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SHORT COMMUNICATION

Assessing the effects of sodium on fire ant foraging in the field and colony growth in the laboratory

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Abstract. 1. Sodium is an essential dietary element and preferential foraging for high concentrations of sodium by inland herbivorous and omnivorous ants suggests it may be limiting. If so, increased sodium availability through altered deposition and anthropogenic sources may lead to increased colony and population growth and cascading ecological impacts.

2. For red imported fire ants, *Solenopsis invicta* Buren, the present study tests: (i) whether colonies from coastal and inland sites differ in their responses to NaCl baits; and (ii) whether supplemental NaCl increases growth of fire ant colonies in the laboratory.

3. Fire ants in inland sites with low sodium deposition responded roughly an order of magnitude more strongly to high concentrations of NaCl baits than did fire ants in coastal sites with high sodium deposition.

4. Laboratory colonies of fire ants, however, showed no signs of sodium limitation or benefits of increased sodium. The link between behavioural responses to baits in the field and effects on colony growth deserves further investigation to assess the ecological impacts of altered sodium availability.

Key words. Ants, salt limitation, *Solenopsis invicta.*

Introduction

Sodium is vital for the physiological functioning of all animals (Frausto da Silva & Williams, 2001) but is often rare in the environment (Kaspari *et al.*, 2008, 2009). Because sodium deposition varies geographically and is largely determined by distance from the coast, coastal ecosystems typically have higher sodium deposition than do inland ecosystems (National Atmospheric Deposition Program, 2011). This geographic variation in sodium deposition influences the sodium-seeking behaviour of animals. For instance, preferential foraging for sodium by ants tracks sodium availability in the environment; thus, coastal ants respond less strongly to sodium baits than do inland ants (Kaspari *et al.*, 2008). The implications of changes in sodium deposition by altered patterns of precipitation due to climate change have been suggested to have profound consequences on the carbon cycle by influencing populations of ants and other insects that can directly or indirectly affect leaf litter decomposition in inland carbon pools such as the Amazon (Kaspari *et al.*, 2008, 2009; Dudley *et al.*, 2012). However, there is a gap between behavioural responses to sodium baits and the assumed benefits to ants of increased sodium deposition. While preferential foraging for sodium by ants is extensively documented (Vail *et al.*, 1999; Kaspari *et al.*, 2008, 2010; O'Donnell *et al.*, 2010; Arcila Hernández *et al.*, 2012; Chavarria Pizarro *et al.*, 2012), the effect of sodium supplementation on ant colony growth is untested.

In this study, we examine the gap between the behavioural responses of ants to sodium baits in field studies and presumed benefits of sodium for colony growth by using red imported

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fire ants, *Solenopsis invicta* Buren (hereafter 'fire ants'), as a model organism. Fire ants are an ideal species for this work because their distribution spans regions of high and low sodium deposition (National Atmospheric Deposition Program, 2011) and laboratory colonies can be readily reared for experimentation (Porter, 1989). Fire ants are an invasive species, widely established in the southeastern United States and several regions throughout the world (Tschinkel, 2006). They are a numerically dominant species in disturbed areas in their introduced range. If sodium does indeed affect ant abundances, then there is potential for increased ecological and economic impacts of fire ants with sodium inputs (impacts of fire ants are reviewed in Wojcik *et al.*, 2001; Tschinkel, 2006). An added level of interest is that fire ants are trophic generalists with flexible trophic positions ranging from omnivorous to carnivorous (Tillberg *et al.*, 2006, 2007; Resasco *et al.*, 2012). Kaspari *et al.* (2008) found that carnivorous ants, which presumably receive sufficient sodium through their prey, have a weaker response to salt baits than do herbivorous and omnivorous ants; therefore it is uncertain whether fire ants can meet their sodium demands solely from animal prey.

To examine the link between positive behavioural responses of ants to sodium baits in field studies and the hypothesized colony growth benefits of sodium-seeking behavior, we: (i) conducted behavioural bait trials to determine whether fire ants show stronger preferences for high concentrations of NaCl in inland sites than coastal sites; and (ii) reared colonies of fire ants in the laboratory to test whether supplemental NaCl at different concentrations increased colony growth, which would ultimately be related to increased alate production, an important fitness component (Tschinkel, 1993). Based on previous work (Kaspari *et al.*, 2008), we predicted that fire ants in inland sites would have stronger preferences for NaCl than fire ants in coastal sites. Because ants have shown affinity to sodium in other studies (Vail *et al.*, 1999; Kaspari *et al.*, 2008, 2010; O'Donnell *et al.*, 2010; Arcila Hernández *et al.*, 2012; Chavarria Pizarro *et al.*, 2012) we also predicted that supplemental sodium would result in increased colony growth.

