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# Differential bait preference and rate of attraction by Argentine ants (*Linepithema humile* Mayr) at freshwater and saltwater marsh sites in southern California

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**Abstract.** Ants are a type of foraging insect species which harvests food resources based on availability. When ants locate food resources that are scarce within their habitat, they tend to be more strongly attracted to that food resource. This study used protein, carbohydrate and control based baits to examine if there was a deficiency in resources demonstrated by the ants at two different wetland habitats. We sampled Argentine ants (*Linepithema humile* Mayr) within the saltwater and freshwater marshes of Ballona Wetlands in Los Angeles, CA. We found significant differences in the rapid deployment of Argentine ants towards protein baits over carbohydrate and control baits. We saw more Argentine ants at the protein baits in the saltwater marshes than in the freshwater marshes. We propose that a protein limitation exists in both wetland habitats with increased protein limitation in the saltwater marshes.

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## Introduction

Different species of ants live in a variety of habitats where they colonize different areas, ranging from terrestrial, leaf foliage and arboreal habitats (Cogni, Freitas, and Oliveira, 2003). The diet of the ants varies from habitat to habitat because the availability of food resources is different. When presented with rare food resources, ants tend to be more attracted to those types of resources. Ants prefer harvesting the resources that they lack or what the habitat is limiting (Kaspari and Yanoviak, 2001).

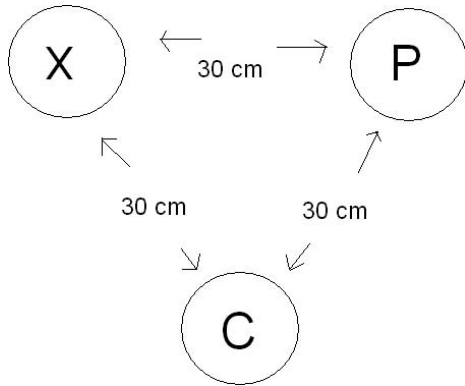
Hahn and Wheeler (2002) performed a study on food preference and foraging behavior of

ants showing that using baits of different nutritional value can determine which resources were and were not limiting for the ants. Terrestrial and arboreal ants have demonstrated differences in their preferences for different baits; terrestrial ants (e.g. *Paratrechina guatemalensis*) preferred carbohydrate-based baits while arboreal ants (e.g. *Ectatomma tuberculatum*) preferred protein baits (Hahn and Wheeler, 2002). A preference for carbohydrate baits suggested that carbohydrate resources were a limiting resource for the ants on the ground, and the protein-based baits were a limiting resource for the ants in trees (Hahn and Wheeler, 2002).

There are habitats, other than terrestrial and arboreal, where there may be differences in food availability for ants. The distribution of food resources in saltwater and freshwater marshes

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**Figure 1.** Bait placement formation (X= control, P= protein, and C= carbohydrates)

varies possibly due to the levels of salinity and soil type (Phleger, 1971). Soil near saltwater marshes can have some level of salt concentration which can affect food resources differently than food resources near freshwater marshes (Phleger, 1971). The soil contents, primarily the salt levels, may contribute to different ant food preferences and foraging behaviors due to the fact that the food resources are much different.

The objective of our study is to evaluate Argentine ant (*Linepithema humile* Mayr) preferences using protein and carbohydrate baits in saltwater and freshwater marshes. According to Hahn and Wheeler (2002), preference for protein and carbohydrate baits reflect deficiencies in ant diet, where in ants will show a preference to the limiting resource in their environment. A depletion of a specific resource, creating a limited resource, leads to a shift in the diet of the ant causing them to move onto the next available resource in that habitat (Tillberg et al., 2007). This shift goes generally from protein resources to carbohydrate resources because the ants prefer protein (Tillberg et al., 2007). We expected to see differences in food preferences for Argentine ants because saltwater and fresh water marsh environments would offer different food resources.

## Materials and Methods

Argentine ants were tested for bait preferences at two different sites: saltwater marsh and

freshwater marsh located in the Ballona Wetlands (Los Angeles, CA). Four randomly selected locations within each site were chosen to conduct the trials. The weather was clear and dry providing ideal conditions for ants. Three petri dishes were placed 30 cm away from each other, forming a triangle (Fig. 1). The three dishes each contained one of three different baits. The first dish contained the control bait, a cotton ball soaked until damp with distilled water. The second dish contained the carbohydrate bait, a cotton ball soaked until damp with honey water. The honey water contained 5% honey (Ralph's generic brand) by weight in a 15 ml squirt bottle. The solution was shaken for approximately 2 min. to ensure thorough mixing. The third dish contained the protein bait, 2 g of Starkist canned tuna in water. During each trial, the number of ants was counted at 2, 6, and 10 min., and a rate of the number of ants per minute was calculated to determine the rate of attraction. We also included counting the ants located in a 2 cm buffer zone around each Petri dish.

