NUTRIENT REGULATION OF AN EXOTIC, UNIDENTIFIED *Paratrechina* sp. (HYMENOPTERA: FORMICIDAE) FOUND IN TEXAS

A Thesis

by

RACHEL ANNE WYNALDA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2008

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Roger Gold
	Spencer Behmer
Committee Members,	Jeffery Tomberlin
	Leon Russell
Head of Department,	Kevin Heinz

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ABSTRACT

Nutrient Regulation of an Exotic, Unidentified *Paratrechina* sp. (Hymenoptera: Formicidae) Found in Texas. (May 2008)

Rachel Anne Wynalda, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Roger Gold
Dr. Spencer Behmer

Colony fitness, size, and reproductive potential are determined by their ability to locate and consume the optimal amounts of various macronutrients. Understanding the nutritional regulation of an ant colony furthers our understanding of their life history and can be used to produce a better baiting system. The "Geometric Framework" was used to conduct experiments determining how *Paratrechina* sp.nr. *pubens* regulated their protein and carbohydrate intake when given two sub-optimal, but complementary food sources, as well as when confined to a single food source. By analyzing how much food they consumed, we can determine how *P.* sp.nr. *pubens* regulates their food intake. Examination of the consumption results when given two choices, showed a preference for carbohydrate rich foods as well as a trend in regulation along a set nutritional trajectory. Further examination of the amount eaten when confined to a single food source, showed a higher consumption rate of the carbohydrate rich foods (p7:c35 and p14:c28). Analysis also showed a narrower range of protein intake when compared to carbohydrate. Accordingly, behavioral data indicate a pattern of consumption following seasonal shifts.

DEDICATION

This thesis is dedicated to my parents, Doug and Wanda Wynalda, to my sister, Rebecca Wynalda, and to my brother, Matthew Wynalda, all of whom encouraged me to pursue my dreams and to never give up. To my mother, I thank you for always being there for me to talk to and for always believing in me. I would not be where I am today without you, and I am eternally grateful.

To my father, I thank you for teaching me two very important lessons. Firstly, the lesson that hard work is the key to success and that if you surround yourself with people who love you, can survive anything. Secondly, to never give up no matter how impossible the situation seems. These two lessons have gotten me far and will be with me for the rest of my life. I only pray that I can instill these lessons in my children one day.

To my sister, I thank you for being my best friend and my inspiration. You have always been the person I look up to, and who I strive to be like. Your unwavering faith in me has encouraged me through tough times, and your sense of humor has brightened some of my darkest days. I cannot ever thank you enough for all you have done for me.

To my brother, I thank you for always being there for me. I could always count on you to cheer me up. I have always admired your incredible work ethic and your ability to find joy in the smallest things.

ACKNOWLEDGEMENTS

I would like to extend my deepest and sincerest thanks to my committee chairman, Dr. Roger E. Gold, for guidance and support throughout this process. Thank you for providing me a wonderful opportunity to be a graduate assistant, as well as providing funds for my graduate career. I would also like to thank my committee co-chairman Dr. Spencer Behmer for your excellent advice, ideas, and support; it was an honor and a privilege working with you. Thank you also, to my committee member, Dr. Jeffery Tomberlin, for your guidance and willingness to include me in your laboratory. I also extend my thanks to my committee member Dr. Leon Russell for taking time from his very busy schedule to help me.

Thanks also need to be extended to everyone at the Center for Urban and Structural Entomology; the past few years have been a pleasure because of you: Dr. James Austin for your advice and wonderful sense of humor; Bill Summerlin for your help and ability to always put a smile on my face; and Laura Nelson for administrative help. The friendships and advice I received from my fellow graduate students, Jason Meyers, Chris Keefer, Tara McGuigan, Aaron Thompson, Stacy Boatright, Adrienne Brundage, Jennifer Pechal, Michelle Sanford, Micah Flores, Barry Furman, and Brian Heintschel were vital to the completion of this degree. I want to say a special thanks to Jason Meyers, Chris Keefer, Jennifer Pechal and Tara McGuigan for always being a source of laughter and support during this process. I would not have been able to get through this without your help.

I am also grateful for the scholarships I have received throughout my time in graduate school. The financial help I was given provided me the opportunity to focus on my research and class work. I am forever grateful for the generous donations from the Robert W. Jenkins, Sr. Memorial Endowed Scholarship, and the Alice Jean and George E. Novy Memorial Endowed Scholarship.

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CHAPTER I

INTRODUCTION

There are over 8,000 ant (Hymenoptera: Formicidae) species have been described worldwide (Klein and Wenner 2001). Like some of the other species in the Order Hymenoptera, ants are eusocial insects, with characteristics of overlapping generations, communal care for their young, a division of labor in which one or more non-reproductive castes are present (Borrer et al. 1992). Ants live in colonies that comprise one (monogyne) or more queens (polygene), males (winged or wingless), and workers (major and minor) (Gullan and Cranston 2005). Ant nests can be found in many different locations, including open soil as mounds, or under objects, such as stones, timber, debris, sidewalks, or slab construction (Hedges 1998, Hedges 2004). Depending on species, colony size can range from a few hundred to over 300,000,000 workers (Kaspari and Vargo 1995). These unique colony characteristics allow ants to occupy many parts of the world excluding cold areas (McGavin 2001). These characteristics also allow them to expand and become established in new regions as invasive species.

Increased globalization and trade has resulted in many ant species being distributed throughout the world (Helms and Vinson 2002). Invasive ant species can out compete or prey on native organisms (Jenkins 1996), and may be ecologically devastating (Clark et al. 1982, Majer 1985, Porter and Savignano 1990, McGlynn 1999).

The ability of invasive ant species to become established depends on many factors. These can include size and fitness of the colonies, the regional environment and

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This thesis follows the style of Journal of Economic Entomology.

temperature, food supply, and the presence of parasites and predators. Other insect species might provide resources and promote establishment of an invasive ant species. For example, the association between the red imported fire ant (RIFA) *Solenopsis invicta* Buren (Hymenoptera: Formicidae), and the invasive mealybug *Antonia graminis* Maskell (Order: Hemiptera) (Helms and Vinson 2002). The mealybug provides honeydew as food for RIFA's which in turn provide protection and shelter for the mealybugs (Helms and Vinson 2002).

Invasive ants are those which establish long-term populations and expand their range into new areas (McGlynn 1999). Established invasive ants can negatively affect native invertebrate and vertebrate species. Some general impacts of invasive ants include their interference with mutualisitic relationships, competition with native ants, or adversely affect the ecosystem through loss of diversity (Holway et al. 2002, McGlynn et al. 1999, and Ness and Bronstein 2004).

The introduction of the crazy ant, *Paratrechina fulva* Mayr, (Hymenoptera: Formicidae) into Columbia almost 30 years ago resulted in the displacement of the native ant fauna, which pertubated throughout the local agro ecosystems (Arcila et al. 2002). Its association with homopteran insects allowed those homopteran populations to increase unregulated which resulted in crop damage (Arcila et al. 2002). This case provided evidence that displacement of native fauna can destabilize the local ecology (Holway et al. 2002). The RIFA can have a mutualistic, as well as negative, relationship with trophobionts, which are insects that produce bodily exudates and include species of mealybugs (Order: Hemiptera), aphids (Order: Hemiptera), treehoppers (Order:

Hemiptera) and scale insects (Order: Homoptera). RIFA's prefer a protein-rich diet and because of this, they will more than likely consume trophobionts due to this need not being met (Ness and Bronstein 2004).

Along with affecting trophobionts, invasive ants can adversely affect certain plants. There are approximately 300 mymecochores, which plants that rely on insects to disperse their seed (Ness and Bronstein 2004). These plants produce lipid rich appendages known as elaiosomes. Mutualistic ants will ingest the elaiosome and disperse the untouched seed. Invasive ants can displace the native seed-spreading ants, and may eat the elaiosome without dispersing or burying the seed (Ness and Bronstein 2004).

Invasive ant species compete with native ants for resources. This characteristic of invasive ant species is the most widely reported direct environmental impact (McGlynn 1999). They displace native ants because of their competitive advantages. They excel at exploiting new resources and recruiting workers, which is achieved by having large numbers of workers or by having workers active both day and night (McGlynn 1999).

Some invasive ant species, like the RIFA, cause numerous problems for humans that interact with them. The RIFA is aggressive and has a painful sting that it can inflict multiple times to an adversary or prey. A sting from the RIFA often results in the formation of pruistic pustules, which if not cared for can result in secondary infections (Deslippe and Guo 2000). In extreme cases where an individual is highly sensitive to the sting they may experience localized swelling, anaphylactic shock, and in rare cases,

death (Rhoades et al. 1989). Preventing the established of these invasive ants prevent the ecological side-effects associated with them.

Suppressing a newly introduced ant species can be accomplished through an understanding of its natural history. Much of this information is not attainable if the species is undescribed. For example, such an ant species, which is thought to be related to *Paratrechina pubens* Forel (Hymenoptera: Formicidae), has been collected in Pasadena, TX U.S.A. It is currently being referred to as *Paratrechina* sp.nr. *pubens.*

Paratrechina sp. nr. *pubens* has caused problems for homeowners and businesses in the Pasadena and Pearland areas of Texas. Although it is displacing the RIFA, which is a pest, homeowners have voiced their desire to have the RIFA instead of this newly introduced species. Preference for the RIFA reflects the nuisances this ant has become. They display an affinity for electrical wiring resulting in shorts and clogging of circuits. There has been a report of *P.* sp.nr. *pubens* invading the electrical wiring of a vehicle which caused the car to ignite when the owner attempted to start it.

Current prevention measures undertaken by local home and business owners have resulted in little success. Although, this ant is not as aggressive as the RIFA, the shear size of the colonies exceeds that of RIFA. One individual reported filling a 189-L trash can with *P.* sp.nr. *pubens* cadavers that accumulated on the floor in his business. There are reports from the Houston, and surrounding areas, that due to ant pressure real estate values have fallen. Rapid colonization of new areas by this ant makes it imperative that effective control methods are developed and implemented. Anecdotal evidence from local pest control operators (PCO) personnel shows that some pesticides

such as, Top Choice® (Fipronil 0.0143%, Bayer Environmental Science, Research Triangle Park, NC) and Termidor® (Fipronil 9.1%, BASF, Research Triangle Park, NC), produce some positive results. Meyers et al. (unpublished data 2007) has shown that populations have the capability to recover quickly from treatment.

The most widely used form of control of ants is a granular bait. The active ingredient is the key to its effectiveness. However, its effectiveness relies on being impregnated in a matrix that attracts the ants (Stanley 2004). Along with being attracted, the ants have to consume sufficient amounts of the formulation and share it with other colony members through trophylaxis to have an impact (Davis and Van Schagen 1993, Klotz and Williams 1996, Collins and Callcott 1998, Lee 2000). Food preferences for protein, carbohydrates, and lipids demonstrated by ant colonies varies depending on the species, size of the particles, and seasonal variations. All of these variables determine the effectiveness of baits against the ant colony (Stanley 2004).

Currently most baits are tailored for the RIFA, which might be the reason for their ineffectiveness in suppressing *P.* sp.nr. *pubens.* Since this ant is considered a new species there have been no studies to determine its dietary preferences. To effectively develop a better baiting system, research needs to examine its biology, life cycle, diet preferences and nutritional regulation mechanisms.

One effective way to investigate the regulation of macro nutrients is the use of a conceptual tool referred to as the "Geometric Framework" (Raubenheimer and Simpson 1993a, 1993b). This experimental design is a state-space model that explores how an animal balances multiple and changing nutrient needs in a multidimensional and variable

nutritional environment (Simpson and Raubenheimer 1993). Most research that has used this approach examines different combinations of the two macro-nutrients most important to insects, which are protein and digestible carbohydrates. By feeding ants on a range of diets with different relative concentrations of protein and carbohydrates, and by examining the amount consumed, the one that promotes optimal growth and development can be determined. This optimal point, inside nutrient space, is referred to as the "*intake target"* (Simpson and Raubenheimer 1993). These nutrients can then be depicted as 'rails', which are a combination of non-nutrient and nutrients that radiate out from a central point (Figure 1.1) (Simpson and Raubenheimer 1993). The angles associated with the rails show the ratio of their constituents.

There are three general methods in which foods can be represented in nutrient space. The first is a balanced food, which means if an insect eats along this rail they reach their intake target without using an alternate food sources (Figure 1.1 A). The second is a suboptimal diet, which prevents the insect from reaching its intake target (Figure 1.1 B). Since there is no regulation mechanism they can employ to reach their intake target they have to determine which nutrient they prefer to have in excess or deficit. If they eat and reach their physiological demand for carbohydrates (point a) they will have a deficit in protein and vise versa for point b (Figure 1.2). If they eat to point c they will not meet their protein or carbohydrate requirements, but they eliminate the possibility of eating nutrient in excess (Figure 1.2). The regulation of nutrient intake is referred to as the *rule of compromise* (Simpson and Raubenheimer 1993). As stated, this rule indicates the relative cost/benefit of overeating one of the nutrients to under-eating

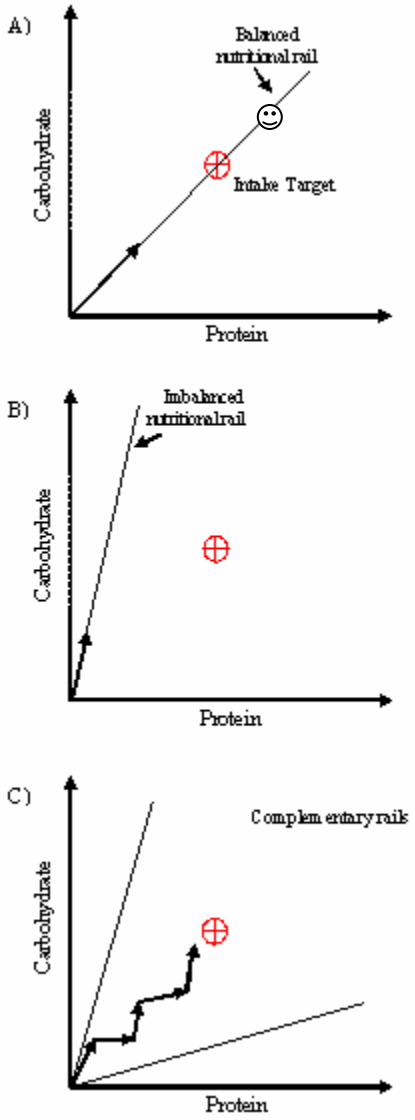

Figure 1.1 Three representations of how the "Geometric Framework" evaluates nutritional regulation. Combinations of nutrients can be represented in nutrient space (e.g., the smiley face in A), while a nutritional rail represents the balance of nutrients (angle represents nutrient ratio). If the ratio is balanced then the intake target can be reached (A). This target cannot be reached when feeding on an imbalanced food (B). If the animal was allowed to feed on two complementary, but suboptimal foods the intake target can be reached by switching between the two foods (C).

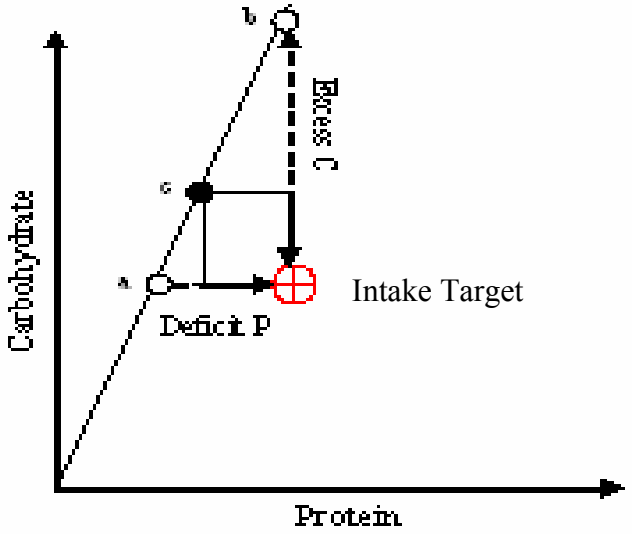

Figure 1.2 Diagrammatic representation of nutritional compromise on an imbalanced food. Feeding to a certain point will satisfy one nutritional need, but suffers a deficit or excess of the other nutrient (point a and b). By feeding to the point c the animal will not meet the requirements of either nutrient, but will avoid eating any extremes of one of the nutrients.

the other. The last method is when the insect encounters two food sources that are considered complementary. This method means that their target intake lies between the two rails. The target intake can then be reached by eating a little from each rail until they have ingested the correct ratio of nutrients (Figure 1.1 C) (Simpson and Raubenheimer 1993).

Analysis of these various nutritional regulation mechanisms gives insight into colony requirements for survival. Currently most of the work being done in the field of diet regulations has focused on the nutritional needs of locust (Orthoptera: Acrididae) species (Behmer et al. 2001). The experiments conducted here represent the first application of the previous research results to *P.* sp.nr. *pubens*. Results generated from these studies will provide a better understanding of its biological functions and be used to produce a better baiting system for its suppression.

