RESEARCH ARTICLE

Salt intake in Amazonian ants: too much of a good thing?

L. M. Arcila Hernández · E. V. Todd · G. A. Miller · M. E. Frederickson

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Abstract Although herbivory is widespread among insects, plant tissues rarely provide the optimal balance of nutrients for insect growth and reproduction. As a result, many herbivorous insects forage elsewhere for particular amino acids and minerals. Recent studies have shown that both herbivory and recruitment to sodium are commonplace among tropical rainforest ants, but little is known about how ants regulate their sodium intake at the individual and colony levels. In social insects, foragers may respond not only to their own nutritional deficiencies but also to those of their nestmates, who may have different nutritional requirements depending on their developmental stage, sex, or caste. Here, we investigate how salt stress among rainforest ants affects their preferences for salt and subsequent survival. We found that ants recruited more to salt than to any other bait type tested, confirming the strong preference for salt of ants in this region. Initially, we observed similarly high recruitment to salt among workers of the arboreal, herbivorous/omnivorous ant species Camponotus mirabilis. However, when provided with unrestricted access to high concentrations of salt, C. mirabilis workers suffered significantly higher mortality relative to controls, suggesting that C. mirabilis workers forage for sodium to the point of toxicity. Nonetheless, surviving workers showed reduced preference for salt at the end

L. M. Arcila Hernández · E. V. Todd · M. E. Frederickson (⊠) Department of Ecology and Evolutionary Biology, University of Toronto, 25 Harbord Street, Toronto, ON M5S 3G5, Canada e-mail: m.frederickson@utoronto.ca

G. A. Miller

Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA of the experiment, so some but not all individuals were able to regulate their salt intake beneath lethal dosages. We discuss how salt intake regulation may depend on colony members other than workers.

Keywords Bamboo · *Camponotus mirabilis* · Herbivory · Insect nutrition · Sodium limitation · Tropical rainforests

Introduction

Herbivorous animals often need more sodium than they can get from the plants they eat (e.g., Arms et al., 1974; Laurian et al., 2008; Kaspari et al., 2008; Dudley et al., 2012). Animals require sodium for many physiological functions, including osmotic regulation and neuromuscular activity, but the sodium content of terrestrial plant tissues is typically orders of magnitude less than that of animal tissues (Stamp and Harmon, 1991; Kaspari et al., 2009, 2010). As a result, many vertebrate and invertebrate herbivores supplement their diets with additional sodium from non-plant sources. In boreal forests, for example, moose (Alces alces) seek out salt pools formed by the accumulation of de-icing salts along roadsides (e.g., Laurian et al., 2008). Similarly, in western Amazonian rainforests, where the present study was conducted, many frugivorous or folivorous mammals and birds visit natural clay licks, apparently to obtain sodium (Emmons and Stark, 1979; Brightsmith and Aramburú Muñoz-Najar, 2004; Bravo et al., 2008; Tobler et al., 2009; Dudley et al., 2012). In arthropods, sodium deficiency is thought to explain why butterflies and other herbivorous taxa 'puddle' on mud, animal feces, and carrion, although nitrogen limitation may also play a role (Arms et al., 1974; Boggs and Jackson, 1991; Smedley and Eisner, 1995, 1996; Molleman et al., 2005; Molleman, 2010).

Recent studies have shown that herbivory is widespread among tropical arboreal ants (Blüthgen et al., 2003; Davidson et al., 2003), raising the possibility that they too may need to forage for sodium. In two seminal papers, Kaspari et al. (2008, 2009) investigated sodium limitation in New World ant assemblages. They found that ants commonly recruit to sodium baits (especially ants in genera or subfamilies with many herbivorous species) and that ant attraction to sodium increases with distance inland (Kaspari et al., 2008, 2009). Specifically, ants in the western Amazon Basin were much more attracted to sodium baits than ants in Florida, Panama, or Costa Rica, where more ocean salt reaches terrestrial ecosystems. Furthermore, when NaCl was added to plots in a rainforest near Iquitos, Peru, the abundance of ants increased sharply (Kaspari et al., 2009). To our knowledge, only two other studies have investigated the sodium requirements of ants. O'Donnell et al. (2010) found that leafcutter ants (Atta cephalotes) in Costa Rica cut and removed more salt-soaked than water-soaked paper baits, although they most strongly preferred the sugarsoaked option, and Kaspari et al. (2010) found that ant recruitment to NaCl baits increased with distance from a salted road at Harvard Forest. Thus, while it is becoming increasingly clear that ants will recruit to sodium in many ecosystems, the consequences of sodium intake for ant nutrition and fitness have received little attention.