We calculated the rate of ants arriving per minute by using the slope formula in Excel 2007. The rates of ants arriving per minute was tested for normality by using a Shapiro-Wilks test (Statistica v. 6) and transformed using the following formula (1).

$$y = \sqrt{\log(x+10)} \quad (1)$$

The differences among the rates of ants arriving per minute were evaluated with respect to bait treatment and site locations using a Two-Way Analysis of Variance (ANOVA; Statistica v. 6). The differences in the rate of ants arriving per minute among bait treatments and site locations were identified using Fisher LSD (Statistica v. 6). The maximum number of ants observed at each bait treatment was tested for normality using Shapiro-Wilks test (Statistica v. 6) and were transformed using Formula (1). Differences in maximum number of ants were evaluated with respect to bait treatment and site locations using a Two-Way ANOVA (Statistica v. 6). We identified differences in maximum number of ants between bait treatments and site locations using a Fisher LSD (Statistica v. 6).

**Table 1.** Results of a Two-Way ANOVA of attraction rate in site location and bait treatment.

Source of variation	df	MS	F	P value
Site	1	0.0028	3.46	0.0794
Bait	2	0.0046	5.60	0.0128
Site & Bait	2	0.0032	3.92	0.0386
Error	18	0.0008		

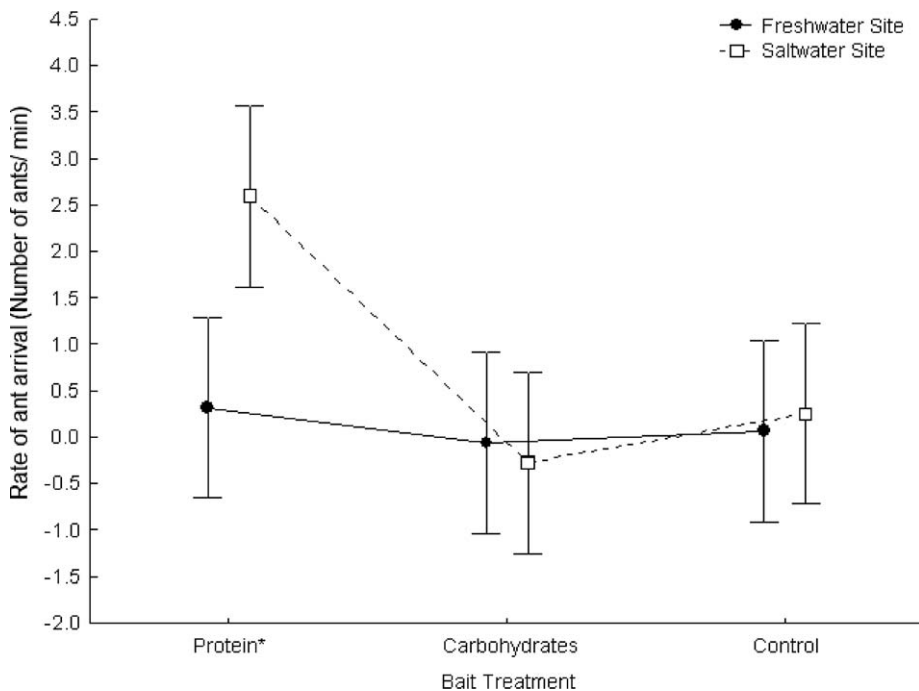
### Results

The rate of Argentine ants arriving per minute was not normally distributed ( $P < 0.05$ ). However, a log transformation resulted in a normal distribution ( $P > 0.05$ ). Site location alone did not explain variation observed in attraction rate ( $P = 0.0794$ ; Table 1). We observed a significant difference between baits and a greater attraction rate at protein baits than any other treatment ( $P = 0.01$ ; Table 1). There was a significant interaction between baits and sites ( $P = 0.03$ ; Table 1). However, we observed a significantly greater attraction rate at the saltwater marsh toward protein baits than at the freshwater marsh (Fisher LSD,  $P < 0.05$ ;

Fig. 2). Site location did not explain variation observed in the maximum number of Argentine ants at bait treatments ( $P = 0.1213$ ; Table 2). We saw a significantly greater maximum number of Argentine ants at protein baits than any other treatments ( $P < 0.001$ ; Table 2; Fig. 3). The interaction term between site location and bait treatment did not explain variation observed in maximum number of Argentine ants ( $P = 0.07$ ; Table 2).