Materials and methods

Sodium bait foraging experiment

We conducted NaCl bait trials in August and September 2012 in two regions of the southeastern United States with contrasting sodium deposition rate (National Atmospheric Deposition Program, 2011). The inland, low-sodium-deposition region was the Savannah River Site (33.3°N, 81.6°W), a National Environmental Research Park, South Carolina (henceforth SC), ~150 km from the coast; it receives ~1.5 kg ha^{-1} yr^{-1} Na^+. The coastal, high deposition region was St Johns Co. (29.8°N, 81.3°W) in northeast Florida (henceforth FL), ~0–25 km from the coast; it receives ≥ ~5 kg ha^{-1} yr^{-1} Na^+. At six replicate sites (> 1 km apart) within each region, we conducted preference trials modified from the protocol of Kaspari *et al.* (2008). However, two sites in FL were not used in the analysis because of lack of fire ants at the baits (SC: $n = 6$ sites; FL $n = 4$ sites).

In each region, we selected sites by searching along roads for fire ant nests. In SC these were in open pine forests; in FL they were adjacent to forests or old fields. At each site we laid out a 100-m transect and placed 1.5-ml plastic, uncapped centrifuge tubes flat on the ground at 1-m intervals along the transect. All tubes were half-filled with cotton wads saturated with a sucrose solution (10% sucrose), deionized (DI) water, or an NaCl solution with one of three concentrations (0.1%, 1%, or 2% NaCl). Tubes placed along a given transect were randomly drawn. Sucrose solutions were used as a measure of ant activity independent of geographic region so that responses to NaCl baits could be scaled by responses to sucrose baits (Kaspari *et al.*, 2008). Thus, all comparisons of fire ant responses among NaCl baits are scaled by site-specific responses to sucrose. Each bait type had 20 tubes within each transect for a total of 100 baits per transect. After 1 h, we picked up the tubes and quickly closed the caps so as to trap the ants within.

We used a two-way ANOVA with type III sum of squares to assess the interaction between geographic region (SC and FL) and NaCl bait concentration (0%, 0.1%, 1%, or 2%) on fire ant recruitment to field baits. The response variable was the proportion of each NaCl bait concentration occupied by fire ants, divided by the proportion of sucrose baits occupied at each site. We checked for normality on Q–Q plots and tested homoscedasticity with Bartlett's test. A square root transformation on the response variable improved heteroscedasticity. We used a Tukey's honestly significant difference (HSD) test to make pairwise comparisons among combinations of geographic region and NaCl bait concentrations.

Colony growth experiment

On 31 May 2012, we collected approximately 150 mated queens in SC after a large mating flight. During the claustral period, we placed queens into individual test tubes with access to DI water. We discarded tubes with queens that had died, had sexual brood or limited worker brood. Seven weeks later, we categorized colonies by the number of workers and brood as either large (27 ± 4.0 SD workers per colony) or small (18.2 ± 4.05 SD workers per colony). Within each size class we randomly assigned colonies to one of four treatments – access to 0% NaCl (DI water), 0.01% NaCl, 0.1% NaCl, or 1% NaCl – while maintaining equal numbers of each size class across treatments. Each treatment received seven replicates of large colonies and eight replicates of small colonies.

Each colony was kept in a fluon-lined, open-top plastic container (Figure S1). Rigorous hygiene procedures were used to eliminate contamination by SINV-3 and other pathogens (Valles & Porter, 2013). Colonies were randomly assigned to plastic bins that held up to 10 containers. Bin bottoms were dusted with talcum powder to prevent cross-contamination in case ants escaped. All treatments received: (i) *ad libitum* sucrose in wicks moistened three times a week with DI water and replaced as often as needed; (ii) *ad libitum* chopped 'superworms' (*Zophobas morio*; reared on a diet of carrots, potatoes, and wheat middlings; Timberline Live