The protein and carbohydrate requirements of ants are comparatively well understood (e.g., Davidson, 1997; Dussutour and Simpson, 2008, 2009; Cook et al., 2010) and studies in this area offer a number of insights into how ants might regulate their intake of sodium. Collecting nutrients in the right amounts and ratios is thought to pose a greater challenge to social than solitary insects, because foragers have to satisfy not only their own needs but also the needs of their nestmates (i.e., other workers, larvae, pupae, sexuals, and the queen or queens), who may have different nutritional requirements. For example, ant larvae typically require more protein than adult workers; a recent study of green-headed ants (Rhytidoponera sp.) found that colonies with larvae maintain a more protein-biased protein-to-carbohydrate (P:C) ratio than colonies without larvae (Dussutour and Simpson, 2009). Interestingly, the same study also found that foraging workers regulated their P:C intake more precisely when their colonies had larvae, suggesting that feedback between larvae and workers inside the nest affects foraging decisions made outside the nest. Similar feedbacks may determine whether ant workers collect sodium, and in what ratios to other nutrients, but at present we do not know how sodium requirements vary with respect to developmental stage, sex, or caste in ants.

Here, we investigated several aspects of salt stress in an ant assemblage in the western Amazon Basin. We began by comparing the recruitment of ants to salt and other baits in the leaf litter and the canopy at our study site. Our approach was similar to previous studies (i.e., Kaspari et al., 2008, 2009), except that we offered both simple baits (salt and sugar) and more chemically and nutritionally complex baits that represented resources that are commonly (bird droppings, extrafloral nectar, protein, and urine) or ostensibly (pollen) harvested by ants in Neotropical rainforests. Next, we investigated whether altering the amount of salt available to one herbivorous/omnivorous, arboreal ant species, the bamboo-specialist Camponotus mirabilis, affected the propensity of workers to forage for salt, as well as worker performance. We maintained experimental micro-colonies of C. mirabilis with or without brood (i.e., larvae and pupae) on high or low-salt treatments for 2 weeks and we monitored how the salt treatment and the presence of immature stages affected the preference of C. mirabilis workers for salt and the survivorship of C. mirabilis workers, larvae, and pupae.

Methods

Study site

This study was conducted at the Centro de Investigación y Capacitación Rio Los Amigos, hereafter "CICRA" ($12^{\circ}34'S$, $70^{\circ}05'W$; elevation ~ 270 m), in the Department of Madre de Dios, Peru. Surrounding the research center is the Los Amigos conservation concession, which comprises 146,000 ha of primary tropical rainforest on a mixture of upland terraces and floodplains. Annual rainfall at Los Amigos is between 2,700 and 3,000 mm, with more than 80 % of the precipitation falling during the October–April wet season (Pitman, 2008). Mean monthly temperatures range from 21 to 26 °C (Pitman, 2008).