### Discussion

In this study, we found that the number of Argentine ants attracted to bait locations was greatest at the protein bait compared to the



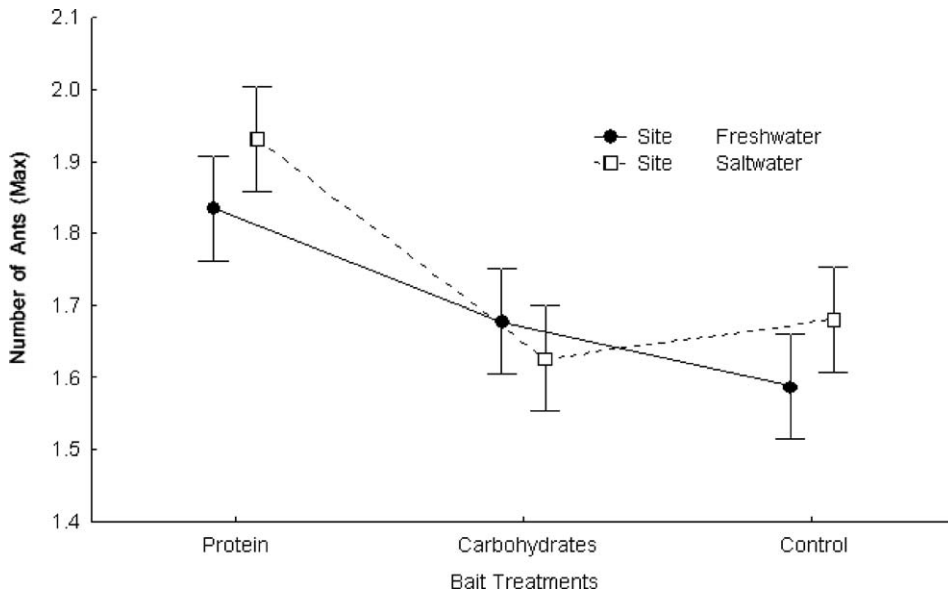
**Figure 2.** Differences among n rate of Argentine ant arrival in bait treatment and site locations verse attraction rate of Argentine ants. (Bars denotes 95% confidence interval, asterisks\* denotes  $P < 0.05$ )

**Table 2.** Results of a Two- Way ANOVA of maximum number of Argentine ants in site location and bait treatment.

Source of variation	df	MS	F	P value
Site	1	0.0127	2.64	0.1213
Bait	2	0.1544	32.27	0.0000
Site & Bait	2	0.0134	2.92	0.0798
Error	18	0.0048		

control and carbohydrate bait (Fig. 3). The carbohydrate and control attraction rates did not vary significantly between the saltwater and freshwater locations. On the other hand, the attraction rates of the protein bait varied significantly between the saltwater marsh and the freshwater marsh locations. Protein baits were favored most in the saltwater marsh by Argentine ants (Fig. 2). Kaspari and Yanoviak (2001) found in their study that the canopy ants compared to the ground ants preferred meat baits. Canopy ants had a preference for protein sources because of the limited source of protein available in the canopy. But for terrestrial ants, there was no preference towards any specific bait. Our study found an attraction to protein by Argentine ants which may reflect a limitation in nitrogen in their diet. The Argentine ants were not attracted to the carbohydrates because it was not limiting in the environment (Pierce, 1985;

Tillberg et. al, 2007). An increase in the rate of attraction at the protein baits may indicate a lack of protein in the diet. Based on the differences in rates of Argentine ants arriving per minute, we observed a protein limitation in saltwater marsh compared to the freshwater marsh habitat. Bluthgen and Fielder (2004) found that preference for proteins by ants depends on the community hierarchy and interspecific competition present at the food resource locations. The ants that are competitively superior tended to forage for nectar based food because they contained higher sugar and amino acid concentration. The Bluthgen and Fielder (2004) study found that interspecific competition played a role in bait preference among different ant species. We propose investigating interspecific competition to evaluate the protein deficiencies we saw in the saltwater marsh compared to the freshwater marsh. The study of nutrient limita-



**Figure 3.** Differences among maximum number of ants in bait treatment verse maximum number of Argentine ants present.

tions is critical to the competition of resources among species. In turn, competition tends to lower the overall fitness of all the species involved causing a much greater increase in interspecific competition among species for these limited resources.

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