Pet Food, Marion, Illinios); (iii) *ad libitum* supplemental NaCl solution or DI water (treatments) provided in test tubes plugged with cotton; and (iv) nesting tubes partially filled with DI water held in place with a cotton plug. We selected superworms because their sodium content is only about one-third of that in domestic crickets, *Acheta domesticus* [Finke (2002); J. Resasco, unpublished: $0.33\,\mathrm{mg\,g^{-1}}$ (0.033%) vs. $0.97\,\mathrm{mg\,g^{-1}}$(0.097%)], and because fire ant colonies cannot be suitably reared on vegetable or artificial diets (e.g. Bhatkar & Whitcomb, 1970; Straka & Feldhaar, 2007; Dussutour & Simpson, 2008; Porter 1989; S. D. Porter, unpublished). NaCl solutions were replaced every 2–3 weeks before more than half of the water had evaporated, to minimise the effect of altered concentrations. After 11 weeks, we measured fresh biomass of entire colonies (queen, workers, and brood) to the nearest 0.01 g.

We used a two-way ANOVA to test the effect of NaCl supplementation on fire ant colony mass. Initial colony size (small or large) and NaCl concentration (0%, 0.01%, 0.1%, and 1%) were factors. Again, we checked for normality on Q–Q plots and tested homoscedasticity with a Bartlett's test. No data transformation was required.

At the conclusion of the colony growth experiment, we sampled 30–50 workers from each colony and combined them by treatment. Sodium content analysis, using inductively coupled plasma determination for elemental analysis using method AOAC 985.01, was performed at ABC Research Laboratories, Gainesville, Florida (www.abcr.com).

Results and Discussion

We found a highly significant interaction between geographic region and NaCl bait concentration on response of fire ants to baits in the field (Fig. 1; $F_{3,32} = 13.36$, $P < 0.0001$). A *post hoc* Tukey's test showed that at low concentrations (0% and 0.1% NaCl), fire ant recruitment to baits did not differ between regions (all $P > 0.99$). However, at high concentrations (1% and 2% NaCl), fire ants in SC but not FL showed a strong preference for NaCl baits. Response to high-concentration baits in SC was roughly an order of magnitude greater than for the same baits in FL (all $P < 0.01$) and for lower-concentration baits (0% and 0.1%) at both sites (all $P < 0.001$). In FL, response did not differ among concentrations (all $P > 0.67$). Although limited in spatial coverage, these results support the expectation that the response of ants to sodium is inversely related to the rate of sodium deposition (Kaspari *et al.*, 2008). These results also suggest that at least inland fire ant populations do not meet their sodium needs from animal prey.

Despite the differences in response to NaCl in the field, we found that NaCl supplementation had no effect on colony growth (Fig. 2; $F_{3,44} = 0.25$, $P = 0.86$). There was no interaction between initial colony size and treatment ($F_{3,44} = 0.32$, $P = 0.81$). Eight colonies were eliminated during the course of the experiment because they failed to grow due to queen mortality or unknown pathologies. Final sample sizes were: 14 colonies at 0% NaCl; 14 colonies at 0.01% NaCl; 13 colonies at 0.1% NaCl; and 11 colonies at 1% NaCl. Eliminated colonies were not significantly related to salt treatments (Fig. 2; Fisher's exact test $P = 0.49$) and their exclusion or inclusion did not change the interpretation of the results (with inclusion of eliminated colonies as zeroes: $F_{3,56} = 0.70$, $P = 0.56$). Analysis of workers after the conclusion of the experiment revealed that sodium content in the laboratory colonies did not increase with increasing NaCl treatment concentration (0.86, 0.70, 0.74, and $0.91\,\mathrm{mg\,g^{-1}}$ for 0%, 0.01%, 0.1%, and 1% NaCl, respectively).

