles at low density (LS) were significantly larger in the low than in the high [Na] treatments (Fig. 3). Beetles at high density (HS) were significantly smaller than beetles at low density, and, similar to mosquitoes at high density, were not affected by [Na]. Neither [Na], scirtid density, nor their interaction had a significant effect on survival. Scirtid survival was between 70% and 90% for all treatments.

Sodium Concentration

Final [Na]s of the low and high [Na] treatments were significantly different for both scirtids and mosquitoes (Table 2). The low [Na] treatments averaged

around 50 μM Na throughout most of the experiment, but increased to up to 120 μM for some microcosms (Table 1). The high Na treatments averaged 268 μM for the scirtid microcosms and 348 μM for the *A. triseriatus* microcosms through week 7, by which time over 90% of the mosquitoes had emerged.

There was a density effect in the final week [Na] in the scirtid microcosms (Table 2). The final [Na] in the high density (HS) microcosms was significantly lower ($238.06 \mu\text{M} \pm 7.71$) than the [Na] in the no insect (NI) microcosms ($272.25 \mu\text{M} \pm 10.35$). There was also an interaction in the final week [Na] in the mosquito microcosms. Although Tukey's pairwise comparison test did not show any differences among Na treatments at either mosquito density, the trend was that microcosms with high densities of mosquitoes at lower initial [Na] had lower final concentrations of sodium than microcosms with no insects.

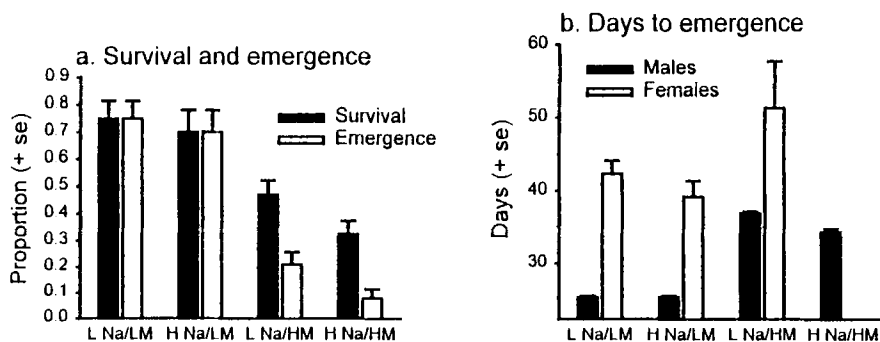


Figure 2. (a) Mean proportion of *A. triseriatus* that survived and emerged for each treatment. (b) Average of the median day of emergence from the four replicates of each treatment for *A. triseriatus*. L Na = low [Na], H Na = high [Na], LM = low mosquito density, HM = high mosquito density.

DISCUSSION

The [Na] and densities used here are well within the ranges observed in the field for central Pennsylvania treeholes (Paradise 1997). In fact, high [Na]/low density and low [Na]/high density treatment combinations reflect the inverse correlation between [Na] and insect densities in natural treeholes (Paradise and Dunson 1997b). This, and the fact that the high Na/low density estimate of population growth is positive, while that of low Na/high density is negative, indicates that in natural treeholes, populations in the former condition will probably grow faster, or at least females will have a higher reproductive potential, than populations in the latter condition. This supports our hypotheses concerning effects of [Na] and density on mosquitoes.

Under low density conditions, female mosquitoes showed an increase in dry mass at higher sodium concentrations, supporting previous results at an even lower density (70/L; Paradise and Dunson 1998b). However, unlike the previous study, male mosquitoes also showed an increase in dry mass with an increase in [Na], possibly because the present experiment had a lower low [Na] (0 μM initial [Na] compared to 100 μM in Paradise and Dunson 1998b). These results suggest that

females are more sensitive to changes in [Na] and that males are also affected under some conditions. Reductions in growth when sodium is low may be caused by increased energy required to obtain sodium, or general effects on growth caused by limited Na, and smaller adult size can affect fitness of those individuals (Livdahl 1982, Leonard and Juliano 1995). Considering the low inputs of sodium from precipitation and stemflow, and the high variability in treeholes (Carpenter 1982, Paradise and Dunson 1998a), conditions of low sodium are likely to occur and inhibit mosquito growth. However, low [Na]s are often associated with high densities of mosquito larvae (Peterson and Chapman 1969, Paradise and Dunson 1997b), and the [Na]s used here had no effect on growth at high mosquito densities, indicating that another factor is limiting growth at that point.