CHAPTER II

PROTEIN AND CARBOHYDRATE DIET PREFERENCE BY *Paratrechina* sp.nr. *pubens*

OVERVIEW

Nutritional needs are an important parameter determining the fitness of *Paratrechina* sp.nr. *pubens* colonies. Most experiments have examined the attractiveness and effectiveness of bait matrixes and not explained exact nutritional needs of various ant species. The "Geometric Framework" was used to determine nutrient regulation of *Paratrechina* sp. nr. *pubens* when given two sub-optimal, but complementary, food sources. Regulation of food intake was determined by examining its consumption rate from both sub-optimal diets e. Its foraging behavior was determined through three 4 h observation periods conducted during the 15 d experiments (replicated 6 times). *Paratrechina* sp. nr. *pubens* demonstrated a preference for carbohydrate rich foods as well as a trend in regulation along a set nutritional trajectory (*nutritional rail)*. Accordingly, behavioral data indicate a pattern of consumption following seasonal shifts.

INTRODUCTION

Ants consume a variety of diets that provide the nutrients needed for energy, maintenance, growth and reproduction. Together these nutritional factors play a role in determining colony fitness (Hughes 1993). Central to their fitness is the colonies allocation of protein and carbohydrates. Acquisition of proteins is essential to ants because of there use in reproduction and development of eggs and larvae (Scherer 2007).

In contrast, carbohydrates serve as sources of energy for activities, such as foraging and defense of the colony (Scherer 2007). However, foods rarely contain these macronutrients in the required proportions. Colonies presented with various forging and food opportunities have to make a nutritional choice that ultimately determines their survival. By studying how foragers of different ant species regulate their nutrient intake, insights can be gained into why they neglect or prefer particular food items. This information can then be used to understand how the colony regulates its specific nutritional needs (Howard 1987, Voelkl et al. 1999, Kay 2004).

Currently research examining dietary preferences of ants has focused on the attractiveness of baits (Stanley and Robinson 2007). Although these studies have provided an idea of what matrix and nutrient combination is preferred by different ant species, they did not reveal anything about their exact dietary needs. Matrixes usually just have a primary classification (ex. protein or carbohydrate) but do not give a specific amount of protein or carbohydrates. One limitation of these studies, as well as other ant nutritional evaluations, is they only examine the attractiveness of foods when the ants are presented with single food choice (Stephens and Krebs 1986) even though in nature ants have access to a multitude of various substrates.

One approach for analyzing the optimal amounts of protein and carbohydrates needed by ant species is to examine their foraging behavior within the context of the 'Geometric Framework' (Raubenheimer and Simpson 1993a). Briefly, this approach allows the organism of interest to demonstrate the nutrient proportions that are most preferred (Behmer et al. 2001). This balance, called an 'intake target' (Raubenheimer

and Simpson 1993a), reveals the amounts of nutrients (e.g. protein and carbohydrate) ingested by an animal over time, which is useful because it can be used to conceptualize the relative significance an organism gives to various nutrients. Implementing the intake target concept also can be conducted with animals constrained to imbalanced foods (Raubenheimer and Simpson 1999, Simpson and Raubenheimer 2000). This approach also differs from most because it makes no *a priori* assumptions about the animal's nutritional needs. Although most research using this approach has been conducted on plant-feeding insects (Simpson and Raubenheimer 2000, Behmer et al. 2001, Behmer et al. 2003), it can easily be applied to a range of insects, including ants, which are regulating nutrient intake at both the individual and colony level.

In this study, I examine the preferences and associated foraging behaviors of *Paratrechina* sp.nr. *pubens* ant colonies for foods that differ in protein and carbohydrate ratios. By observing the foraging behavior and consumption rates of different artificial foods with known nutrient profiles, I was able to assess whether, and to what extent, this ant species actively defend a protein-carbohydrate intake target.

MATERIALS AND METHODS

Insects: *Paratrechina* sp.nr. *pubens* were collected in Pearland, TX (GPS coordinates: N 29°33.518, W 095° 20.531). Finding colony locations was accomplished by examining known nesting habitats. These sites included under fallen tree limbs, in leaf litter, or by digging approximately 50.8- 305 mm into the soil. Once colonies were located, they were sight identified to be the appropriate species and then shoveled along with the dirt or debris present into a bucket (22 L) that had its sides treated with baby

powder (approx. 1 oz) to prevent the ants from escaping. Colonies were then transported to the Center for Urban and Structural Entomology, Texas A&M University, College Station, TX. The colonies were removed from the dirt/debris in the bucket by a water dripping method which is frequently used to separate ant colonies. This method was done by placing the dirt/ debris under a faucet, which slowly dripped (approx. 2 drips per second; 1 drip = 0.125 ml) water into the bucket. At the top of the dirt pile a Petri dish (8.5 cm, h= 1.5 cm), half way filled with plaster of paris, was placed and acted as an artificial nest. Occasionally one artificial nest would not be large enough to hold the entire colony. When this happened multiple petri dishes were placed in the bucket. When the water rose the colony would move up the mound of dirt and into the artificial nests. Once the colony was inside the nests they were removed and placed together into a plastic box (30.5 x 16.5 x 8.9 cm). The inside walls of the boxes was treated with Fluon® (Polytetrafluoro-ethylene, ICI Fluoropolymers INC, Exton, PN, U.S.A.) to prevent the ants from escaping. Inside these plastic boxes two water sources were presented to the ants. One source was a 75 ml glass jar with a plastic top and cotton wick (Braided Rolls made by Richmond Dental, Charlotte, NC) filled with water. This source provided moisture. The other source was a plastic container (5.5 x 4.5 x 2 cm) filled with cotton balls soaked with a 20% honey water solution. This source provided carbohydrates (Chapman 1998). A prey source was also included in each colony box and consisted of approximately five to six dead crickets placed in a plastic weight boat (5.5 x 4.5 x 2 cm) (Orthoptera: Gryllidae). The water, sucrose, and crickets were checked daily and replenished when needed. The colonies were maintained in a growth

chamber (Elliott-Williams model: Conviron 8601) at 30±2°C, 12:12 light: dark, and 60% RH.

Over the course of the experiment, six separate colonies were collected and processed using the methods previously described. Each colony represented a replicate. Colonies were collected from December 2006 until May 2007 (Table 2.1). For purposes of this experiment December through February were considered winter months and March through May were spring/summer months. This definition of seasons was done in order to make possible correlations between shifts in behavior and seasonal cycles.

Experimental Foods: The experimental foods used were a dry, granular, chemically defined matrix and were prepared as described in Behmer et al. (2001). Variations of protein and digestible carbohydrate gave rise to the following three combinations of protein (p) and carbohydrate (c) expressed as a percent (%): p7:c35, p28:c14, and p35:c7. No known previous work had been done to determine the combination that is nutritionally optimal for this ant species. These three diets represented extreme protein-carbohydrate ratios that alone might be suboptimal for ants. Together, however, these foods were complementary, and ants were able to eat from both foods, thus providing the opportunity to optimally regulate their protein-carbohydrate intake. All three matrixes had equal total amounts of protein plus carbohydrate, and also contained identical proportions of the other ingredients, including indigestible cellulose powder (Table A-1).

Table 2.1. Dates when *Paratrechina* sp.nr. *pubens* were collected from the field (Pearland, TX).

Replicate	Dates of Collection
1	12/2/2006
2	1/3/2007
3	2/2/2007
4	3/2/2007
5	4/1/2007
6	4/28/2007

Experimental Ant Colonies and Test Arenas: Collected colonies remained in their original containers for 3-6 d before sub-colonies were removed to make the experimental treatments. A sample of specimens from a colony was used in an experiment. These experimental ant colonies consisted of one functional queen, 250 workers, and approximately 10 mg of brood. Each colony was housed in a glass test tube (1.6 x 15 cm) that served as both a water source and nest. The tube was filled half way with distilled water with a cotton plug inserted to keep the water from spilling out. Experimental arenas (Figure 2.1a) consisted of two separate plastic boxes (9 cm high x 16.5 x 30.5 cm) set adjacent to each other. Both boxes had their sides treated with Fluon ® in order to prevent the ants from escaping. One box contained the ant colony, and the other contained the experimental foods (described below). Within the food box, dishes of food were equally spaced 3.8 cm from the base of the bridge and 7 cm and 9 cm from the box walls (Figure 2.1b).

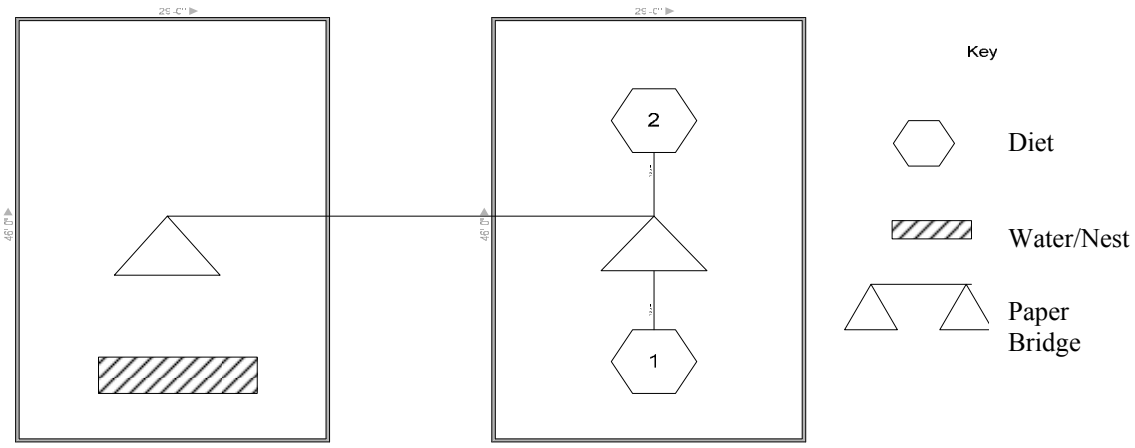

Figure 2.1.a . Diagrammatic representation of experimental set-up for the choice test. Rectangles depict the plastic boxes used to house the experimental colony, and the experimental diets. Rectangle with lines through it shows where the water source/ nest were located. Pentagons with numbers one and two represent the location of each dietary dish (1 = p7:c25, 2 = p35:c7 for mix one; or 1 = p7:c35, 2 = p28:c14 for mix two). The triangles with the line connecting them represents where the paper bridge was located. Figure 2.1 b shows the box to scale.

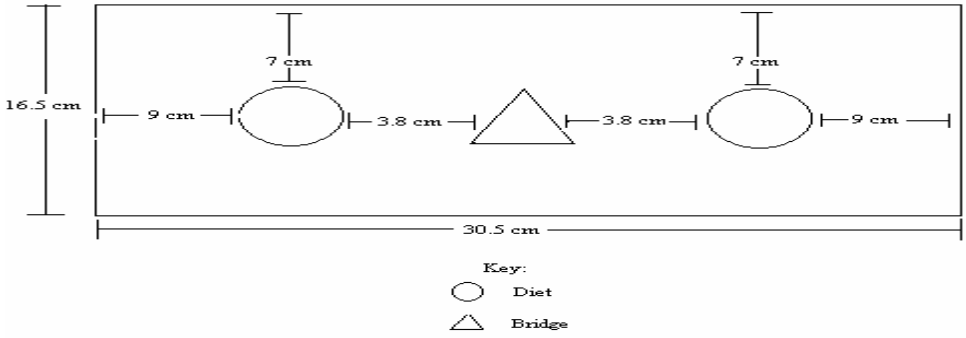

Figure 2.1. b. Diagrammatic representation of the exact location of each diet within the *diet box* relative to the walls and bridge. The triangle represents the plastic tube stopper that was used as the base of the bridge. The two circles represent the two diets used in each choice-test treatment.

The colony box and diet box were connected to each other via a paper bridge (Figure 2.2), which was 45.7 cm by 2.5 and made of printing paper (Sparco Brand, Atlanta, GA). This bridge was used because in house studies demonstrated that this type of bridge was sturdy and allowed for easy observations of foraging behaviors. Measuring from either end, approximately 11cm up, the bridge was bent to form two 90° angles (Figure 2.2). At the base of each end of the bridge, a Plastic tube stopper weighing approximately 11 g was placed to anchor the bridge upright throughout the entire experiment. When the bridge was added to the arenas, one base was placed in the center of the colony box and the other base was placed in the center of the diet box, and centered between the two food dishes.

Experimental Protocol: These experiments consisted of two treatments. The first treatment paired p7:c35 with p35:c7 (Mix 1), while the second paired p7:c35 food with p28:c14 food (Mix 2). Two treatments were needed to insure that nutrient protein-carbohydrate intake was not the outcome of random feeding, in which ants ate equally from the two food dishes in the arena, regardless of protein-carbohydrate ratio of the available foods.

Each experimental colony was deprived of food 15 h prior to initiating the experiment. Prior to the initiation of the experiments, one gram of food was allocated to the respective plastic weighing dishes (pentagon shaped dish: 1 cm high x 2.5 x 2.5 cm) and placed under a heating lamb for 15 h to allow the diet to equilibrate to a room temperature and humidity level. Diets were again weighed to the nearest ten

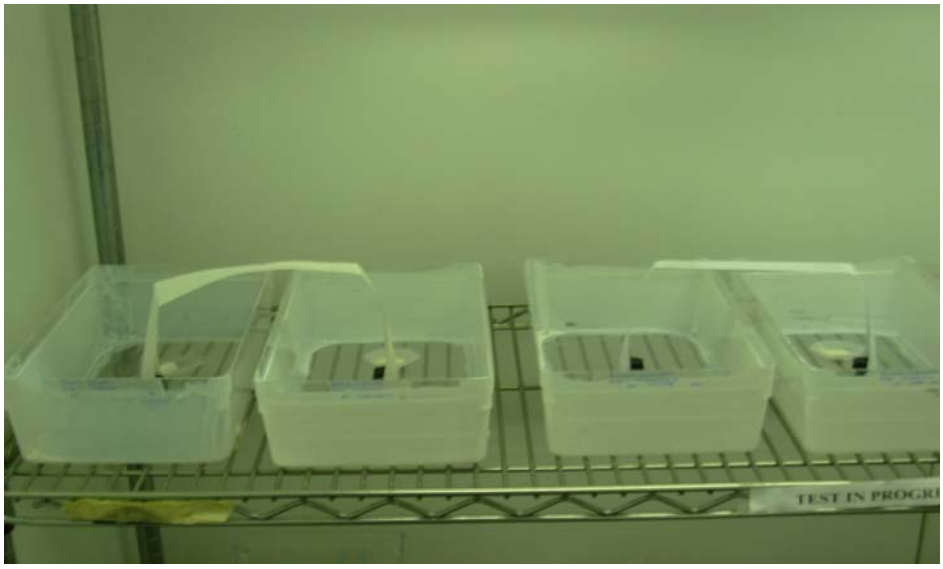

Figure 2.2. Visual representation of experimental set up.

thousandths of a gram after the drying period, and placed in the diet box. The bridge then was added to connect the two boxes and initiate the experiment.

The colonies were allowed to feed for seven days, after which each food dish was removed and replaced with a fresh, pre-weighed dish of the same food type. Removed food dishes at day 7 and 15 were placed under the heating lamps for 15 hr and then reweighed.

In total, the p7:c35 + p35:c7 treatment was replicated with each colony, while the p7:c35 + p28:c14 treatment was replicated with four of the colonies representing each month except December and January due to unavailability of appropriate diets.

Throughout the course of the 15 d experiment, daily counts over the number of dead ants were taken (dead ants classified as no longer having any life signs). These counts were made to determine if there was any correlation between mortality and consumption.

Ant Behavior: Four foraging ants were removed prior to the initiation of each trial and marked in order to record their foraging behavior. Selected ants were removed and placed on a chill table and a mark placed on the dorsum of their abdomen. A toothpick was used to mark the ants with one of four colors of paint (red, white, orange, and green). Paints used were Nissen® Metal Marker in a Bottle: Permanent Paint Marker (Nissen, Glenside, PA). They were selected due to being oil-based and being less toxic to ants (Wojoik et al. 2000). Once marked, they were observed in a plastic box (9 cm high x 16.5 x 30.5 cm) for approximately 10 min to ensure the paint was dry and did not hinder their movements. If the paint was shown to hinder their movements, the ant was not

used in the experiment and another forager was marked. Marked foragers were returned to their original colony.

Observations were made on 1, 7, and 14 d of the experiment. Each observation consisted of recording ant foraging behavior every 10 min for 4 h between 0900 and 1300 hr. Information recorded included the location and activity of the marked foragers, the number of foragers on the diets, bridge, and in the diet box. Along with the intensive observations daily moribund ants were counted and removed to prevent cannibalism.

Statistical Analysis: A multivariate analysis of variance (MANOVA) was used with the statistical package SPSS 15.0. For MANOVA analyses, the Pillai's trace, the MONOVA test statistic that has the greatest robustness was used to analyze the consumption of the diets. Comparison between amounts eaten within a treatment was analyzed using the non-parametric Wilcoxon Signed Ranks Test, again with the statistical package SPSS 15.0. Mortality data was analyzed using analysis of variance (ANOVA) with the statistical package SPSS 15.0.

RESULTS

Mortality: Figure 2.3 shows the mean ant mortality ($\pm$ $_{SEM}$) and ant mortality from 0-7 d (Figure 2.3a), 8-15 d (Figure 2.3b), and for these two periods combined (Figure 2.3c).