Bait preferences of leaf litter and canopy ant assemblages

In July–August 2010, we investigated the preferences of ants in the leaf litter and in the canopy for a variety of baits. We prepared eight types of baits: salt (table salt, i.e., iodized NaCl), sugar (table sugar, i.e., sucrose), protein (whey protein powder, Interactive Nutrition, Inc.), urine (collected fresh from a mammal), extrafloral nectar (EFN), bee pollen (Selva Natural), bird droppings (collected fresh from a chicken), and water (as a control). We used bird feces and mammal urine because canopy ants often recruit to these substances (e.g., Powell, 2008; Yanoviak et al., 2008; Kaspari et al., 2009) and they may be among the most common naturally available sources of salt in this ecosystem. Similarly, extrafloral nectar is a widely available sugar source that attracts many rainforest ants (Blüthgen et al., 2000); reports of possible palynivory in tropical arboreal ants prompted us to also include pollen as a bait type (Baroni Urbani and de Andrade, 1997; Davidson et al., 2003). For all bait types except EFN, we added 2 g of the ingredient to 30 mL of distilled water. Because collecting EFN proved challenging, we instead cut 12 extrafloral nectaries from Inga plants and added the nectaries in their entireties to 30 mL of distilled water. When available, we used information from product labels or published sources (i.e., Bankir et al., 2007) to estimate the sodium content of our bait types; sodium concentrations were approximately 26 mg/mL in the salt baits, 0 mg/mL in the sugar baits, 0.1 mg/mL in the protein baits, 0.14-0.22 mg/mL in the urine baits, and 0 mg/mL in the water baits. We do not know how much sodium was in the bird droppings, extrafloral nectar, or pollen we used, but they were probably much lower (EFN, pollen) or similar to (bird droppings) the sodium concentration in the urine baits. We saturated pieces of cotton with one of the eight bait solutions and transferred them to test tubes. We then taped eight test tubes, each with a different bait type, together in random order to form bait sets.

We sampled ants on 12 trees growing in floodplain and upland forests at CICRA. Trees were separated from each other by at least 10 m and were large enough to support climbing equipment. For each tree, we placed three bait sets on the leaf litter at the base of the tree and three bait sets in the canopy ($\sim 6-20$ m from the ground). After an hour, we trapped all the ants present in the tubes by sealing the tubes with additional cotton and tape. We sampled the ants on each tree once in the morning and once again in the afternoon, using different bait sets each time. After collection, we froze the test tubes for at least 12 h to kill the ants and then counted the total number of ants in each tube.

Preference for sodium in an herbivorous/omnivorous ant, *C. mirabilis*

Bamboo-dominated forest grows in patches throughout the Los Amigos concession and elsewhere in the western Amazon Basin (Nelson, 1994; Griscom and Ashton, 2003). In these patches, several Camponotus (Formicidae: Formicinae) species make their nests inside the hollow culms of bamboo (Guadua spp.; Davidson et al., 2006a, b). We focused on one of these bamboo specialists, C. mirabilis, which has polydomous colonies that occupy clusters of adjacent stems of live bamboo (Davidson et al., 2006b). The diet of C. mirabilis consists mostly of honeydew from coccids (Coccidae), "supplemented by occasional prey" (Davidson et al., 2006b); herbivory/omnivory in C. mira*bilis* is further supported by a nitrogen isotope ratio (δ^{15} N) intermediate between that of chewing herbivores and that of predators (Davidson et al., 2003). C. mirabilis is thus similar to many other tropical arboreal ants, which feed mostly on honeydew or extrafloral nectar but take prey opportunistically (Davidson et al., 2003). All the bamboospecialist *Camponotus* tend coccids that have recently been described as the new species *Cryptostigma guadua* (Kondo and Gullan, 2004), and we observed numerous coccids in the ant-occupied culms of the bamboo we collected.

Camponotus mirabilis collection and set-up of micro-colonies

In June–July 2011, we collected large fragments of five C. mirabilis colonies that we found along the trail system at Los Amigos. For each colony fragment, we cut open several live bamboo culms occupied by C. mirabilis and collected the workers and brood. We established four micro-colonies from each of the five C. mirabilis colonies. Each microcolony comprised either (1) ten workers or (2) ten workers, ten pupae, and ten larvae. In all cases, the ten workers were made up of seven minor and three major workers; in other respects, we chose workers haphazardly from the colony fragments we collected. The larvae and pupae in the microcolonies with immature stages ranged in size, but we deliberately avoided selecting larvae or pupae fated to become reproductives. We housed each micro-colony in a small, lidded plastic container with the center of the lid replaced with fine mesh for airflow. We provided each micro-colony with a tent made from transparent red plastic, which blocked light; workers piled brood and congregated beneath the tent. Micro-colonies were kept at CICRA away from direct sunlight and under ambient temperature and humidity conditions for the duration of the experiment.