Contrary to our expectations, [Na] had the opposite effect on scirtid dry mass. At higher [Na], scirtids had a lower mean body mass than at lower [Na]. Although unexpected, these results are not completely surprising. While both scirtid and mosquito densities are inversely correlated with [Na], scirtids, unlike mosquitoes, colonize low sodium microcosms ($\sim 100 \mu\text{M}$) with higher density than high sodium microcosms ($\sim 300 \mu\text{M}$; Paradise and Dunson 1997b). The differing effects of [Na] on mosquitoes and scirtids may stem from differences in their feeding ecology, even though both insects may obtain sodium from anal papillae (Wigglesworth 1938, Treherne 1954). *A. triseriatus* larvae feed primarily on microorganisms and obtain some nutrients from the treehole water (Stobbert 1960, Merritt et al. 1992), while scirtid beetles feed mainly on leaf litter (Barrera 1996a, Paradise and Dunson 1997a). There is evidence that some microbial populations have higher growth under high [Na] conditions (Paradise and Dunson 1997b), and this would benefit mosquitoes. However, it may be a detriment to scirtids, since many microbes are also feeding on leaf litter, and microbial feeding could decrease quantity and quality of leaf litter. Differences in the natural history of the insects could also play a role. *A. triseriatus* generally overwinters as eggs and has a short larval life cycle; larvae are not tolerant of desiccation (Bradshaw and Holzapfel 1988). Scirtids have a much longer larval life cycle (Barrera 1996a) and can probably withstand a higher level of desiccation, as evidenced by their presence in treeholes with little or no standing water (Paradise 1997).

High larval densities had a greater impact on the insect populations than did [Na], having a significant negative effect on most response variables. The profound effect of density on insect growth is not surprising, as it has been well documented in various *Aedes* species (Barrera 1996a, Hard et al. 1989, Broadie and Bradshaw 1991, Leonard and Juliano 1995, Barrera 1996b). Decreased growth rate is a general response to stress in food-limited environments (Leonard and Juliano 1995). High [Na] here does not compensate for increased density, indicating that sodium is no longer a limiting factor. Other alternative explanations for the density effect on growth are interference by direct contact, chemical inhibition, and concentration of waste products (Broadie and Bradshaw 1991, Walker et al. 1991, Leonard and Juliano 1995). All have been shown in simulated treeholes and may be involved in the density response of mosquitoes seen here. Both scirtids and mosquitoes are sensitive to the level of leaf litter present, and high densities may limit their per capita food consumption (Fish and Carpenter 1982, Carpenter 1983, Leonard and Juliano 1995, Paradise and Kuhn 1999).

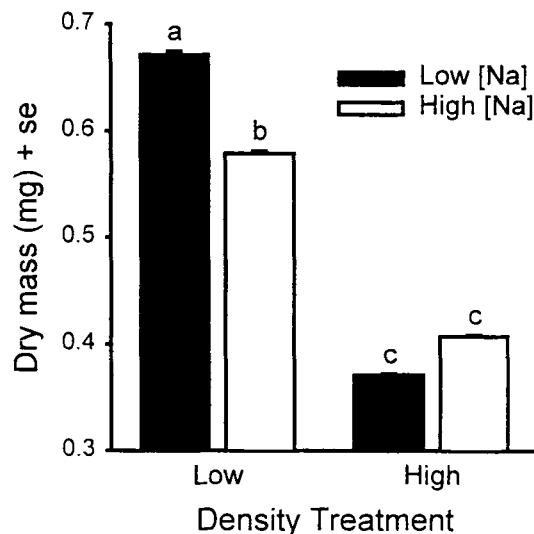


Figure 3. Mean dry mass for scirtids for each treatment combination. Bars with different letters are significantly different.

We observed an effect of insect density on water [Na], where microcosms with high densities had lower [Na] than microcosms without insects, supporting our hypothesis that the insects take up sodium from the treehole water. Indeed, the densities we used in our study were an order of magnitude lower than some densities seen in the field (Paradise 1997), which could result in an even greater depression of [Na]. This hypothesis needs to be tested further in microcosms with higher larval densities of both mosquitoes and scirtids in manipulated treeholes in the field. However, our data indicate that densities and [Na] interact, and that high densities of these animals could be reducing [Na] via uptake through anal papillae and ingestion of food. This phenomenon would further complicate the effects of Na, as it would become an even more limiting resource.

The relationship between sodium and density is also related to treehole volume. Large treeholes with high water volumes are correlated with high scirtid and mosquito densities and tend to have low [Na] (Paradise and Dunson 1997b). Larger treeholes have higher densities of larvae and greater species richness than smaller treeholes (Bradshaw and Holzapfel 1983, Sota 1996, Paradise 1997). Since water volume can vary drastically in a treehole over a season (Paradise 1997), large treeholes with more water are less likely to dry out, thereby giving the larvae a greater chance to survive (Bradshaw and Holzapfel 1988). However, the high densities in treeholes are a significant detriment to both mosquitoes and scirtids regardless of the differential effect of sodium. In treeholes that have low densities, low water volume, and high [Na] (the other end of the correlation), we would predict mosquitoes to do well and complete their larval life cycle prior to drying out of the treehole.

Since density and [Na] both have effects on mosquito and scirtid growth, and both factors are correlated with water volume in field conditions (Peterson and

Chapman 1969, Paradise and Dunson 1997b, Paradise 1998), it is of interest to determine how [Na] and water levels interact to affect insect colonization and population growth in treeholes. This may help clarify the relationships among treehole size, insect densities, and [Na]. More detailed experiments on the role of, and the effects of, [Na] on microbes and the treehole food web are also needed. However, the effects of Na on growth of treehole insects is both species-specific and density-dependent.

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