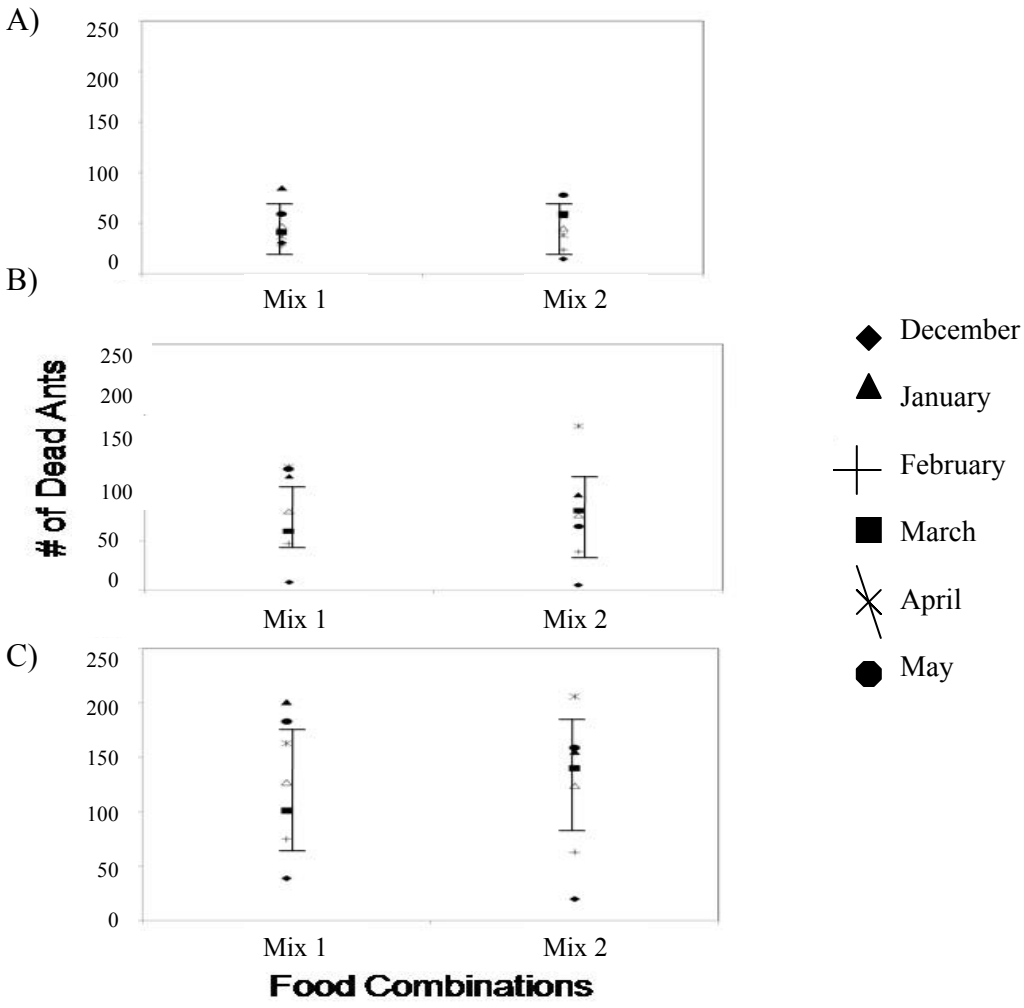

Figure 2.3: Number of dead ants counted out of the 250 live ants used to start the experiment. Numbers are for Mix 1 and Mix 2 for each replicate (i.e. each month), with the mean amounts moribund ($\pm_{SEM}$) represented by open triangle symbol (△). (A) Represents the mortality amounts for days 0-7 (B) days 8-15 (C) days 0-15.

Analysis over the mean number of dead ants counted shows no statistical difference for any time period (Table 2.2).

Food Selection: The mean weight (mg) of the diet consumed from each food dish over days 0-7 (Figure 2.4a), days 8-15 (Figure 2.4b), and for these two periods combined (Figure 2.4c). When the patterns of consumption (Figure 2.4) from the two food dishes were compared for each time period, no statistical differences were observed (Table 2.3). For all time periods, and for each treatment, more carbohydrate rich matrix (p7:c35) was eaten relative to the protein-rich matrix (p35:c7 or p28:c14) (Table 2.4). Figure 2.3 and table 2.4 shows that the majority (85%) of the food was consumed during the first 7 days.

Statistical analysis of the amount of matrix consumed in Mix 1 and Mix 2 (i.e. p7:c35 vs. p35:c7 and p7:c35 vs. p28:c14, respectfully) was conducted using the Wilcoxon Signed Ranks Test. Results (Table 2.5) showed that the comparison of Mix 1 (p7:c35, p35:c7) were not significant, but there was a trend approaching significance ($P < 0.1$) for the p7:c35 food during the two time periods (days 0-7 and 8-15; $p = 0.093$, $p = 0.068$, respectfully). When consumption was summed over these two periods, ants showed a significant preference ($P \leq 0.05$) for the p7:c35 food over the p35:c7 food. With respect to Mix 2 (p7:c35 + p28:c14), there was no statistically difference in amount eaten between the two food dishes for either time period (days 0-7 or 8-15; Table 2.5). However, when these two time periods were combined, a trend towards significance was observed ($P = 0.068$) with the p7:c35 food being preferred over the p28:c14 food (Table 2.5).

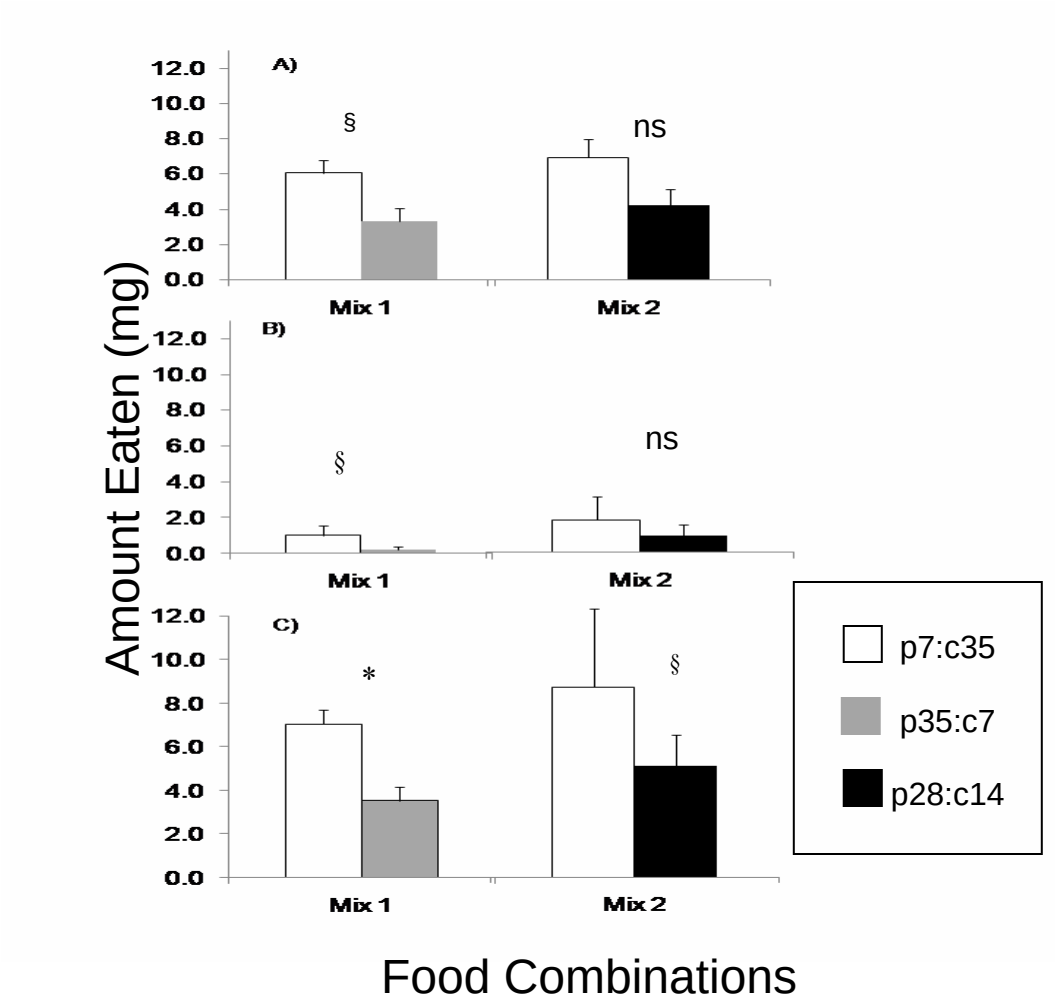

Figure 2.4. Mean weight of food eaten (mg ± $_{SEM}$) when *P.* sp.nr. *pubens* was provided two nutritionally distinct foods. Panel (A) represents the mean amounts eaten over days 0-7, (B) days 8-15 and (C) over the entire experiment (0-15d). The white bars represent the p7:c35 food, the grey bars the p35:c7 food and the black bars the p28:c14 food. * Represents the combinations that had statistically different amounts eaten ($P< 0.05$). § Represents the combinations that were not significant at the $P < 0.05$ level but show a trend at the $P < 0.1$ level;. ns= Not Significant.

Table 2.2. ANOVA analysis over the number of dead ants counted for each time period. Compares the number of dead ants in treatment 1 (Mix 1= p7:c35 and p35:c) to the amount dead in treatment 2 (Mix 2= p7:c35 and p28:c14).

ANOVA				
Treatment	df	Mean Square	F-Value	P-Value
Day 0-7	5	30.1	0.007	0.936
Day 8-15	5	6.8	0.013	0.911
Day 0-15	5	56.3	0.021	0.888

Table 2.3. MANOVA analysis when comparing the total amount of diet consumed (mg) in each treatment (i.e. p7:c35 + p35:c7 compared to p7:c35 + p28:c14). Results show the amount consumed by *Paratrechina* sp.nr. *pubens* during the time periods of 0-7, 8-15 and 0-15 days.

MANOVA			
Effect	df	F-value	*P*-value
Treatment			
Days 0-7	2,7	0.194	0.828
Days 8-15	2,7	1.054	0.398
Days 0-15	2,7	0.451	0.654

Table 2.4 Mean amount eaten by *P.* sp.nr. *pubens.* Table represents the mean amount eaten (mg) for Mix 1 and Mix2 for days 0-7, 8-15 and 0-15. Numerical representation of Figure 2.3 (bar graph).

Mean Amount Eaten (mg)				
Day	Mix 1		Mix 2	
	p7:c35	p35:c7	p7:c35	p28:c14
Day 0-7	6.047	3.297	6.550	3.975
Day 8-15	1.017	0.233	1.775	0.900
Day 0-15	7.063	3.533	8.325	4.875

Table 2.5 Wilcoxon Sign Rank Test compares the mean amounts consumed (mg) by *Paratrechina* sp.nr. *pubens* within each mix, and during each time period (i.e. 0-7, 8-15 and 0-15 d). Mix 1 compares the mean amount of the proteitn: carbohydrate ratio for the p7:c35 diet consumed to the mean amount of p35:c7 diet consumed. Mix 2 compares the mean amount of p7:c35 diet consumed to the mean amount of p28:c14 diet consumed.

P. sp.nr. *pubens* non-parametric results for all mixes						
Wilcoxon Sign Rank Test						
Test	Days 0-7		Days 8-15		Days 0-15	
Statistics	Mix 1	Mix 2	Mix 1	Mix 2	Mix 1	Mix 2
Z value	-1.682 (a)	-1.461 (a)	-1.826 (a)	-1.342 (a)	-1.992 (a)	-1.826(a)
P value	0.093	0.144	0.068	0.180	0.046*	0.068

a Based on positve ranks

* Statistically different ($P < 0.05$). Indicates a significate differences in the amount of each diet consumed for Mix 1 (with more of the p7:c35 diet consumed.

Nutrient Intake: Figure 2.5 shows a bi-coordinate plot of the amounts of protein and carbohydrate consumed over days 0-7 (Figure 2.5a), days 8-15 (Figure 2.5b) and for these two time periods combined (Figure 2.5c). Comparison of the mean amounts consumed (mg ± $_{SEM}$) of protein and carbohydrate for each experimental time unit was conducted using multivariate analysis of variance, and results indicated there was no significant difference between treatments in terms of the amounts of protein and carbohydrate consumed for either time period, or when the time periods were combined (Table 2.6).

Behavior: Data were collected for the feeding and foraging behaviors of the ant during the first 4 h of the light phase (0900-1300 h) on 1, 7 and 14 d, and these data are shown in Figure 2.6. Inspection of these figures showed that foraging to the diet box had the highest numbers, although the amount of time spent on the either of the food dishes was relatively low. Time spent on the two food dishes is shown more clearly in Figure 2.7. Inspection of this figure reveals that ants spent more time on the p7:c35 food dishes (D1) in each treatment, relative to the alternative food dish (D2).

Along with intensive observations made of the forging behavior of the colony, observations were made of the four painted forgers located in each experiment (Table 2.7). This table represents the number and location of each observation recorded for each painted forager during the six replicates and for each mixture. Observations with respect to the number of foragers on Diets 1 and 2 were removed because this behavior was only observed twice throughout all six replicates. Since observations were made every ten minutes over a 4 h period that allowed for each treatment to have a total of 24

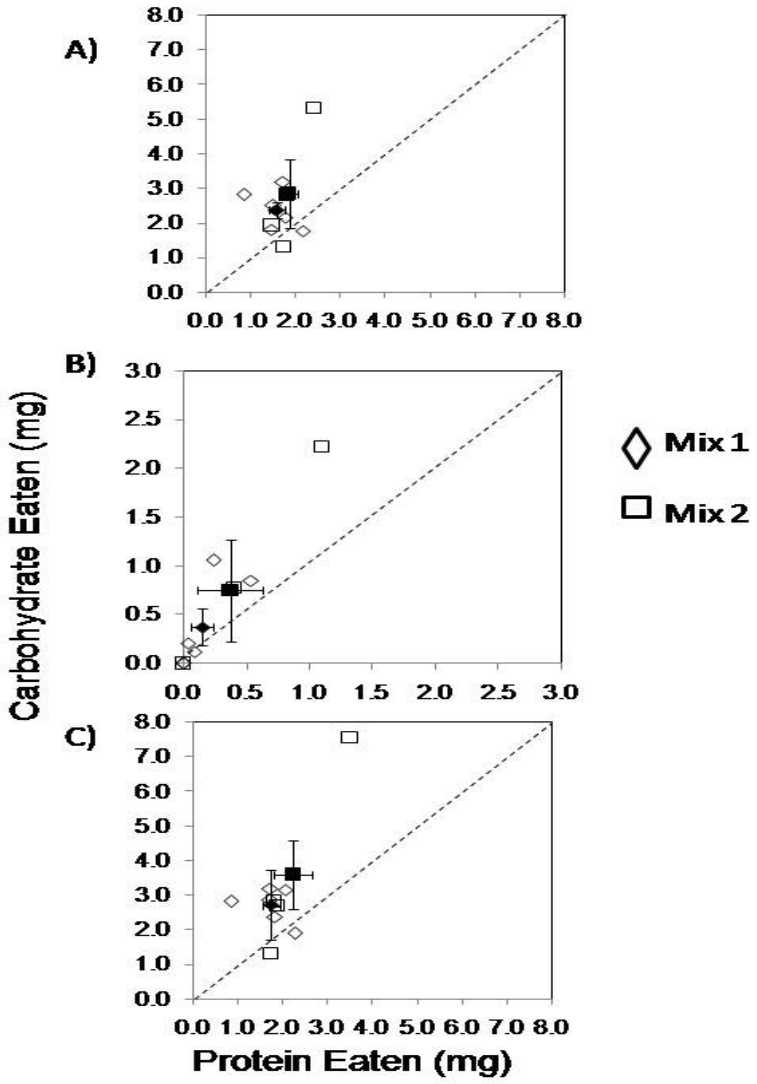

Figure 2.5: Bi-coordinate plots of the mean amount of protein and carbohydrate eaten (mg ± $_{SEM}$) for each experimental treatment (Mix 1 and 2). (A) Represents the mean amounts for days 0-7 (B) days 8-15 (C) days 0-15. Closed square or diamond symbols represent the mean amount eaten for mix 1 (p7:c35 and p35:c7) and mix 2 (p7:c35 and p28:c14) respectfully. - - - - : represents a 1:1 nutritional rail. Note that the scale axis is smaller on graph B than the A and C graphs. The values were to low to have on the same scale length as A and C.