Camponotus mirabilis preference tests

Before starting the experiment, we tested the preferences of C. mirabilis workers for distilled water, a 10 % solution of table sugar, and a 10 % solution of table salt. Note that a 10 % solution of table salt has a sodium concentration of about 39 mg/mL. After setting up the micro-colonies, we starved them overnight and then chose three workers at random from each micro-colony. For each worker, we put one drop of each solution in an equilateral triangle in a Petri dish, added the ant, and then allowed it to settle for 60 s. Every 5 s for the next 2 min, we recorded whether the ant was touching the water droplet, the salt solution droplet, the sugar solution droplet, or none of these. We did not distinguish between instances in which an ant remained at a droplet from one observation to the next and those in which an ant moved away and returned to a droplet between observations. At the end of each test, we converted the number of times the ant was touching each droplet to a preference score; for each test solution, the minimum score was 0 (the ant was not touching the test solution at any 5 s interval) and the maximum score was 100 (the ant was

touching the test solution at every 5 s interval). We returned workers to their micro-colonies after testing their initial preferences for the test solutions. We re-used Petri dishes for subsequent trials, washing them thoroughly with soap and water and drying them completely between uses. At the end of the experiment, we also tested the preferences of between one and three of the surviving workers (if there were any) from the micro-colonies, using the same protocol.

Experimental addition of salt

All micro-colonies were fed an artificial diet that we modified from Straka and Feldhaar (2007) by replacing the amino acids in their holidic diet with whey protein powder. Briefly, each 100 mL of diet contained 1 g agar, 20 g sucrose, 2.595 g whey protein powder (Interactive Nutrition, Inc.), and 0.270 g Vanderzant vitamin mixture for insects (Sigma). The whey protein powder we used to make the artificial diet contained a small amount of sodium (1.5 mg/g) as well as potassium (5 mg/g) and calcium (5 mg/g); we did not add other salts or minerals. We transported Petri dishes filled with artificial diet to CICRA in a cooler and stored them at approximately -4 °C. Every 3 days, we provided each micro-colony with one level scoop of diet (~ 0.2 mL), measured using a micro-spoon, on a square of aluminum foil; the ants did not consume all of the food we gave them and remaining food was removed at the next feeding.

We provisioned the colonies with water ad libitum. We placed two cotton-filled bulbs made from plastic disposable pipettes in each micro-colony. The low-salt treatment group had both bulbs filled with distilled water, whereas the high-salt treatment group had one bulb with distilled water and the other bulb filled with a 10 % solution of table salt (iodized NaCl). We repeatedly observed workers visiting both types of bulbs during the experiment, but we did not quantify intake rates. The cotton was replaced if it became so wet that the ants could not enter and drink easily, or if it was filled with debris or mold.

We maintained the treatments for 2 weeks and examined each micro-colony every 3 days for survivors. We counted and recorded the number of dead workers, and also the number of live workers, checking against previous counts to make sure that none had escaped. We also recorded the number of dead larvae and pupae. We included missing larvae and pupae in the totals for dead larvae and pupae, since they could not escape and we presumed that their adult sisters had eaten them. We removed all the dead ants and any debris during each examination.

Statistical analysis

We square-root transformed all count data before statistical analysis to improve normality and then used mixed model ANOVAs to test for treatment effects. For each baited tree, we summed the number of ants in the three morning and three afternoon tubes of the same bait type for leaf litter and canopy tubes separately. We then analyzed the number of ants at each bait type, with tree (1-12) as a random effect, forest stratum (leaf litter, canopy) and bait type (bird droppings, extrafloral nectar, pollen, protein, salt, sugar, urine, water) as fixed effects, and forest stratum by bait type as an interaction effect.

For data from the preference tests, colony (1-5) was a random effect, salt (high/low), brood (present/absent), and test solution (water, sugar, salt) were fixed effects, and salt by test solution and brood by test solution were interaction effects. Similarly, we compared the number of workers that survived to the end of the experiment among treatments with colony (1-5) as a random effect, salt (high/low) and brood (present/absent) as fixed effects, and salt × brood as an interaction effect. All of the larvae and pupae had died by the end of the experiment (apparently cannibalized by workers), so we compared the mortality rates of larvae and pupae in the high- and low-salt treatments using survival analysis. For significant effects in the ANOVA models, different levels were compared with Tukey's HSD tests. Statistical analyses were carried out in JMP[®] 9.0.0.