Sodium-seeking behaviour is widespread among animals, including insects. While the behavioural pattern of sodium-seeking and its physiological role are well understood, fitness benefits remain poorly studied (Molleman, 2010). Results from the two components of our study seem contradictory: although fire ants in regions of low sodium deposition showed strong patterns of sodium-seeking, experimental colonies reared under

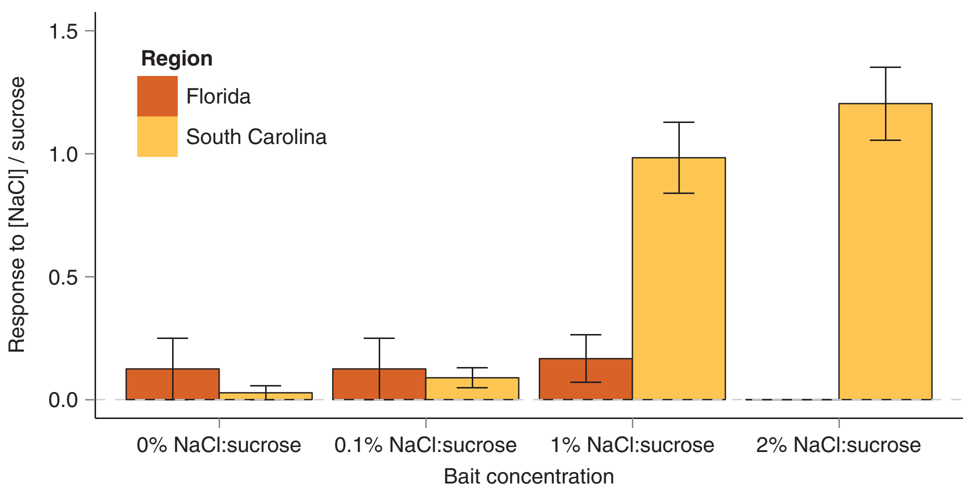

Fig. 1. Mean (±1 SE) ratio of fire ant activity at NaCl baits of increasing concentration [0% (DI water), 0.1%, 1%, and 2%] to 10% sucrose bait activity at high-sodium-deposition sites in Florida ($n = 4$) and low-sodium-deposition sites in South Carolina ($n = 6$).

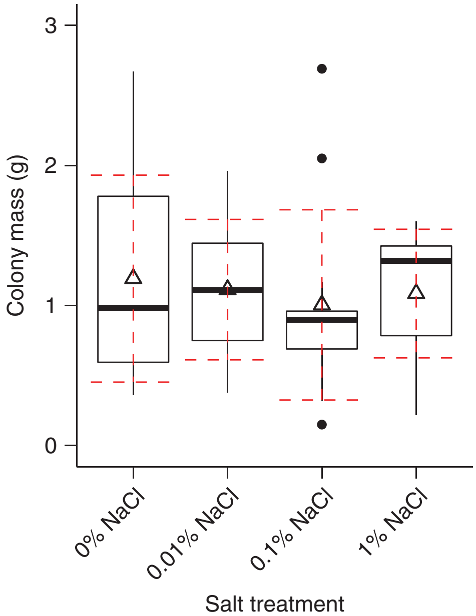

Fig. 2. Box and whisker plot of laboratory-reared, experimental fire ant colonies with salt solution supplements of 0% NaCl (deionized water; $n = 14$ colonies), 0.01% NaCl ($n = 14$), 0.1% NaCl ($n = 13$), and 1% NaCl ($n = 11$). Boxes represent the 25% and 75% percentiles of the distribution; bold horizontal lines through the boxes indicate medians; solid lines (whiskers) extend to 1.5 times the interquartile range. Treatment means are represented by triangles and SD bars are dashed.

low sodium conditions showed no sign of sodium limitation. The explanation may be that experimental colonies were able to fulfil their sodium requirement through consumption of superworms or by ingesting their own fecal material as a concentrated source of sodium (M. Kaspari, pers. comm.). Alternatively, sodium may benefit ants in ways we did not measure (e.g. worker longevity, alate production, immune function). Nevertheless, our results show that increasing sodium availability did not increase colony growth rate. In agreement with this finding, experimentally added dietary sodium did not increase survivorship or reproduction in Japanese beetles (*Popillia japonica*; Stamp & Harmon, 1991). However, sodium supplementation does benefit growth or reproduction in crickets (*Acheta domesticus*; McFarlane, 1991) and butterflies (*Papilio glaucus*; Lederhouse *et al.*, 1990). Future work should be directed at rearing ant colonies on synthetic sodium-free and sodium-supplemented diets, and at field manipulations of sodium to better isolate any growth or reproduction benefits to ants and other consumers. Such work will help to assess the implications of altered sodium deposition on inland ants and on the carbon cycle.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12089

Figure S1. Photograph of one experimental fire ant colony.

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