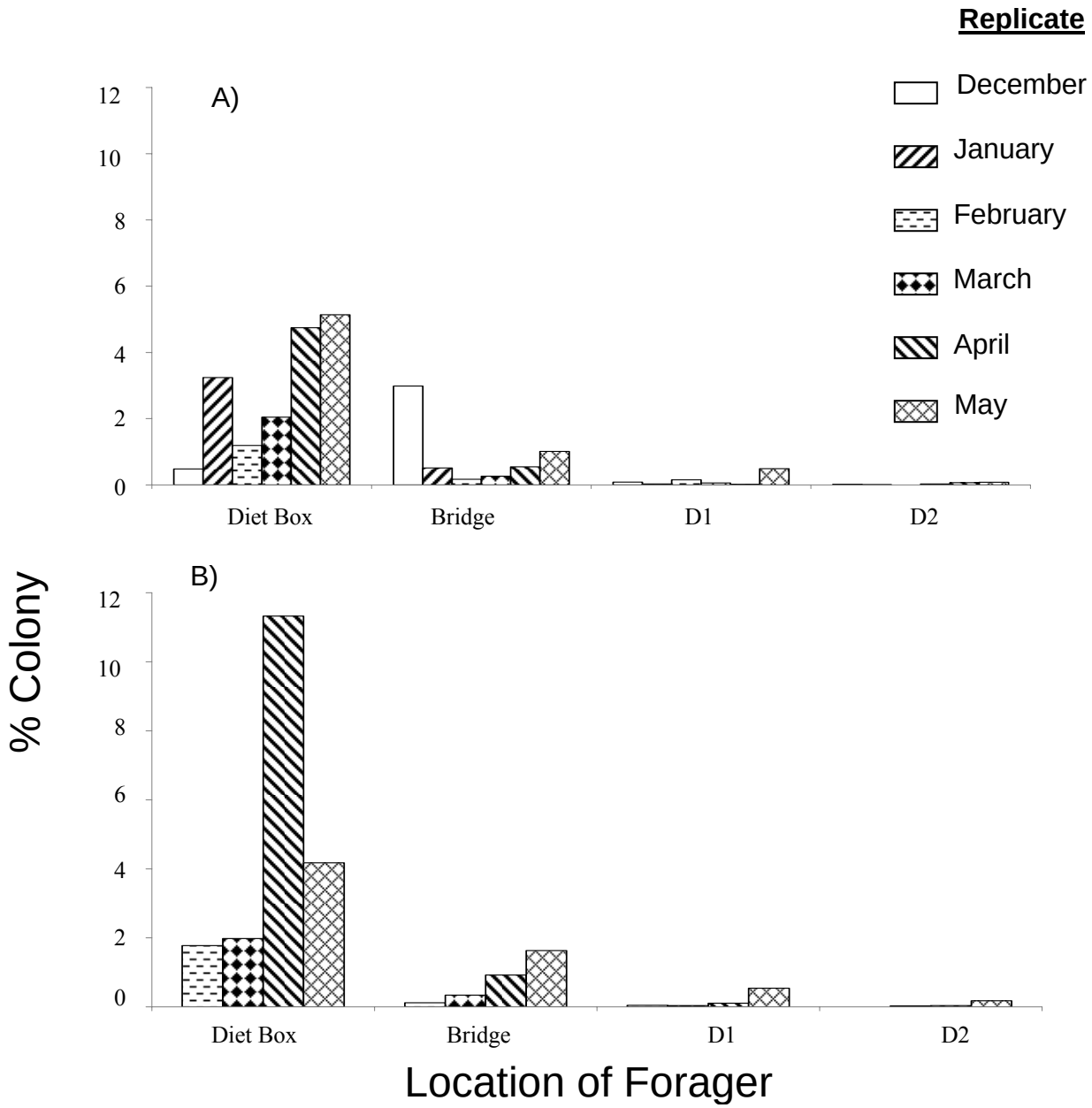

Figure 2.6. Bar graph represents the percent of the colony that spent time at different locations or performing different activities. Each bar/ pattern corresponded to a different replicate (see key). *Box* indicates the ant was observed in the box containing the two diets. *Bridge* = on the bridge connecting the two boxes. *D1* = located on diet 1 (p7:c35). *D2* = located on diet 2 (p35:c7). A) Data collected for replications of Mix 1. B) Data collected for replications of Mix 2.

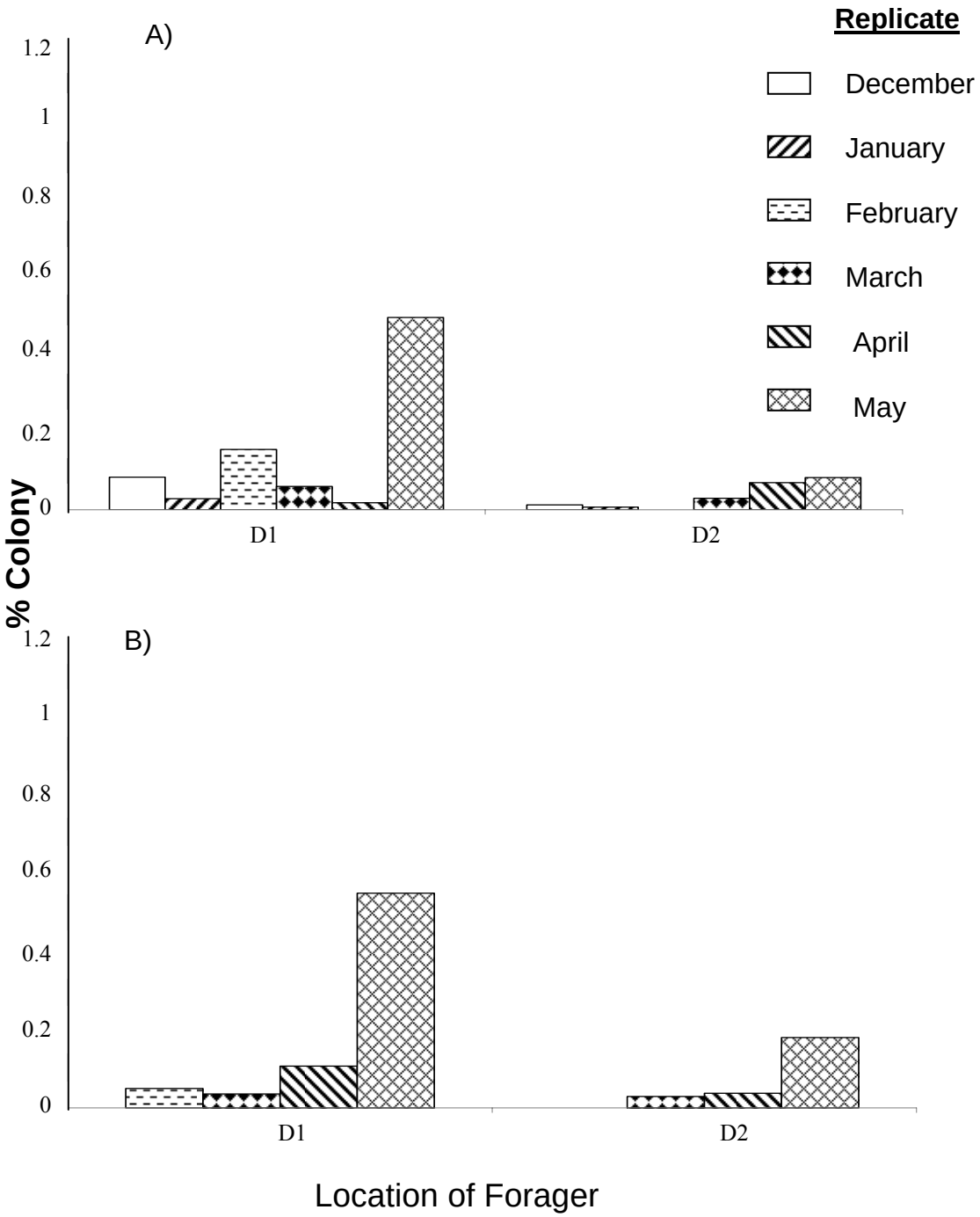

Figure 2.7. Bar graphs, representing the percent of the colony observed on Diet 1 (D1, p7:c35), or Diet 2 (D2, p35:c7 or p28:c14 for mix 2). A) Observations for Mix 1. B) Observations for Mix 2.

Table 2.6. MANOVA analysis of protien-carbohydrate intake for *Paratrechian* sp.nr. *pubnes* during the choice experiments. Test compares the amount of protein and carbohydrate consumed for treatment 1 (Mix 1= protein: carbohydrate ratios of: p7:c35 and p35:c) to the amount consumed for treatment 2 (Mix 2= p7:c35 and p28:c14).

MANOVA

Effect	df	F-value	*P*-value
Treatment			
Days 0-7	2,7	0.470	0.643
Days 8-15	2,7	0.504	0.624
Days 0-15	2,7	0.608	0.571

Note that none of the treatments showed a significant difference.

Table 2.7. Sum of the observations made for marked foragers during three intensive observation periods for each replicate (Painted foragers: A= white, B=red, C=orange, D=green). Note: Observations were made if the forager was on the different diets, but over all the replicates this behavior was only recoded twice so these numbers were removed from the table.

Location	Mix 1 (p7:c35, p35:c7)					Mix 2 (p7:c35, p28:c14)				
	A	B	C	D	Mean†	A	B	C	D	Mean†
December Replicate										
Nest	64	71	71	72	69.5	**	**	**	**	**
Colony	8	1	1	0	4.25	**	**	**	**	**
Bridge	0	0	0	0	0	**	**	**	**	**
Diet Box	0	0	0	0	0	**	**	**	**	**
January Replicate										
Nest	72	72	48*	43*	58.8	**	**	**	**	**
Colony	0	0	0	0	0	**	**	**	**	**
Bridge	0	0	0	1	0.25	**	**	**	**	**
Diet Box	0	0	0	2	0.50	**	**	**	**	**
February Replicate										
Nest	72	45	72	72	65.25	72	72	43*	72	64.75
Colony	0	3	0	0	0.75	0	0	5	0	1.25
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	24	0	0	6	0	0	0	0	0
March Replicate										
Nest	72	0*	48*	48*	42	72	72	72	69	71.25
Colony	0	0	0	0	0	0	0	0	3	0.75
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
April Replicate										
Nest	48*	72	72	72	66	72	72	72	48*	66
Colony	0	0	0	0	0	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
May Replicate										
Nest	72	69	67	48*	64	48*	72	72	72	66
Colony	0	3	5	0	2	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0

*Foragers that did not survive to the end of the experiments. **No replicates for Mix 2. † Mean number of observations for the four painted foragers being observed.

observations. When the three intensive observation periods were summed, there were 72 total observations made for each treatment. If the total number of observations was less than 72 it denotes the painted ant died before the end of the experiment. Location of the painted ants included the nest (the glass tube with the cotton plug), box (box containing the diet), and the bridge. No trends in foraging behavior can be determined from these observations.

DISCUSSION

Survival and performance of an ant colony is dependent on their acquisition of required nutrients; however, colony needs are dynamic and may change depending on whether it is growing or maintaining current population levels.

In this experiment, I presented ants with two nutritionally imbalanced but complementary food sources (one with a low protein-carbohydrate ratio and one with a high protein-carbohydrate ratio), and measured their food consumption and protein-carbohydrate intake. A previous study showed that when insects have relative easy access to carbohydrates they prefer protein, whereas species with greater access to protein prefer carbohydrates (Kay 2004). In the current study ants were provided a choice between high protein and high carbohydrate diets, so it was likely that their consumption patterns and protein-carbohydrate intake would have reflected actual needs rather than a simple preference based on a single class of nutrient.

Throughout the experiment counts of ant mortality were made (Figure 2.3). There was mortality but since they were no statistical difference in the mean number of

dead ants (Table 2.2) for all replicates our focus was on the amount of each matrix consumed. Diet removed from the diet dish, resulting in a decrease in weight, is considered consumed. Results from the consumption data indicated that ants having access to two complementary, suboptimal foods, would distribute their feeding in such a manner that more of the high carbohydrate food was eaten, which is not unexpected since carbohydrate rich foods are used as a principle metabolic fuel (Grover et al. 2007), while proteins are used preferentially for growth (Grover et al. 2007).

Two possibilities may explain the observed preference for carbohydrates. The first is that carbohydrates are essential for invasive ants. Studies have shown that the use of carbohydrate rich foods contribute to competitive performance (Grover et al. 2007), and *P.* sp.nr. *pubens* is an invasive and aggressive ant species. Perhaps preference for carbohydrates is a characteristic of this species and is a contributing factor to its ability to invade and become established in new areas.

Carbohydrate preference could also be related to its cannibalistic behavior. This idea of cannibalism, as a means for supplementing carbohydrates, was first put forth by Dlussky and Kupinaskaya (1972). They suggest that ant colonies will eat the brood *in lue* of an absent nutrient. Since little is known about *P.* sp.nr. *pubens* colony behavior, and based on observations made during this experiment; this hypothesis did not appear to be the reason for their nutrient selection. It is believed that the best explanations for increased carbohydrate consumption was due to other factors, and reflect the colonies needs rather than the result of cannibalism.

Food consumed by the colony revealed the majority of their food was consumed during the first 7 d. The lowered consumption during days 8-15 could be the result of accumulated mortality in the experimental colonies (Figure 2.3). During each replicate, mortality tended to increase as the experiment progressed and increased mortality data would have reduced the amounts of diet consumed. Alternatively, colonies may have collected enough food and were storing the excess. However, nothing is known about this ant species' foraging and storage behaviors. Since foraging is a relatively inexpensive activity (in terms of energy expended) (Fewell 1988, Baroni-Urbani and Nielsen 1990) the colony could have collected enough food, and stored it in the nest, to support their activity levels at a point in time. Research has also shown that some ant species store excess fat and pass it on to colony members through lipid-rich oral secretions (Hahn 2006). It could be common practice to forage heavily until enough food is stored up and thus reducing later foraging activates. Further studies into foraging and storage behavior would have to be conducted to determine if this was the case. Based on personal observations, increased mortality seems the most likely explanation for reduced food consumption with time.

Ants in this study tended to ingest more carbohydrate than protein, and this pattern held when the data were partitioned down into different time periods (days 0-7, 8-15 and 0-15) (Figure 2.5). Although they preferred carbohydrates to protein they did show active regulation between the two diets. This balance in consumption between diets was seen since the regulation points, for each treatment (i.e. Mix 1 and Mix 2), were running along a similar trajectory. If they were feeding randomly in each treatment

it would be expected to see two separate trajectories, not a single trajectory. Also, if it were the case of just wanting carbohydrates they would have fed solely on the p7:c35 diet and not regulated between the two. This regulation between two suboptimal diets showed the actual nutrient selection of the colony. Since this trajectory stayed relatively similar in all time units, it further supported the evidence that this was their preferred protein-carbohydrate intake point.

The determination of the protein-carbohydrate intake, or nutrient selection, for ant colonies was the first time this had been done. Although there has been research done on ant dietary needs (Stanley and Robinson 2007, Boaretto et al. 2003), they have not evaluated their nutrient selection, rather have focused on diet selection. Research conducted by Stanley and Robinson examined the attractiveness of food that was classified as high protein or high carbohydrate, not the exact protein-carbohydrate needs of the colonies. Their research on *Paratrechina longicornis* Latreille, showed they preferred protein rich tuna when presented a choice between various diets and baits (Stanley and Robinson 2007). These other baits/ diets examined included Amdro® (BASF Corporation, Research Triangle Park, NC), Boric acid + water, Maxforce® (Bayer Environmental Science, Research Triangle Park, NC), sugar water, and Xstinguish® (Bait Technology Ltd., North Harbour, Auckland) all of which had a primary nutrient class (i.e. Lipid, Protein, or Carbohydrate). In comparison, *Atta capiguara* Goncalves, was not attracted to sugar or artificial sweeteners when given a choice from multiple sugar substances including sucrose, fructose, lactose, or glucose (Boaretto et al. 2003).

The Argentine ant, *Linepithema humile,* Mayr has been shown to exhibit dietary shifts (Abril et. al 2007) over time. Research over seasons showed that during times of reproduction the colonies consumption of protein rich prey (i.e. other insects) increased (Abril et al. 2007), while during other periods their consumption of energy rich sugars, obtained from tending aphids, increased (Abril et al. 2007). Increases in energy rich sugar collection corresponded to increased colony activity since they had to tend to new brood and collect enough food to support the growing colony.

Behavioral data support the hypothesis of seasonal dependent foraging. Trends shown in Figure 2.6 showed the majority of the foraging activities were to the diet box, for in both treatments. When seasonality (i.e. December, January, and February = Winter; March, April, and May = Spring/ Summer) was considered (Figure 2.5), there was a trend of increasing foraging behavior as time changes. This trend can be seen in the incremental increase of foraging behavior as the colonies move towards the warmer seasonal months. Since these replicates were not repeated over the same seasons no clear conclusion can be drawn; however, this increased foraging, because of seasonal changes, could be the result of warmer months being the typical time for larval and pupal production (Thomas 2003). Future research over multiple seasons could show the seasonality effects in more detail.

Using a bridge to connect the treatment boxes served as a means to observe foraging behavior and allow the assumption that individuals on the bridge were collecting food. Numerous studies have centered on the use of a bridge (Dussutour et al. 2004a, Dussutour et al. 2004b, Dussutour et al.2005) to demonstrate foraging for food,

and allowed the ants the shortest path to the food (Beckers et al. 1992). This design ensures that both diets were equal distances from the nest and equal opportunity to feed on either diet. One limitation of these methods is the observations were made every 10 minutes and not continuously which could result in a forager being counted twice. A suggestion for future research would be to make continuous observations. Another area of focus was the number of ants foraging on the diet dishes. Observations made over this behavior shows a preference for diet 1 (p7:c35), which supports the preference for carbohydrate rich foods.

Behavioral observations on a selective number of individuals (painted foragers) (Table 2.7), were made, but unfortunately the data do not show any trends other than the majority of their time was spent inside the nest. The majority of the observations recorded shows the painted foragers in the nest and could be due to the painted foragers not being true foragers. To avoid these situations in the future, it would be useful to select confirmed foragers. The data collected on individuals in the current study are not ideal for any statistical analysis, but it does provide insight for future research.

This experiment showed the experimental approach of the Geometric Framework can be applied to the study of ants and can be conducted at the colony level. The experiments also gave us a better understanding of the nutritional regulation of *P.* sp.nr. *pubens* and may lead to the production of a better baiting system. This system can in turn help control the ever evolving problem of this invasive ant species.