Results

Bait preferences of leaf litter and canopy ant assemblages

We collected a total of 4,003 ants at the baits, of which almost half (1,859 ants, or 46 %) were collected at the salt baits. Significantly more ants recruited to the salt baits than to any other type of bait; in fact, recruitment to other bait types did not differ significantly from recruitment to the water controls (Table 1; Fig. 1). Neither forest stratum nor the interaction between stratum and bait type was significant (Table 1), although the *P* values for both were only marginally non-significant (0.10 and 0.08, respectively). Across all bait types, large numbers of *Crematogaster* (2,380 workers, both strata), *Azteca* (976 workers, in the canopy only), *Camponotus* (366 workers, both strata), and *Pheidole*

Table 1 Mixed model ANOVA results for number of ants at baits

Effect	df	SS	F	Р
Tree	11	425.86	6.13	<0.001
Bait type	7	393.00	8.89	<0.001
Stratum	1	17.12	2.71	0.10
Bait type × stratum	7	80.95	1.83	0.08

Results in italics are significant at P < 0.05



Fig. 1 *Box plots* showing numbers of ants at baits in the **a** leaf litter and **b** canopy. *Lines* medians, *boxes* 75th and 25th percentiles, *whiskers* 90th and 10th percentiles, and *dots* outliers. Numbers of ants at bait types marked with *different letters* are significantly different at $\alpha = 0.05$ according to Tukey's HSD tests. The results of Tukey's HSD tests are shown in **b** only but apply to ants in both strata

(116 workers, in the leaf litter only) recruited to our baits; these four genera accounted for over 95 % of bait visitors.

Camponotus mirabilis preference tests

Camponotus mirabilis workers initially preferred the salt solution to the sugar solution and visited both more than they visited the distilled water droplets (Table 2; Fig. 2a). After 2 weeks on the artificial diet, however, the surviving ants had switched their preference to the sugar solution, and visits to the salt solution droplets were statistically indistinguishable from visits to the water droplets (Table 2; Fig. 2b). In the final preference tests, there was also a statistically significant interaction between salt treatment and test solution preference (Table 2). Workers in the high-salt treatment maintained a stronger preference for salt and a weaker preference for sugar than workers in the low-salt

Table 2 Mixed model ANOVA results for preference tests

Effect	df	SS	F	Р
Initial preference				
Colony	4	2.727	0.572	0.683
Salt	1	0.890	0.747	0.389
Brood	1	1.525	1.279	0.260
Test solution	2	66.358	27.816	<0.001
Salt \times test solution	2	0.329	0.138	0.871
Brood \times test solution	2	1.048	0.439	0.645
Final preference				
Colony	4	2.430	1.060	0.382
Salt	1	0.132	0.230	0.633
Brood	1	1.727	3.014	0.086
Test solution	2	15.014	13.099	<0.001
Salt \times test solution	2	4.527	3.950	0.023
Brood \times test solution	2	1.514	1.321	0.273

Results in italics are significant at P < 0.05

treatment, although both groups still chose sugar over salt (Fig. 2b). However, this result should be interpreted with caution because we tested the final preferences of only six workers in the high-salt treatment, as almost all workers in this treatment had died by the end of the experiment (Fig. 3).

Ant survivorship

More *C. mirabilis* workers survived in micro-colonies in the low-salt treatment and in micro-colonies with brood (Table 3; Fig. 3). Pupae died faster than larvae, but there were no differences in the mortality rates of larvae or pupae between the salt treatments (mean \pm SE days to death for larvae on high salt = 7.22 ± 0.26 , on low salt = 7.28 ± 0.29 , log-rank test: $\chi_1^2 = 0.055$, P = 0.814; mean \pm SE days to death for pupae on high salt = 5.86 ± 0.28 , on low salt = 5.58 ± 0.28 , log-rank test: $\chi_1^2 = 0.454$, P = 0.500).

