Nutritional Ecology of the Carpenter Ant *Camponotus pennsylvanicus* (De Geer): Macronutrient Preference and Particle Consumption

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(ABSTRACT)

The nutritional ecology of the black carpenter ant, *Camponotus pennsylvanicus* (De Geer) was investigated by examining macronutrient preference and particle consumption in foraging workers. The crops of foragers collected in the field were analyzed for macronutrient content at two-week intervals through the active season. Choice tests were conducted at similar intervals during the active season to determine preference within and between macronutrient groups. Isolated individuals and small social groups were fed fluorescent microspheres in the laboratory to establish the fate of particles ingested by workers of both castes.

Under natural conditions, foragers chiefly collected carbohydrate and nitrogenous material. Carbohydrate predominated in the crop and consisted largely of simple sugars. A small amount of glycogen was present. Carbohydrate levels did not vary with time. Lipid levels in the crop were quite low. The level of nitrogen compounds in the crop was approximately half that of carbohydrate, and exhibited seasonal dependence. Peaks in nitrogen foraging occurred in June and September, months associated with the completion of brood rearing in *Camponotus*.

In choice tests, foragers demonstrated a preference for sucrose, fructose, and glucose, the most common honeydew sugars. Sucrose was preferred over other sugars in laboratory and field tests. Consumption rates peaked at a concentration of 20%. Casein

hydrolysate and processed fish products stimulated the most feeding in choice tests of protein foods. Though a variety of lipids of plant and animal origin were offered in both field and laboratory tests, they were generally ignored.

No effect of time was observed during choice tests of macronutrient preference. Overall, nitrogenous food was collected four-fold more intensively than carbohydrate, in contrast to the results obtained from examinations of the crop contents. These data suggest that accessible nitrogen is limited in the environment.

Workers readily consumed fluorescent microspheres 0.5 - 45 µm diameter. Forty-five µm microspheres were excluded from the crop. Particles 3 - 10 µm reached the crop, but were never seen in the mid- or hindguts of either major or minor workers. They also filled the infrabuccal pocket, where they were compacted into pellets. It is thought that the proventriculus contains such particles in the foregut, where they are eventually filtered from the ingluvium.

Microspheres 1 µm or less were difficult to observe in the infrabuccal pocket, suggesting that they are not as effectively sequestered as larger particles. Microspheres smaller than 1 µm were seen in the mid- and hindgut of both worker castes, indicating that particles of this size are immune to the proventricular filter. Caste exerted an effect at one µm diameter, the threshold of filtering efficiency. One µm microspheres consumed by minor workers were detected in the mid- and hindgut, whereas one µm microspheres were never detected beyond the proventriculus in major workers.

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DEDICATION

I dedicate this dissertation to my husband, Christoph Leser,
whose steadfast shoulders have helped me time and again
out of deep water.

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CHAPTER 1. INTRODUCTION

Although the ants account for only 2% of all insect species described to date, it is estimated that they constitute more than half of the world's insect biomass (Hölldobler and Wilson 1994). At present, the number of species worldwide is approximately 9,500, but the true number may eventually reach 20,000 or more (Hölldobler and Wilson 1990, Hölldobler and Wilson 1994). The ants are among the most widespread, diverse, and dominant life forms, particularly in the tropics (Tobin 1993). They are found in deserts, on some of the highest mountains, and on all but the most remote islands around the world (Went et al. 1972, Brown 1973, Hölldobler and Wilson 1990). Locally, ants may be the primary predators, soil turners, or seed dispersers in a given habitat (Beattie 1985, Hölldobler and Wilson 1990).

Much of the nutritional ecology of the Formicidae remains uncertain. The current debate regarding the ants' general trophic level demonstrates that even the most fundamental conclusions regarding feeding behavior are still tentative. Wilson (pers. comm. in Tobin 1993) views the ants as primary consumers first and foremost. Stradling (1987) maintains that the ants are a carnivorous group that supplements its diet with other foods such that most species of ants are omnivorous. An omnivorous group as large and diverse as the ants would weaken Gordon's (1956) assertion that "truly omnivorous species are relatively rare in nature." Carroll and Janzen (1973) claim that most ant species are scavengers rather than predators. Tobin (1993) disputes the view of ants as fundamentally carnivorous, a category in which he includes both predators and scavengers; he believes that the ants, by virtue of their abundance, must be "the dominant primary consumers in most temperate and tropical ecosystems." Tobin (*op. cit.*) does concede that omnivory is likely to be more important than current data suggest; his data indicate that 52.8% of ant genera are omnivorous.