CHAPTER III

NUTRITIONAL REGULATION OF *Paratrechina* sp.nr. *pubens* WHEN PROVIDED A SINGLE NUTRIENT SOURCE

OVERVIEW

Colony fitness, size, and reproductive potential are determined by their ability to locate and consume the optimal amounts of various macronutrients. Understanding the nutritional regulation of an ant colony furthers our understanding of its life history and can be used to produce a better baiting system for its suppression. We used the "Geometric Framework" to conduct experiments determining how *Paratrechina* sp.nr. *pubens* regulated their protein and carbohydrate intake when confined to a single food source with a known protein-carbohydrate ratio. By analyzing how much they consumed it can be determined which diet they preferred to consume, as well as which nutrients they prefer to have an excess or deficit of. In addition to examining their compensatory mechanisms, their foraging behavior was examined through three 4 hr observation periods for each 15 d experiment. Examination of the amount eaten showed a higher consumption rate of the carbohydrate rich foods (p7:c35 and p14:c28), with the p14:c28 having the most consumption as well as the lowest ant mortality data. It was also observed that the colonies more tightly regulated their protein intake when compared to carbohydrates.

INTRODUCTION

Acquisition of required nutrients is essential to the survivability of an ant colony. Central to this is the consumption of protein, which is utilized for growth and reproduction, as well as carbohydrates that are needed for energy (Scherer 2007). Colony fitness, size, caste and reproductive capacity all depend on locating and ingesting the required amounts of these macronutrients (Hughes 1993, Cassill and Tschinkel 1995).

Little data on the amount of macronutrients collected by ant species is available (Tschinkel 2006). And no reported studies have examined food consumption when macronutrient content is known. Furthermore, little information on seasonal shifts as it relates to nutrient needs of ant colonies and the effects of these shifts on ant colonies is known (Abril et. al 2007).

Most work done in the area of ant nutrition focused on the attractiveness and effectiveness of bait matrixes for their suppression (Stanley and Robinson 2007). Development of matrixes as baits for fire ants (*Solenopsis invicta* Hymenoptera: Formicidae), have been studied the most. Different ant species have varying nutritional needs. Therefore, baits that are attractive to fire ants would not translate into attractiveness to another species. Insight can be gained into why ant species neglect or prefer particular food items by studying their foragers as it relates to regulation of their nutrient intake. This information can then be used to understand how the colony is regulating its specific nutritional needs (Howard 1987, Voelkl et al. 1999, Kay 2004).

One approach for analyzing the optimal proteins and carbohydrates needed by ant species is to examine their foraging behavior within the context of the 'Geometric

Framework' (Raubenheimer and Simpson 1993a). Briefly, this approach allows the organism of interest to demonstrate the proportions of nutrients that are most preferred (Behmer et al. 2001). This balance, called an 'intake target' (Raubenheimer and Simpson 1993a), reveals the amounts of nutrients (e.g. protein and carbohydrate) ingested by an animal over a given period of time. Determination of the intake target is achieved when an organism is given a choice between two sub-optimal, but complementary food choices. Colonies are able to achieve their intake target by eating between the two complementary foods.

P. sp.nr. *pubens* will be forced to consume foods that are imbalanced and thus the intake target cannot be reached because complementary foods or nutritionally balanced foods are unavailable. Lack of optimal food will force the colony to make a nutritional decision to under-eat some nutrients, while over-eating others. By examining these consumption patterns it makes it possible to determine the nature of a colonies nutritional compromise (Raubenheimer and Simpson 1998). Although most research using this approach has been conducted on plant-feeding insects (Simpson and Raubenheimer 2000, Behmer et al. 2001, Behmer et al. 2003), it can easily be applied to a range of insects, including ants, which are regulating nutrient intake at both the individual and colony level.

In this study, I examine how *Paratrechina* sp.nr. *pubens* ant colonies compromise their protein and carbohydrate intake when given access to only one food source. By observing the foraging behavior and consumption rates of different artificial

foods with known nutrient profiles I was able to assess whether, and to what extent, this ant actively defends a protein-carbohydrate intake target.

MATERIALS AND METHODS

Insects: *Paratrechina* sp.nr. *pubens* were collected in Pearland, TX (GPS coordinates: N 29°33.518, W 095° 20.531). Finding colony locations was accomplished by examining known nesting habitats. These sites included, but were not limited to, under fallen tree limbs, in leaf litter, or by digging approximately 50.8- 305 mm into the soil. Once colonies were located, they were sight identified to be the appropriate species and then shoveled along with the dirt or debris present into a bucket (22 L) that had its sides treated with baby powder (approx. 1 oz) to prevent the ants from escaping. Colonies were then transported to the Center for Urban and Structural Entomology, Texas A&M University, College Station, TX. The colonies were removed from the dirt/debris in the bucket by a water dripping method which is frequently used to separate ant colonies. This method was done by placing the dirt/ debris under a faucet, which slowly dripped (approx. 2 drips per second) water into the bucket. At the top of the dirt pile a Petri dish (d=8.5 cm, H= 1.5 cm), half way filled with plaster of paris, was placed and acted as an artificial nest. Occasionally one artificial nest would not be large enough to hold the entire colony. When this happened multiple petri dishes were placed in the bucket. When the water rose the colony would move up the mound of dirt and into the artificial nests. Once the colony was inside the nests they were removed and placed together into a plastic box (30.5 x 16.5 x 8.9 cm). The inside walls of the boxes was treated with

Fluon® (Polytetrafluoro-ethylene, ICI Fluoropolymers INC, Exton, PN, U.S.A.) to prevent the ants from escaping. Inside these plastic boxes two water sources were presented to the ants. One source was a 75 ml glass jar with a plastic top and cotton wick (Braided Rolls made by Richmond Dental, Charlotte, NC) filled with water. This source provided moisture. The other source was a plastic container (5.5 x 4.5 x 2 cm) filled with cotton balls soaked with a 20% honey water solution. This source provided carbohydrates (Chapman 1998). A prey source was also included in each colony box and consisted of approximately five to six dead crickets placed in a plastic container (5.5 x 4.5 x 2 cm) (Orthoptera: Gryllidae). The amount of water, sucrose, and crickets were checked daily and replenished when needed. The colonies were maintained in a growth chamber (made by Elliott-Williams model: Conviron 8601) at 30±2°C, 12:12 light: dark, and 60% RH.

Over the course of the experiment, six separate colonies were collected and processed using the methods previously described. Each colony represented a replicate. Colonies were collected from December 2006 until May 2007 (Table 3.1). For purposes of this experiment December through February are considered winter months and March through May are spring/summer months. This definition of seasons was done in order to make possible correlations between shifts in behavior and seasonal cycles.

Experimental Foods: The experimental foods used were a dry, granular, chemically defined matrix and were prepared as described in Behmer et al. (2001). Variations of protein and digestible carbohydrate gave rise to the following six combinations of protein (p) and carbohydrate (c): p7:c7, p7:c35, p14:c28, p21:c21, p28:c14, and p35:c7

(all values are expressed on a percentage dry weight basis). Results from Chapter II showed evidence that *P.* sp.nr. *pubens* prefers a carbohydrate rich diet. Also, the analysis of protein and carbohydrate consumption when given two food choices (Chapter II) shows the ants eating along a nutritional rail very similar to a p14:c28 diet. These six diets represent an array of varying protein- carbohydrate ratios, and all but the p14:c28 diets are currently being considered suboptimal. With no choice of the diet provided to them, they should start to show a regulation which prefers a deficit or excess of carbohydrates or proteins. All six foods had equal total amounts of protein plus carbohydrate and, therefore, also contained identical proportions of the other ingredients, including indigestible cellulose powder (Table A-2).

Experimental Ant Colonies and Test Arenas: Collected colonies remained in their original colonies between 3-6 d before members were removed make the experimental treatments. A sample of specimens from a colony was used in an experiment. These experimental ant colonies consisted of one functional queen, 250 workers, and approximately 10 mg of brood. Each colony was housed in a glass test tube (1.6 x 15 cm) that served as both a water source and nest. The tube was filled half way with distilled water with a cotton plug used to keep the water from spilling out.

Experimental arenas (Figure 3.1a) consisted of two separate plastic boxes (9 cm high x 16.5 x 30.5 cm) set adjacent to each other. Both boxes had there sides treated with Fluon ® in order to prevent the ants from escaping. One box contained the ant colony, and the other contained the experimental foods (described below). Within the

food box, dishes of food were equally spaced 3.8 cm from the base of the bridge and 7 cm and 9 cm from the box walls (see Figure 3.1b).

The colony box and diet box were connected to each other via a paper bridge (Figure 3.2), which was 45.7 cm by 2.5 and made of printing paper (Sparco Brand, Atlanta, GA). This bridge was used because in house studies demonstrated that this type of bridge was sturdy and allowed for easy observations of foraging behaviors. Measuring from either end, approximately 11cm up, the bridge was bent to form two 90° angles (Figure 3.2). At the base of each end of the bridge, a Plastic tube stopper weighing approximately 11 g was placed to anchor the bridge upright throughout the entire experiment. When the bridge was added to the arenas, one base was placed in the center of the colony box and the other base was placed in the center of the diet box and 3.8 cm away from the diet.

Experimental Protocol: This experiment consisted of six treatments. These included the p7:c7, p7:c35, p14:c28, p21:c21, p28:c14, and p35:c7 diet. Along with these combinations a control was used, and consisted of crickets that were ground up by using a hand held coffee grinder. These were used to determine if the amount the ants were eating was because of the diets or if it was normal for any food presented to them. These treatments were needed to demonstrate that nutrient protein-carbohydrate is regulated to the point of defending their intake target and to determine which macro nutrient they prefer to have in deficit or excess.

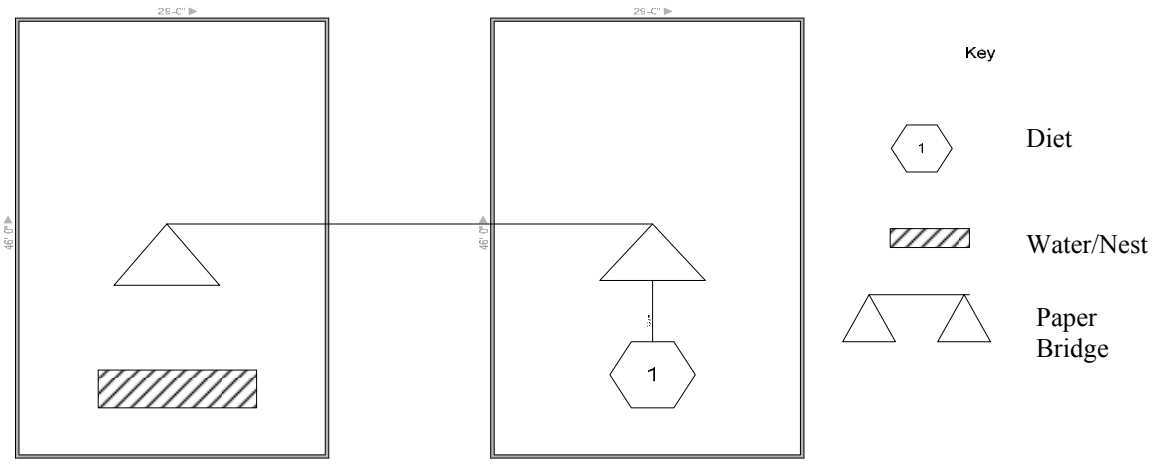

Figure 3.1 a. Diagrammatic representation of experimental set-up for the no-choice test. Rectangles depict the plastic boxes used to house the experimental colony, and the experimental diets. Rectangle with the lines through it shows where the water source/ nest were located. Pentagon represents the location of the dietary dish. The triangles with the line connecting them represents where the paper bridge were located. Figure 3.1b shows the boxes to scale.

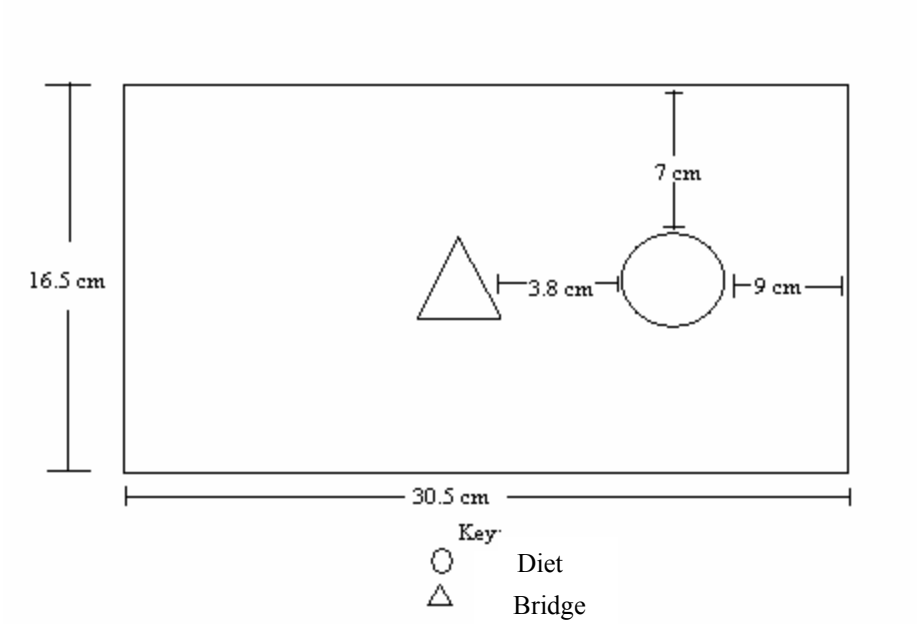

Figure 3.1 b. Diagrammatic representation of the exact location of the diet within the *diet box* relative to the walls and bridge.

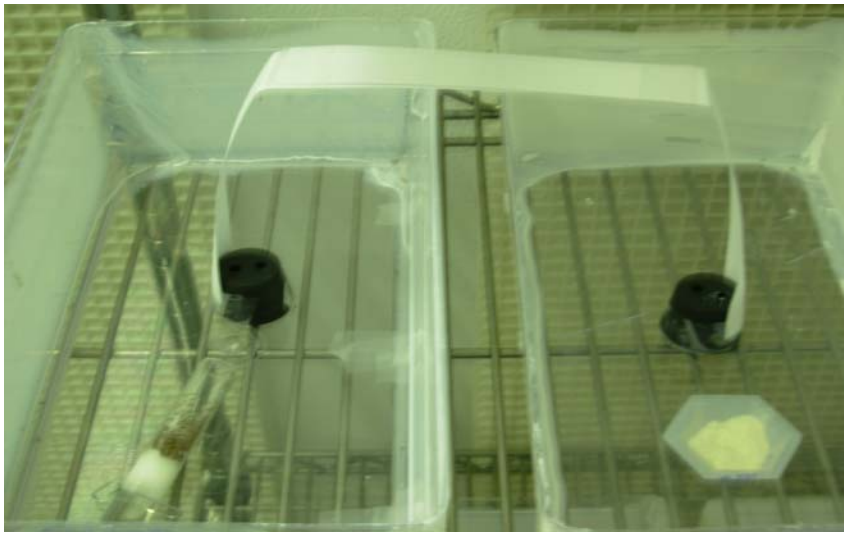

Figure 3.2. Visual representation of experimental set up.

To ensure a uniform level of hunger at the start of each replicate, each experimental colony was deprived of food for 15 h. Prior to the start of this starvation period, 1.0 g of food was weighed out and allocated to their respective plastic dishes (pentagon shaped dish: L 2.5 x W 2.5 x H 1 cm). After which they were placed under a heating lamb for 15 h, which allowed the diet to equilibrate to a constant humidity level. At the end of the 15 h the diets were weighed again (at the 0.0001 g level), and then placed in the diet box. Next the bridge was added, thus connecting the two boxes. This marked the start of the experiments.

The colonies were allowed to feed for 7 days, after which each food dish was removed and replaced with a fresh, pre-weighed dish of the same food type (using the same protocol as described above). The food dishes that had been removed were placed under the heating lamps for 15 hours and then reweighed. At the end of the experiment (day 15), the two food dishes were removed, placed under the heating lamps for 15 h, and then re-weighed.

In total, each treatment (p7:c7, p7:c35, p14:c28, p21:c21, p28:c14, and p35:c7) had six replications and each replication used a colony collected that month (Table 3.1). Throughout the course of the 15 d experiment, mortality counts were taken every day. These counts were made to determine if there was any correlation between mortality and consumption.

Ant Behavior: Four foraging ants were removed prior to the initiation of each trial and marked in order to record their foraging behavior. Selected ants were removed and

Table 3.1. Collection dates for *Paratrechina* sp.nr. *pubens* from the field (Pearland, TX).

Replicate	Dates of Collection
1	12/2/2006
2	1/3/2007
3	2/2/2007
4	3/2/2007
5	4/1/2007
6	4/28/2007

placed on a chill table and a mark placed on the dorsum of their abdomen. A toothpick was used to mark the ants with one of four colors of paint (red, white, orange, and green). Paints used were Nissen® Metal Marker in a Bottle: Permanent Paint Marker (Nissen, Glenside, PA). They were selected due to being oil-based and being less toxic to ants than other markers (Wojoik et al. 2000). Once marked, they were observed in a plastic box (9 cm high x 16.5 x 30.5 cm) for approximately 10 min to ensure the paint was dry and did not hinder their movements. If the paint was shown to hinder their movements, the ant was not used in the experiment and another forager was marked. Marked foragers were returned to their original colony.

Observations were made on 1, 7, and 14 d of the experiment. Each observation consisted of recording ant foraging behavior every 10 min for 4 h between 0900 and 1300 hr. Information recorded included the location and activity of the marked foragers, the number of foragers on the diets, bridge, and in the diet box. Along with the intensive observations daily moribund ants were counted and removed to prevent cannibalism.