Discussion

Ants in the Peruvian Amazon like salt. When offered a wide range of baits, ants preferred salt to all other choices and only salt baits attracted significantly more ants than water controls (Fig. 1). We even observed workers trying to remove salt-soaked cotton from test tubes. This preference likely reflects the much higher sodium content of the salt baits relative to other bait types (i.e., 26 mg/mL of sodium in the salt baits, compared to 0.14–0.22 mg/mL in the next most sodium-rich bait type, urine). The large number of ants that recruited to the salt baits suggests that salt is a key limiting nutrient for ants in this region. Nonetheless, following 2 weeks in high-salt conditions, *C. mirabilis*



Fig. 2 *Box plots* showing *C. mirabilis* worker preferences for droplets of water and 10 % solutions of sugar and salt **a** before the experiment was initiated (N = 60 workers) and **b** after the micro-colonies had been maintained in low (N = 26 workers) and high (N = 6 workers) salt treatments for 2 weeks. *Lines* medians, *boxes* 75th and 25th percentiles, *whiskers* 90th and 10th percentiles, and *dots* outliers. Worker preferences for test solutions marked with *different letters* are significantly different at $\alpha = 0.05$ according to Tukey's HSD tests

workers reduced the time they spent in contact with salt solutions (Table 2; Fig. 2), suggesting satiety. However, workers experienced higher death rates in high compared to low-salt conditions (Table 3; Fig. 3), so satiety did not arise fast enough to prevent mortality in all workers.

Kaspari et al. (2008) found greater salt limitation among ants in 'green' food webs (i.e., in abundant vegetation) than among ants in 'brown' food webs (i.e., in leaf litter). However, we did not find a statistically significant difference in salt preference between ants in the canopy and the leaf litter, although the trend was in the predicted direction and the P value for the interaction between stratum and bait type was only marginally non-significant (Table 1). A larger sample size might have revealed a difference in bait preferences between strata, or perhaps ants in this region are strongly salt stressed in both green and brown food webs.

In our micro-colony experiment, we found that saltsatiated *C. mirabilis* workers reduced their foraging for salt,



Fig. 3 Numbers of surviving *C. mirabilis* workers at the end of the experiment. Each *symbol* is a micro-colony; *open symbols* presence of brood, *filled symbols* absence of brood, *circles* high salt, and *triangles* low salt. The symbols for micro-colonies having the same number of surviving workers are offset slightly to make them visible. See Table 3 for treatment effects

Table 3 Mixed model ANOVA results for worker survival

Effect	df	SS	F	Р
Colony	4	0.996	0.566	0.692
Salt	1	14.089	32.027	<0.001
Brood	1	2.727	6.200	0.028
Salt \times brood	1	0.104	0.237	0.636

Results in italics are significant at P < 0.05

but not before many workers in the high-salt treatment had died. Shortly after we collected them, *C. mirabilis* workers preferred salt to both sugar and water (Fig. 2a), but after 2 weeks on artificial diet, they switched their preferences in favor of sugar and they visited droplets of salt solution and water equally (Fig. 2b). We observed this change in preference among ants in both the low-salt and the high-salt treatments, suggesting that even the ants in the low-salt treatment were receiving enough sodium to meet their needs. Ants in the low-salt treatment had access to two potential sources of sodium, through their artificial diet or through rampant cannibalism of their immature nestmates. Cannibalism is known to efficiently address nutritional needs, particularly with regard to salt and protein (e.g., Simpson et al., 2006).

Although the initial preference of *C. mirabilis* workers for salt suggests that they or their nestmates suffer from salt deficiency under natural conditions, workers with unrestricted access to salt solution suffered higher mortality (Fig. 3). Thus, it seems likely that either sodium itself, its chloride anions or the iodine present in small quantities in table salt is directly toxic to C. mirabilis workers at certain doses. The question then becomes: why cannot (or do not) these ants regulate their salt consumption? Ants might have an innate preference for salt if it usually indicates high protein content in a food item (although only salt and not protein itself attracted large numbers of ants in our bait study). Perhaps C. mirabilis never encounters salt in the form or concentrations we used and thus has not evolved to regulate its intake within non-toxic limits. Iodine occurs naturally in sea salt (which is predominantly NaCl), albeit at lower concentrations than in iodized table salt (Dasgupta et al., 2008); however, the Peruvian Amazon is not near oceanic salt sources. To determine whether sodium itself can be toxic to ants, future studies could monitor the survivorship of ants offered a range of sodium concentrations in a variety of anion backgrounds (e.g., NaCl, Na₃PO₄, Na₂SO₄, etc.). Note that Kaspari et al. (2009) demonstrated that ant recruitment to NaCl is driven by Na⁺ and not by Cl⁻ by showing that ants prefer NaCl, NaNO₃, Na₃PO₄, and Na₂SO₄ to KCl, MgCl₂, and CaCl₂, but this study did not investigate the survivorship of ants that ingested these compounds.

Alternatively, the absence of a queen, other reproductive ants, or simply larger numbers of workers in our experimental micro-colonies could have impinged on the ability of workers to regulate salt intake appropriately. When we collected the *C. mirabilis* colony fragments, they contained numerous alate ants, both males and females, so perhaps the workers' initial preferences for salt reflected the reproductive status of the colonies we used. Workers in these colonies may have collected large amounts of salt 'expecting' to provide it to their reproductive sisters and brothers or other ants back at the nest, only to be cut off from these nestmates by our experimental intervention.

In some solitary insects, sodium is thought to be particularly important to reproductive stages. Among Lepidoptera, adult butterflies and moths, but not their caterpillars, forage for sodium, and males often 'puddle' more than females (Arms et al., 1974; Boggs and Jackson, 1991; Smedley and Eisner, 1995, 1996; Molleman, 2010). One hypothesis to explain this sex difference is that the greater flight activity of male lepidopterans increases their sodium demands (Arms et al., 1974; Molleman et al., 2005). Molleman (2010) used the fact that ants forage for sodium as one piece of evidence to discount the notion that insects need a lot of sodium to support flight, saying "that ants used sodium baits even though they do not fly (Kaspari et al., 2008) also suggests that the neuromuscular activity hypothesis may not be the explanation [for puddling]." But most reproductive ants do fly, of course, and workers may forage for sodium not for themselves, but to supply their reproductive nestmates with sodium for their upcoming mating flights. Interestingly, this hypothesis is consistent with the finding that adding NaCl "double[d] ant densities in a Peruvian forest (Kaspari et al., 2009), and based on a threefold increase in queens, likely nest densities..." (Kaspari et al., 2010, p. 546).

In contrast to recent research on P:C regulation in ants (Dussutour and Simpson, 2009), we did not find that the presence of brood improved workers' ability to regulate sodium intake. In the final preference tests, there was no significant interaction effect between brood presence and test solution preference, suggesting that the presence of larvae and pupae in the micro-colonies did not change the preferences of C. mirabilis workers for salt, sugar, and water at the end of the experiment. There was also no significant interaction of brood presence and salt treatment on worker survival. However, workers cannibalized most of the larvae and pupae in the micro-colonies in the first week of the experiment, so there may have been transient effects of brood presence that had attenuated by the end of the experiment. Brood presence did reduce worker mortality, likely because workers benefitted from cannibalizing larvae and pupae. This result suggests that there were macronutrients or minerals available in larvae and pupae that were not supplied in the right amounts or ratios in the artificial diet to maximize worker survival. The increased survivorship of workers in micro-colonies with brood was independent of salt treatment (Table 3), so sodium was probably not the limiting nutrient responsible for this pattern. Instead, it is more likely that workers cannibalized brood because the carbohydrate-rich artificial diet that we fed the micro-colonies lacked sufficient protein or specific amino acids. Alternatively, workers may have cannibalized brood in response to the absence of queen-laid eggs in our micro-colonies (e.g., Endler et al., 2004).

Overall, this study adds to the small but growing literature on the use of salt by ants. We encourage further research into how sodium affects the performance/fitness of ants at the individual and colony levels and how sodium requirements vary across developmental stages, sexes, and castes in ants.

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