Statistical Analysis: To analyze the consumption of the diets, counts over number of dead ants, as well as consumption adjusted for mortality I used analysis of variance (ANOVA) with the statistical package SPSS 15.0.

RESULTS

Mortality: The number of dead ants as well as the mean amount of morbid ants ($\pm$ $_{SEM}$) for each treatment and for each replicate (i.e. collection months) over the 15 d experiments is provided in Figure 3.3. When the mean number of dead ants from each

treatment was compared (number of dead out of 250 alive ants), no statistical differences were observed (Table 3.2). However, there were more dead ants counted in the April ants than any other month. Ants that fed on the p14:c28 diet had the fewest number of dead ants when compared to the other experimental diets (p14:c28 mean number of dead ants = 91; p7:c7=132; p7:c35=168; p21:c21=115; p28:c14=99; p35:c7=120). On average, the number of dead ant units counted in April and May was the highest number at the conclusion of the 15 d experiment (Figures 3.3 and 3.4). Figure 3.4 also shows a trend with increasing mortality as the month changes.

Consumption: Figure 3.5 shows the mean amount consumed from each treatment over days 0-7 (Figure 3.5a), days 8-15 (Figure 3.5b), and for these two periods combined (Figure 3.5c). When the pattern of consumption from these treatments was compared for each time period, no statistical differences were observed for the parametric test (Table 3.3). The carbohydrate rich diets (i.e. p7:c35 and p14:c28) was consumed in the greatest amount relative to the protein rich foods (p28:c14, p35:c7). The 1:1 ratio diets (p7:c7, and p21:c21) had the lowest consumption amounts. The amounts of the control matrix consumed were comparable to the other diets.

Comparison of the amount consumed and the mortality data shows when mortality increases the amount consumed decreases (Figure 3.6). These graphs show the

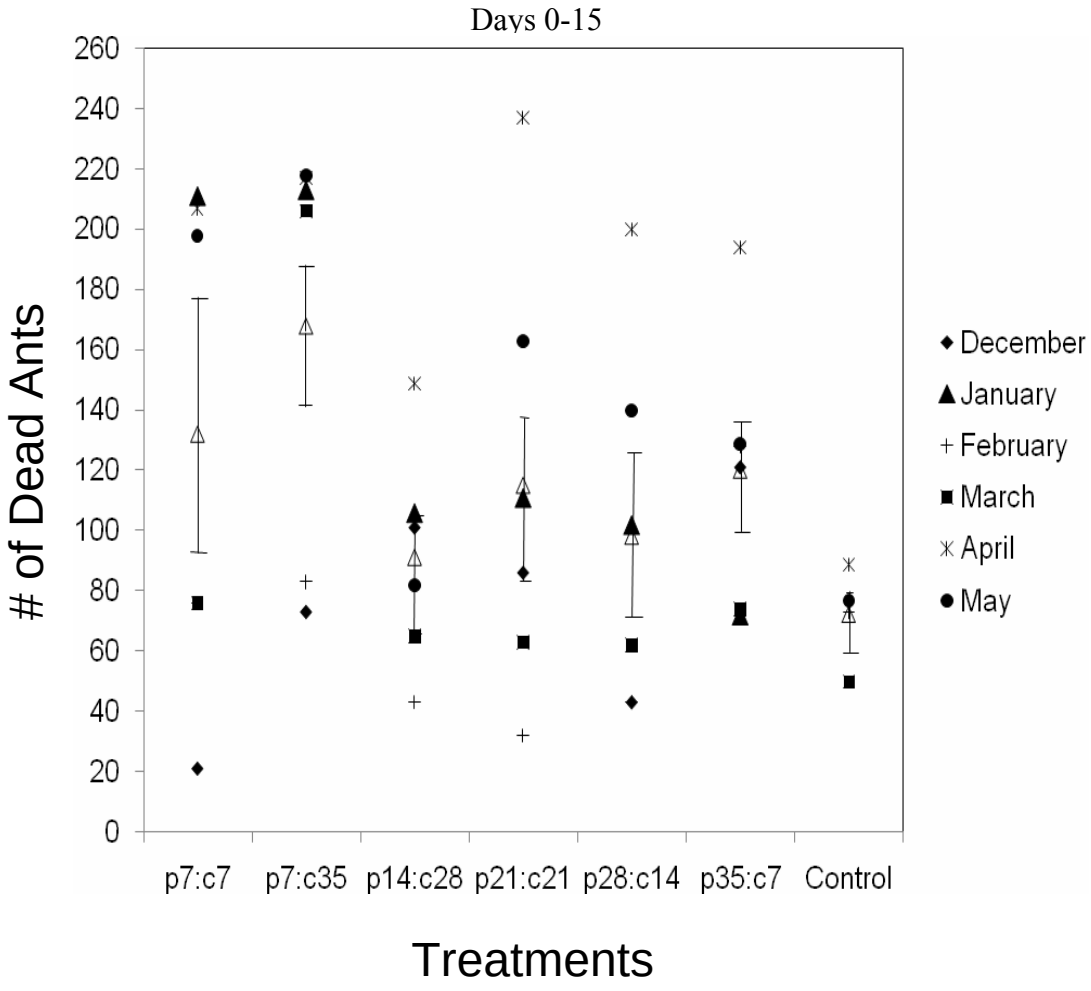

Figure 3.3. Bi-coordinate plot representing the mean number (± $_{SEM}$) of dead ants counted at the end of each 15 d experimental treatment for six replicates. [Note: 'p' stands for protein; 'c' stands for carbohydrate.]

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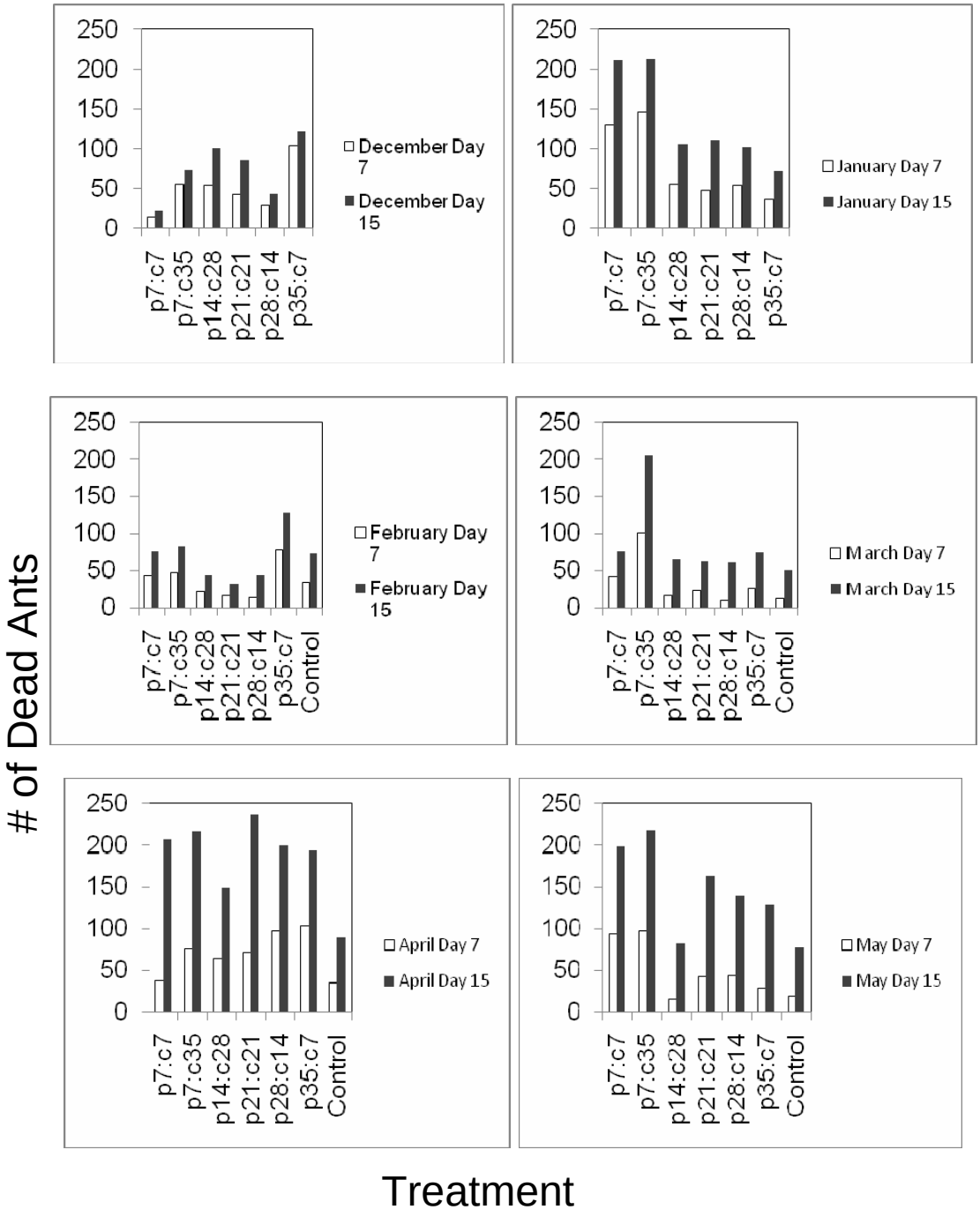

Figure 3.4. Bar graph representing the number of dead ants counted for each monthly replicate. White bars represent the number of dead ants counted during days 0-7, while the black bars represent the counts for the entire experiment (0-15 d).

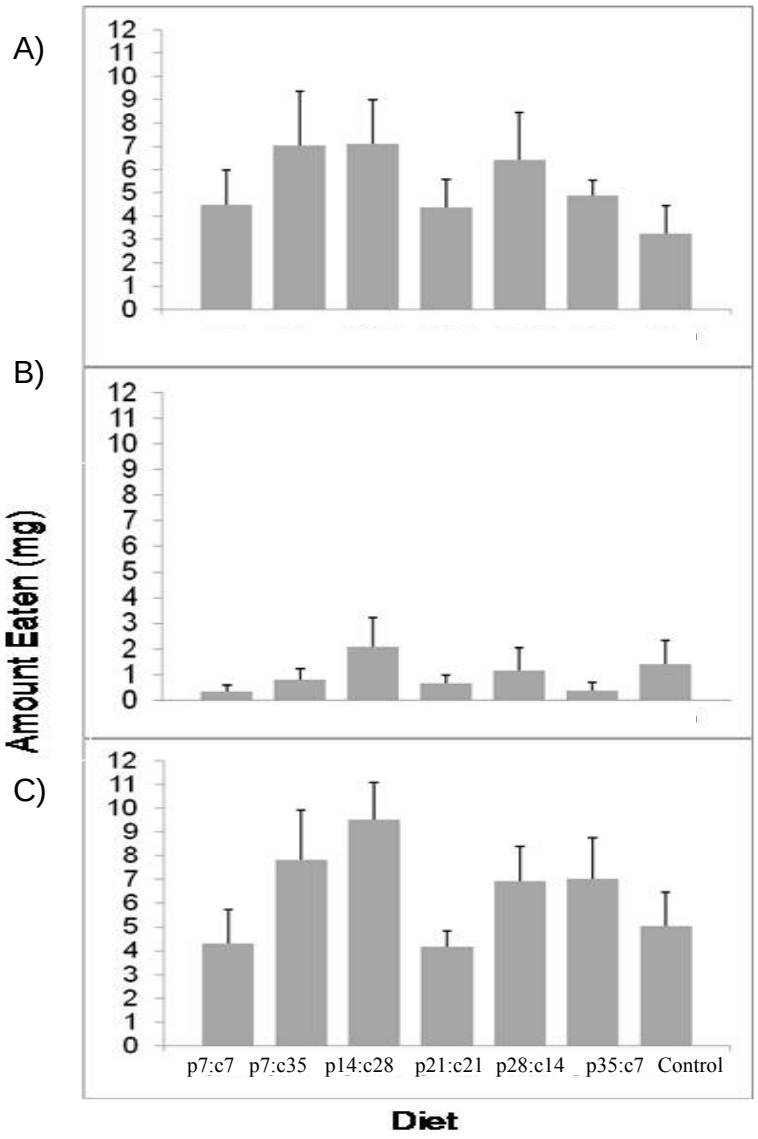

Figure 3.5. Mean amount of food consumed (mg ± $_{SEM}$) when *P.* sp.nr. *pubens* was provided a single food choice. Panel (A) represents the mean amount eaten over days 0-7, (B) the amounts eaten over day 8-15 and (C) the amounts eaten over the entire experiment (days 0-15). p = protein; c = carbohydrate.

Table 3.2. Analysis of mortality ($\pm_{SEM}$) using ANOVA.

ANOVA				
Treatment	df	Mean Square	F-Value	P-Value
Day 0-15	6	5205.919	1.383	0.250

Table 3.3. Analysis of amount consumed ($\pm_{SEM}$) using ANOVA.

ANOVA				
Treatment	df	Mean Square	F-Value	P-Value
Day 0-7	5	9.584	0.167	0.688
Day 8-15	5	2.557	1.029	0.419
Day 0-15	5	22.080	1.627	0.187

p21:c21, p28:c14, and p35:c7 diets had similar consumption and mortality data. Each graph shows the mortality vs. consumption for each month replicated. Analysis of these months shows that April had the highest mortality numbers, and some of the lowest consumption numbers (Figure 3.6). While the carbohydrate rich diets (p7: c35, and p14: c28) were consumed at consistently higher rates (Figure 3.6).

Figure 3.7 shows a bi-coordinate plot of the amounts of protein and carbohydrate consumed by the ants for each day of each treatment. Consumption results from Chapter II are also shown on each graph (light grey points) and were used as a comparison between Chapter II choice test and Chapter III no-choice test.

Consumption of protein and carbohydrate was adjusted for mortality for each treatment during each day of the experiment (Figure 3.8). Points depict the amount of protein and carbohydrate potentially eaten per ant. Morality was adjusted by taking the amount consumed for each treatment and dividing that number by the number of ants alive at the end of the experiment. When the consumption patterns for each treatment were compared no statistical difference was observed (Table 3.4).

Behavior: Data were collected for the feeding and foraging behaviors during the first 4 h of light phase (0900-1300 h) on days 1, 7 and 14 (Figure 3.9). Inspection of these figures showed that the majority of the colony was foraging in the diet box, although the amount of time spent on the experimental diets was relatively low when compared to other foraging behavior. Along with intensive observations made of the forging behavior of the colony, observations were made of the four painted foragers located in each experiment. Table 3.5 summarizes the number and location of each observation

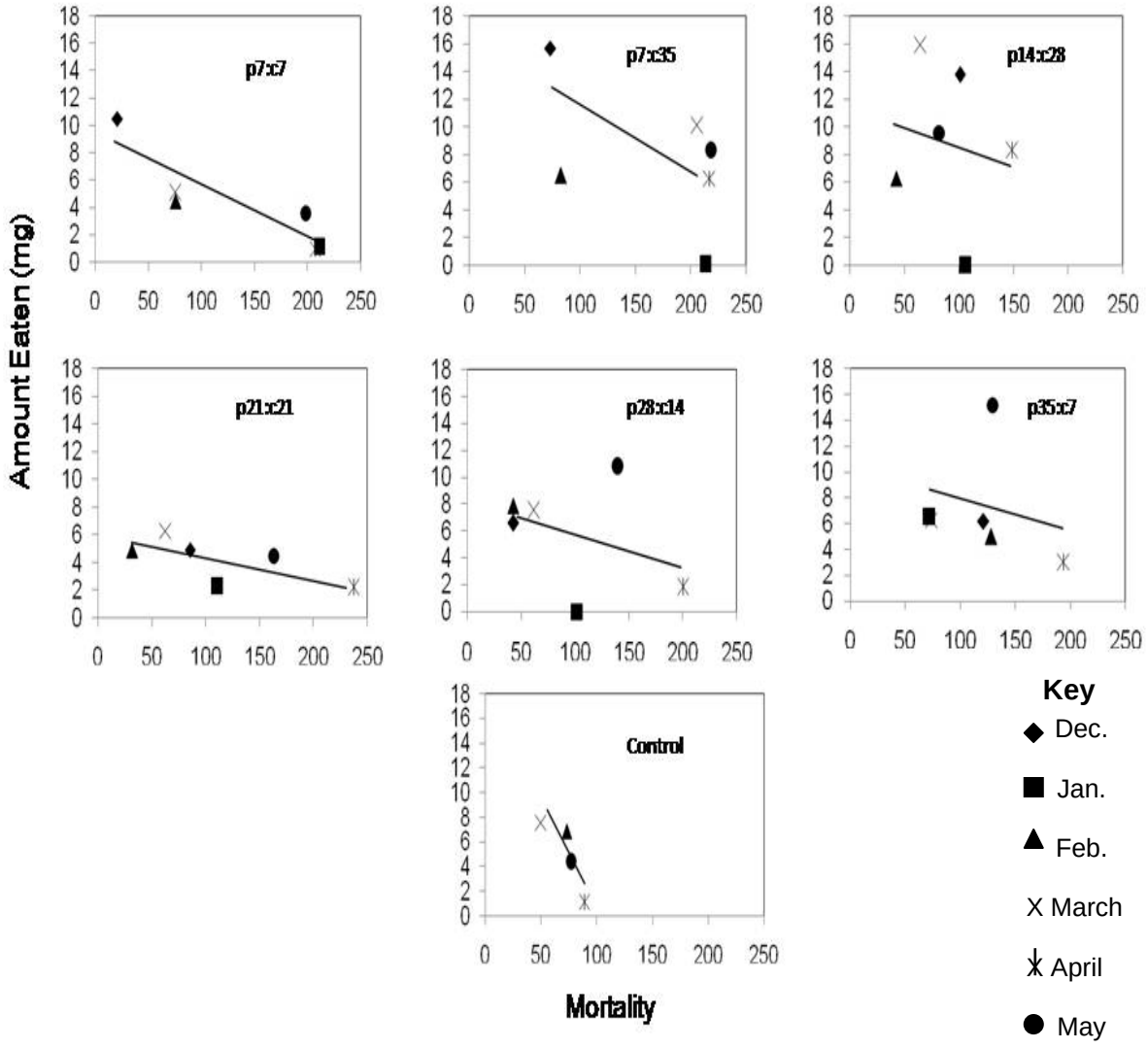

Figure 3.6. Scatter plot of number of dead ants counted versus the amount eaten (mg) for each treatment. Symbols (see key) represent the different replicates (months). Lines represent a negative linear trend as the number of dead ants increases the amount consumed decreases.

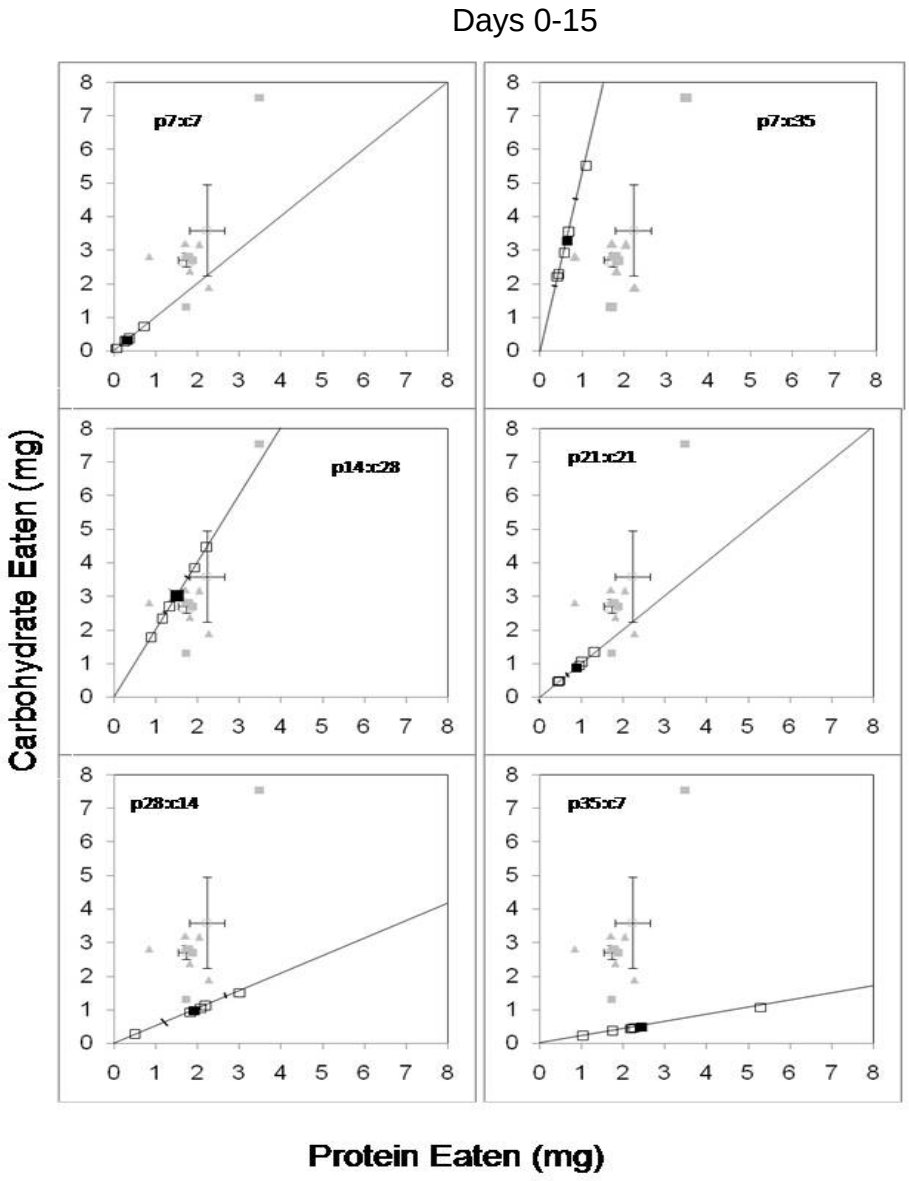

Figure 3.7. Bi-coordinate plots of the amount of protein and carbohydrate eaten (mg ± $_{SEM}$) for the six nutritional diets examined in the no-choice experiments. All plots depict amount eaten for the entire experiment (days 0-15). Closed symbols represent the mean amount eaten for all replicates, while open symbols represent amounts eaten for separate replicates. Solid lines depict the *nutritional rail* each diet lies on. Light grey triangles and squares = Chapter II data.

Adjusted for Mortality Day 0-15

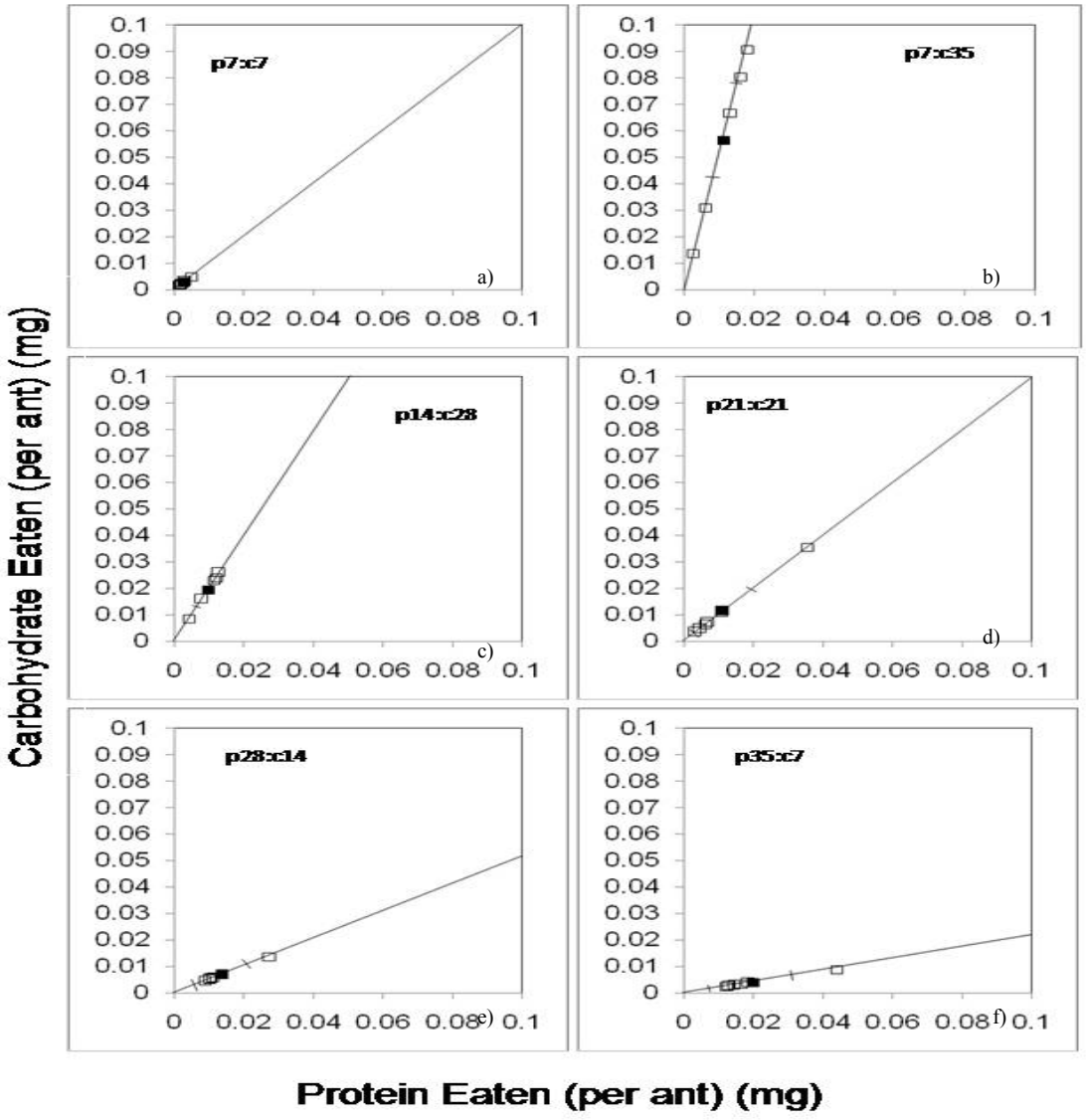

Figure 3.8. Bi-coordinate plots of the amount of protein and carbohydrate eaten (mg ± $_{SEM}$) when adjusted for mortality. Axes depict amount consumed per ant. (A) Represents days 0-7 (B) days 0-15. Each line represents the *nutritional rail* each diet lies on. Closed symbols depict mean amount consumed, while open symbols represent amount consumed for each replicate.

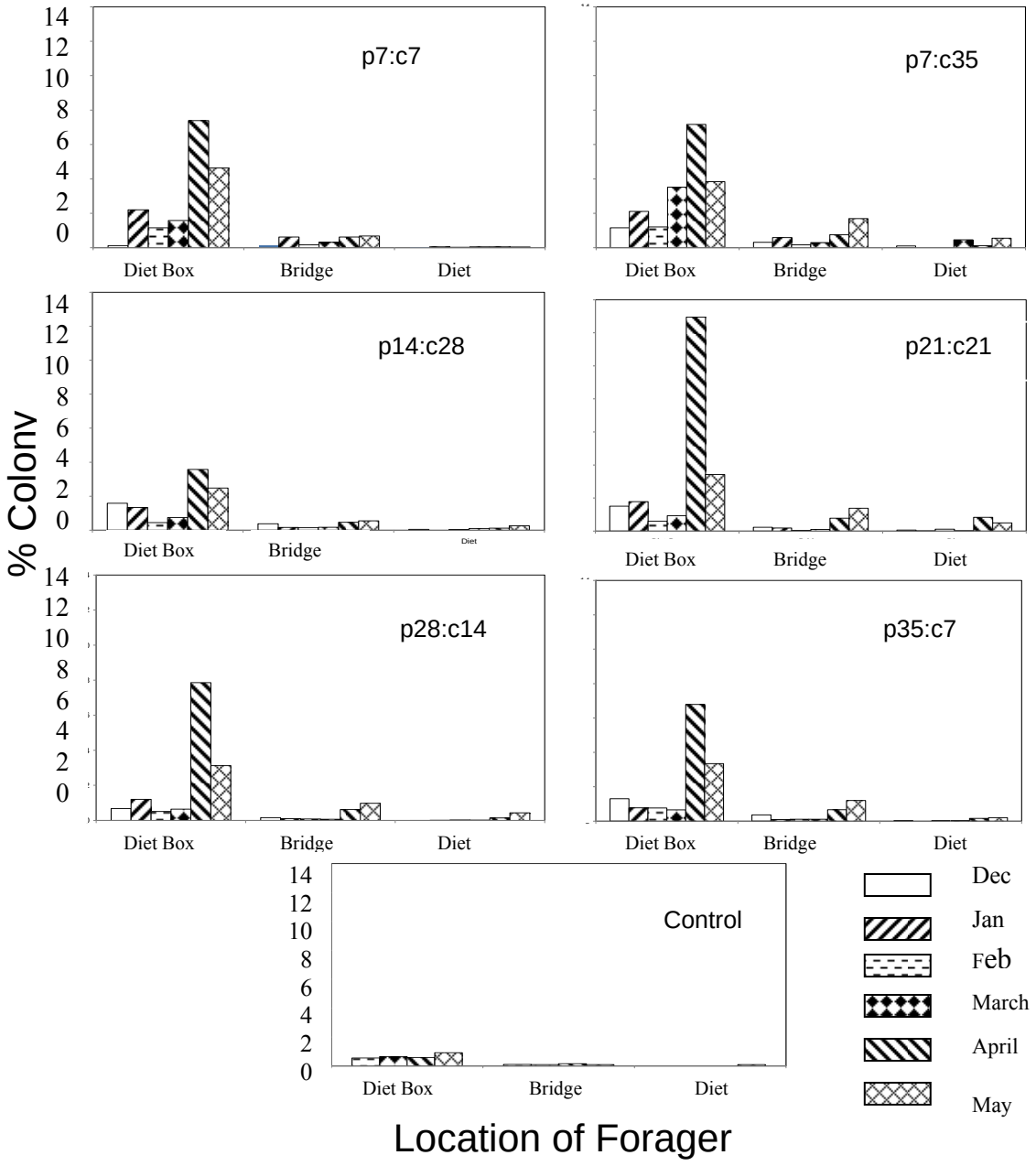

Figure 3.9. Bar graph represents the percent of the colony that spent time at different locations or preforming different activities. Each pattern correspondes to a different replicate (see key). *Diet box* indicates the ant was obsereved in the box containing the two diets. *Bridge* indicates ants that were observed on the paper bridge connecting the two boxes. *Diet* represents the number of ants located on the no-choice experimental diet.

Table 3.4. Analysis of consumption once adjusted for mortality ($\pm_{SEM}$) using ANOVA.

ANOVA					
Treatment		df	Mean Square	F-Value	P-Value
	Amt Consumed (per ant)	5	0.007	2.370	0.065

recorded for each painted forger during the six replicated and for each treatment. [Observations with respect to the number of foragers on the treatment diets were removed because this behavior was never recorded for all six replicates.] No trends in forging behavior can be determined from these observations.

DISCUSSION

Colony fitness, size, and reproductive capacity all depend on foragers acquiring needed nutrients. Central to this is the consumption of the macronutrients, protein and carbohydrate. Changes in the amount of macronutrients needed depended on whether the colony was actively growing or just in a maintain mode.

This experiment, I presented ants with one of six foods and measured consumption. Previous studies demonstrated that when insects have relative easy access to carbohydrates they prefer protein, whereas species with greater access to protein prefer carbohydrates (Kay 2004). In the current study, however, ants were only given access to one food choice with no opportunity to regulate their diet between two food sources, so it was likely that consumption reflects how they regulate protein and carbohydrate when they are in excess or deficit (Simpson and Raubenheimer 1993).

Throughout the experiment counts were made over the number of dead ants (Figure 3.3). Examination of this figure shows the p14:c28 diet had, on average, the lowest number of dead ants when compared to the other experimental diets. Reduced

Table 3.5. Sum of the observations made for each painted forager during three intensive observation periods for each replicate (Painted forgers: A= white, B= red, C= orange, D= green).

Location	p7:c7					p7:c35				
	A	B	C	D	Mean†	A	B	C	D	Mean†
December Replicate										
Nest	70	72	70	23*	58.75	71	67	71	23*	58
Colony Box	2	0	1	1	1	1	5	1	1	2
Bridge	0	0	1	0	0.25	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
January Replicate										
Nest	31*	70	36*	24*	40.25	23*	48*	72	7*	37.5
Colony Box	5	0	3	0	2	1	0	0	17	4.5
Bridge	2	0	2	0	1	0	0	0	0	0
Diet Box	10	2	7	0	4.75	0	0	0	0	0
February Replicate										
Nest	48*	46*	23*	24*	35.25	72	72	72	72	72
Colony Box	0	2	1	0	0.75	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
March Replicate										
Nest	24*	72	72	72	60	72	72	48*	2*	48.5
Colony Box	0	0	0	0	0	0	0	0	22	5.5
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
April Replicate										
Nest	0*	72	72	72	54	72	72	48	72	66
Colony Box	0	0	0	0	0	0	0	24	0	6
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	24	0	0	0	6	0	0	0	0	0
May Replicate										
Nest	72	72	72	72	72	72	48*	48*	48*	54
Colony Box	0	0	0	0	0	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0

Table 3.5 Continued

December Replicate

Location	p14:c28					p21:21				
	A	B	C	D	Mean†	A	B	C	D	Mean†
Nest	14*	72	20*	23*	32.25	32*	40*	0*	64	34
Colony Box	7	0	3	1	2.5	4	6	0	7	4.25
Bridge	2	0	0	0	0.5	2	0	0	0	0.5
Diet Box	1	0	1	0	0.5	10	2	0	1	3.25
January Replicate										
Nest	70	72	72	44*	64.5	24*	72	48*	38*	45.5
Colony Box	2	0	0	2	1	0	0	0	2	0.5
Bridge	0	0	0	1	0.25	0	0	0	1	0.25
Diet Box	0	0	0	1	0.25	0	0	0	7	1.75
February Replicate										
Nest	24*	71	48*	72	53.75	24*	24*	7*	72	31.75
Colony Box	0	1	0	0	0.25	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	17	0	4.25
March Replicate										
Nest	72	47*	71	72	65.5	48*	72	72	72	66
Colony Box	0	1	0	0	0.25	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
April Replicate										
Nest	72	72	72	48*	66	72	72	72	48*	66
Colony Box	0	0	0	0	0	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
May Replicate										
Nest	72	72	72	72	72	72	72	72	72	72
Colony Box	0	0	0	0	0	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0

Table 3.5 Continued

December Replicate

Location	p28:c14					p35:c7				
	A	B	C	D	Mean†	A	B	C	D	Mean†
Nest	56	0*	72	0*	32	19*	24*	24*	72	34.75
Colony Box	8	0	0	0	2	5	0	3	0	2
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	8	0	0	0	2	0	0	0	0	0
January Replicate										
Nest	70	24*	14*	17*	31.25	24*	69	71	24*	47
Colony Box	2	0	10	7	4.75	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	24	3	0	0	6.75
February Replicate										
Nest	48*	23*	72	72	53.75	48*	72	20*	72	53
Colony Box	0	1	0	0	0.25	0	0	4	0	1
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
March Replicate										
Nest	48*	72	72	48*	60	48*	72	72	48*	60
Colony Box	0	0	0	0	0	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	0
April Replicate										
Nest	72	72	72	24*	60	48*	9*	24*	72	38.25
Colony Box	0	0	0	0	0	0	14	0	0	3.5
Bridge	0	0	0	0	0	0	1	0	0	0.25
Diet Box	0	0	0	0	0	0	0	0	0	0
May Replicate										
Nest	69	72	48*	48*	59.25	72	72	72	72	72
Colony Box	3	3	0	0	1.5	0	0	0	0	0
Bridge	0	0	0	0	0	0	0	0	0	0
Diet Box	0	0	0	0	0	0	0	0	0	

* Indicate foragers that did not survive to the end of the experiment. †Mean number of observations for the four painted foragers being observed.

mortality supports Chapter II data, which depicted their regulated intake target, which centered around the p14:c28 nutritional rail. For all the treatments the months of April and May had the highest mortality.

Increased mortality during this time could reflect the natural life cycle of *Paratrechina* sp.nr. *pubens.* Research has been conducted over the mean lifespan of various ant species in the field (Höbbdobler and Wilson 1990, Keller 1998), but not over this species. One experiment examining the life history of the ponerine ant, *Harpegnathos saltator* Jerdon, showed that field colonies had a short life span with an average survival of less than half a year (Liebig and Poethke 2004). Further research conducted over the life cycle of *Paratrechina flavipes* Smith (Hymenoptera: Formicidae) showed the maximum life span of workers was estimated around two years (Ichinose 1987).

With limited knowledge of the life history of *P.* sp.nr. *pubens,* it is difficult to draw definitive conclusion as to why they had higher mortality during these two months. Preliminary data showed a trend for increased mortality as the testing period increased (Figure 3.4). Tabulating the number of dead ants over time shows mortality increases with time. January was the sole month that did not follow this trend. Examination of the average temperatures in Houston, TX shows that January had a lower average temperature when compared to any other month (Table 3.6). Cold weather has shown to decrease colony fitness and can affect mortality data (James et al. 2002). Decreased colony fitness can explain why for the month of January mortality counts were high and consumption rates were low (Figure 3.6).

Table 3.6. Average low and high temperatures for Houston, TX (and surrounding areas) for December 2006-May 2007.

Average Temperatures for Houston, TX (2006-2007)		
Month	**Low**	**High**
December	42.8 °F	64.6 °F
January	41.2 °F	62.3 °F
February	44.3 °F	66.5 °F
March	51.3 °F	73.3 °F
April	57.9 °F	79.1 °F
May	66.1 °F	85.5 °F

Along with mortality counts taken, the amount of each experimental diet consumed was recorded. Diet removed from the diet dish, resulting in a decrease in weight, is considered consumed. Consumption amounts are based off the removal of the diet from the dishes, resulting in the diet dish decreasing in weight. Results from the consumption data showed the ants were regulating their intake of the protein rich foods (p21:c21, p28:c14 and p35:c7) (Figure 3.5). Colonies demonstrated having a set threshold for the amount of protein they can ingest and this can be observed in Figure 3.6, 3.7 and 3.8. Further analysis of the results from the consumption data showed that when ants had access to only one food source they showed a slight preference for the carbohydrate rich foods (i.e. p7:c35 and p14:c28), with the ants consuming the p14:c28 more readily (Figure 3.5). Carbohydrate rich foods are used as a principle metabolic fuel for many insects (Grover et al. 2007), while proteins are used preferentially for growth (Grover et al. 2007). As stated in Chapter II, there is a possible explanation for this observed preference for carbohydrates as this macronutrient is essential for invasive ants.

Studies have shown that the use of carbohydrate rich foods contribute to competitive performance (Grover et al. 2007), and *P.* sp.nr. *pubens* is an invasive and aggressive ant species. Perhaps its preference for carbohydrates was a characteristic of this species, and is one that could be a contributing factor to their ability to invade and becoming established in new areas.

Additional comparison of the mean amount of food consumed shows the p7:c7 diet and p21:c21 to be consumed by the ants at a same rate (Figure 3.5). The p7c:7 diet

was used to test for a compensatory mechanism in response to nutrient dilution. Although it had a 1:1 protein-carbohydrate ratio like the p21:c21 diet, its total macronutrient content is only one-third of the other five diets. If ants were able to compensate for dilution, consumption would have been three times as much on the p21:c21 diet to overcome the dilution effect. Not only was the consumption the smallest, this diet resulted in some of the highest mortality among the colonies. Low consumption and high mortality data showed this to be the worst diet out of the six experimental treatments.

Consumption rates on the control diet (ground up crickets) were comparable to the diets containing a total macronutrient content of 42%. The crickets used were considered a prey item and were described as being a source of protein (Scherer 2007). Crickets provided the ants with a source of protein, but a low amount of carbohydrate, it explains why their consumption was similar to the protein rich diets (Figure 3.5).

Comparison of the mean amount of food consumed also revealed that the colonies ate the majority of their food during the first 7 d (Figure 3.5 a). The lowered consumption was measured during days 8-15 (Figure 3.5 b), and could be the result of increased mortality in the experimental colonies (Figure 3.4). During each replicate, mortality tended to increase as the experiment progressed and increased rates of mortality would have seriously depressed the amounts of matrix consumed.

Alternatively colonies may have already collected enough food and were storing the excess. Nothing is known about this ant species foraging and storage behaviors. Since foraging is a relatively inexpensive activity in terms of energy expenditure

(Baroni-Urbani and Nielsen 1990, Fewell 1988,) the colony could have collected enough food, and stored it in the nest, to support their current activity levels. Research has shown that some ant species store excess fat and pass it on to colony members through lipid-rich oral secretions (Hahn 2006). It could be common practice to forage heavily until enough food is stored up and thus reducing later foraging activates. Further studies into foraging and storage behavior would have to be conducted to determine if this was the case for this ant species. Based on personal observations, increased mortality seems the most likely explanation for reduced food consumption with time.

Analysis of the amount of diets consumed versus mortality data shows when mortality increases, consumption decreased (Figure 3.6). This figure further shows that consumption was highest on the high carbohydrate foods and that the p14:c28 had a lower mortality when compared to the other diets. Although the carbohydrate rich foods had more consumption there was a noticeable increase in mortality when examining the p35:c7 treatment (Figure 3.6). One explanation for this observation may be an increase in cannibalism. Research has shown that when foods fed to ants do not have that species required amount of protein, cannibalism within that colony increases (Aron et al. 2001). Since the colonies could not regulate their diet they may have resorted to eating brood or other colony members. Another explanation is the months with some of the highest mortality were March, April and May. Although the colonies show a preference for carbohydrate rich foods, if March and April are the reproductive months they would need an increase in protein consumption to support the production of more brood (Grover et al. 2007). This diet, having the lowest amount of protein available to the

colony, could have caused decreased colony fitness since they were not getting the required nutrients for those months. Until further research is done over the life history definitive conclusions are hard to draw.

Figure 3.6 showed that the protein rich diets (i.e. p21:c21, p28:c14, and p35:c7) had a very similar consumption rate. Consumption rate was more tightly regulated when compared to the carbohydrate rich foods, meaning the ant tried to keep there protein intake below a certain threshold, whereas the carbohydrate rich treatments consumption patterns were observed over a much wider range. This result further demonstrates a preference for carbohydrate foods and a threshold point for protein.

In terms of protein-carbohydrate consumption, Figure 3.7 shows how much they ate and compared it to the regulated intake target obtained from Chapter II (light grey symbols). Each diet runs along a nutritional rail and has been represented by the solid black line in each bi-coordinate plot. Comparison of the Chapter II data and the amount eaten for the p14:c28 treatment showed a very similar consumption. This further supports the hypothesis that a diet close to the p14:c28 ratio is preferred.

Further analysis of Figure 3.7 shows the compensatory mechanism of *P* sp.nr. *pubens,* with the limiting factor being the amount of protein eaten. When comparing the intake for these treatments to the Chapter II intake target they were more tightly regulating their protein intake, thus demonstrating protein to be their limiting nutrient. An example of this can be seen in the p28:c14 treatment where they were eating to the optimal protein amount but are not going over that even though they could consume enough of the diet to reach their optimal carbohydrate intake (Figure 3.7). In summary

they appear to be willing to feed to their optimal carbohydrate point as long as they did not exceed the protein threshold.

Throughout the experiments the numbers of dead ants were counted and Figure 3.8 shows the protein-carbohydrate consumption when the amounts from Figure 3.7 were adjusted for mortality. A striking result is how tightly they regulate their protein compared to carbohydrate consumption. There was a narrow range for the protein consumption, staying between 0.0 - 0.02 mg, while carbohydrate consumption had a much wider range, staying between 0.0 - 0.09 mg. With the only exceptions in Figure 3.8 being panels d, e, and f where the consumption points representing May was above the protein range of 0.02mg. Colonies could be ignoring this protein threshold to gain more carbohydrates. Consuming more carbohydrates could correspond to a higher energy need, due to taking care of new brood and supporting a larger colony. Although these plots represent consumption on an individual level, the comparison of this Figure 3.8 to Figure 3.7 (non-adjusted) shows the consumption patterns are similar to each other, especially with regards to tightly regulating their protein intake. Similar consumption patterns further support the protein-carbohydrate regulation seen in the non-adjusted plots (Figure 3.7).

Dietary preference has been studied before, but has mainly focused on food that was classified as high protein or high carbohydrate. Examination of nutrient regulation when given a food source with known macronutrient content has currently not been conducted in social insects. Previous research focused mainly on the effectiveness and attractiveness of baits. For example, research on *Paratrechina longicornis* Latreille, has

shown they prefer the protein rich tuna when presented a choice between various diets and baits (Stanley and Robison 2007).

Furthermore, research conducted with the Argentine ant (*Linepithema humile* Mayr) demonstrated a dietary shift (Abril et al. 2007) through time. Research over seasons showed that during times of reproduction (May) the colonies consumption of protein rich prey (i.e. other insects) increased (Abril et al. 2007), while in June, their consumption of energy rich sugars, obtained from tending aphids, increased (Abril et al. 2007). Increase in energy rich sugar collection corresponded to increased colony activity since they had to tend to new brood and collect enough food to support the growing colony.

Seasonal dietary trends can start to be observed throughout these treatments. These trends are best seen in the protein rich diets in Figures 3.7 and 3.8. May appears to be associated with an increase in carbohydrate needs. In the protein rich plots (i.e. p21:c21, p28:c14, and p35:c7), for both the non-adjusted and adjusted for mortality plots, the points furthest from the clusters correspond to the month of May. The willingness of the colony to ignore their protein threshold to gain more carbohydrates demonstrates the need for this macronutrient during this time of the year. Since these experiments were not run over multiple seasons no clear conclusions can be drawn, but they do show some evidence of seasonal regulation.

Further analysis of the behavioral data supported the idea of seasonal dependent foraging. Trends shown in Figure 3.9 indicated that the majority of the foraging activities were to the diet box, for all treatments. When seasonality (i.e. Dec, Jan, and

Feb = Winter; March, April, and May = Spring/ Summer) was considered (Figure 3.9) there was a trend in behavior as time changes. This hypothesis can be seen in the incremental increase in foraging behavior as the colonies move towards the warmer months. Again, since these replicates were not repeated over the same seasons, no clear conclusion can be drawn; however, this increased foraging, because of seasonal changes, could be the result of warmer months being the typical time for pupae and larvae production (Thomas 2003). Future research over multiple seasons could show the seasonality effects in more detail.

The use of the bridge to connect the treatment boxes served as a way to observe foraging behavior and allowed us to assume individuals on the bridge were going to collect food. Numerous studies have centered on the use of a bridge (Dussutour et al. 2004a, Dussutour et al. 2004b, Dussutour et al. 2005) to demonstrate foraging for food, and allowed the ants the shortest path to food (Beckers et al. 1992). This ensures that the diets across all treatments were all the same distance from the nest in order make experimental design uniform, thus controlling for possible foraging variables. One limitation of these methods is that the observations were made every 10 minutes and not continuously. The chance of counting a forager twice increases because of this limitation. A future suggestion would be to make continuous observations ant to track the number of ants foraging to the diet dishes.

Behavioral observations on a selective number of individuals (Table 3.5) were made, but unfortunately this data does not show any trends, other than the majority of their time was spent inside the nest. Low variation of foraging behavior could be due to

the painted foragers not being true foragers. To avoid these situations in future observational studies, it would be useful to select confirmed foragers. The data collected on individuals in the current study were not ideal for any statistical analysis, but it does give good suggestions as to what to do for future research, such as confirming foragers as well as making continuous observations.

This experiment showed the experimental approach of the Geometric Framework can be applied to the study of ants, and can be conducted at the colony level. The experiments also gave us a better understanding of the nutritional regulation of *P.* sp.nr. *pubens* and may lead to the production of a better baiting system. This system can in turn help control the ever evolving problem of this invasive ant species.

CHAPTER IV

CONCLUSION

This research has provided some insight into the biology and nutrient needs of *Paratrechina* sp.nr. *pubens*. Here I used the experimental approach of the "Geometric Framework" to explore protein-carbohydrate regulation. I hypothesized that having a colony choose between two sub-optimal, but complementary, foods would result in its feeding between the two in order to defend a particular protein-carbohydrate intake target. Analyses of the data show that the colonies were foraging approximately twice as much carbohydrate as protein. Results from no choice experiments indicated that protein rich foods (p28:c14, p35:c7, p21:c21) had less consumption in comparison to carbohydrate rich foods (p7:c35, p14:c28). Furthermore, the no-choice experiments indicate they perform best on foods containing twice as much carbohydrate as protein.

Prior to my study the "Geometric Framework" approach had primarily been used on insect herbivores. Working with social insects presents a unique set of challenges compared to studying nutrient regulation in individual insects, since social insects regulate nutrients to reflect the needs of the colony and not individual needs. Ultimately my study demonstrated the ability of this approach to work resulting in more insight into specific nutrient needs of targeted ant species. Future research can apply this approach, and the results gained from it, to develop a better and more attractive bait matrix that is ant species specific. Currently most baits are tailored towards the preferences of the RIFA, and now a frame work exists that can be used to target different species more

specifically. Also, by replicating this experimental design over multiple season a more defined seasonal dietary shift can be observed, which will also help develop a more targeted baiting system.

Future research should examine the life span of this species to determine if mortality data reflect poor nutritional diets or are part of their natural life cycle. Another area of future research would be to refine the design of my experiments by examining different artificial diets as they relate to ant foraging. Currently these diets have been tailored to grasshoppers since most work has been conducted with these insects. The diets tested had low macronutrient content as well as high levels of cellulose. Although the ants consumed these diets, comparing them to diets with a lower cellulose level would confirm their attractiveness. Further research needs to be done to determine if this species is in fact feeding on moribund individuals from the colony. Furthermore, studies examining different active ingredient as part of the artificial diets in order to produce an optimal baiting system for this ant species need to be conducted.

A number of outstanding questions still remain. Results from my experiments demonstrated the ability to study nutrient regulation of social insects at the colony level, specifically their nutritional needs and the mechanisms they employ in terms of regulating protein and carbohydrate intake. The results from this study provide a potentially big step towards developing species-specific baits, as well as expanding our knowledge of the biology of this insect.

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APPENDIX A

Table A-1. Nutritional content used for each experimental diet.

	p7:c35	p28:c14	p35:c7
Cellulose (g)	108	108	108
Casein (g)	8.4	33.6	42
Peptone (g)	2.8	11.2	14
Albumen (g)	2.8	11.2	14
Sucrose (g)	35	14	7
Dextrin (g)	35	14	7
Linoleic Acid (ml)	1.1	1.1	1.1
Cholesterol (mg)	1100	1100	1100
Chloroform (ml approx.)	80	80	80
Wesson's Salt (g)	5	5	5
Ascorbate (mg)	550	550	550
Vitamin Mix (mg)	360	360	360
20% ethyl alcohol (ml approx)	250	250	250

Table A-2. Nutritional content used for each experimental diet used in the no-choice experiments.

	p7:c7	**p7:c35**	**p14:c28**	**p21:c21**	**p28:c14**	**p35:c7**
Cellulose (g)	164	108	108	108	108	108
Casein (g)	8.4	8.4	16.8	25.2	33.6	42
Peptone (g)	2.8	2.8	5.6	8.4	11.2	14
Albumen (g)	2.8	2.8	5.6	8.4	11.2	14
Sucrose (g)	7	35	28	21	14	7
Dextrin (g)	7	35	28	21	14	7
Linoleic Acid (ml)	1.1	1.1	1.1	1.1	1.1	1.1
Cholesterol (mg)	1100	1100	1100	1100	1100	1100
Chloroform (ml approx.)	80	80	80	80	80	80
Wesson's Salt (g)	5	5	5	5	5	5
Ascorbate (mg)	550	550	550	550	550	550
Vitamin Mix (mg)	360	360	360	360	360	360
20% ethyl alcohol (ml approx)	250	250	250	250	250	250

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