The problem for nutritional ecologists studying ants is twofold. First, there are large gaps in our knowledge regarding their feeding habits. A fair amount is known about certain ants, such as wood ants (*Formica* spp.), which are beneficial predators in European forests; fire ants (Solenopsis spp.), which are major urban and agricultural pests in the southern U.S.; and species having unusual or highly specialized feeding habits, such as leaf-cutting ants (*Atta* and *Acromyrmex* spp.) (Hölldobler and Wilson 1990). In contrast, our understanding of the feeding habits of some of the largest genera, such as *Camponotus*, *Crematogaster*, and *Pheidole*, is quite limited (Tobin 1993). In some cases, the literature consists to a large extent of historical anecdote and unmethodical observation. For example, although carpenter ants are generally considered to be a predatory group (Ayre 1967, Sanders 1970, Hansen and Akre 1985, Sanders and Pang 1992), the only carpenter ant in North America for which documented, indisputable evidence of predatory capabilities exists is *Camponotus herculeanus* (L.) (Ayre 1963a, 1963b). Similarly, the inability of ants, and of all adult aculeate Hymenoptera, to ingest solids is so axiomatic that statements in the literature to that effect are often unaccompanied by citation. Efforts to establish the quantifiable limits of this assertion have been made only recently and for only three species (Eisner and Happ 1962, Quinlan and Cherrett 1978, Glancey et al. 1981).

Second, some studies frequently cited in discussions of foraging and feeding may be flawed, as the following examples demonstrate. A key study of *Formica rufa* L. by Skinner (1980) purports to show the primary importance of honeydew in the diet. Not only does the author consider "building material" transported by foragers as "solid food," but he fails to test for nitrogen compounds in the crops of returning foragers, instead assuming all crop contents to be honeydew. In a well-cited study by Smith (1944), protein-rich diets appear to be detrimental to *Camponotus pennsylvanicus* larvae. That study contains numerous errors, but the most important may be his use of a casein slurry in water as a protein source. Casein is not readily soluble in water and crystalline foods are not likely to be consumed by the ants. In the same study, Smith (*op. cit*.) attempts to

demonstrate the importance of vitamins by substituting vitamin-free casein and removing yeast from the diet. Yeast is, in fact, a good source of protein (Souci et al. 1990). A study by Retana et al. (1988) has been cited by Tobin (1993) as evidence of the importance of herbivory in *Camponotus*. The choice test designed by Retana et al. (*op. cit*.), however, compared honey, which carpenter ants are known to consume, to foods high in fat, salt, or grains, none of which are known to be attractive to them.

An impressive list of foods acceptable to carpenter ants has been compiled. Different species have been observed feeding on honeydew from aleyrodids, aphids, coccids, fulgorids, lycaenids, membracids, and psyllids (Pricer 1908, Funkhouser 1915, Gotwald 1968, Levieux 1975, 1977, Levieux and Louis 1975, Retana et al. 1988); the sap of ash trees, "pie-plant", lilac shrubs, and others (Pricer 1908, Gotwald 1968, Levieux 1975, 1977, Levieux and Louis 1975); the juices of well-ripened fruit (Smith 1965); nectar (Levieux 1977, Retana et al. 1988, Rico-Gray and Sternberg 1991); and live and dead arachnids, myriapods, collembolans, odonates, orthopterans, mantids, homopterans, isopterans, coleopterans, lepidopterans, hymenopterans, and dipterans (Ayyar 1935, Ayre 1963a, 1963b, Sanders 1964, Gotwald 1968, Levieux and Louis 1975, Levieux 1977, Busher et al. 1985, Dejean 1988). They will also feed on a wide variety of household foods, including sugar, honey, syrup, molasses, jam, jelly, preserves, pears, apples, oranges, melons, fermented raisins, angel cake, breads, raw and cooked meats, liver, grease, boiled eggs, milk, and Coca Cola (Smith 1965, Dukes 1982, Smith and Whitman 1992, Klotz pers. comm.). Ironically, food preference and nutrition has not been investigated beyond this extensive and very specific list of foods. Normally, the first step toward understanding dietary requirements of an organism is to distinguish important nutrients from important foods (Dadd 1985). This groundwork is lacking for *C. pennsylvanicus*, the black carpenter ant.

Of equal importance yet often forgotten are the mechanical properties of a food, which must be compatible with the animal's feeding apparatus (Schmidt-Nielsen 1983).

In the adult stage, the ants, like all adult aculeate Hymenoptera, are mandibulate insects that are restricted by anatomy to feeding on liquid foods. The mouthparts and infrabuccal pocket appear to be the key structures, acting in concert to filter solids from the material consumed during feeding and grooming (Janet 1895, 1905 in Eisner and Happ 1962). In ants at least, the proventriculus may also play a role (Eisner and Wilson 1952, Roche and Wheeler 1991). Scant quantitative data exist regarding the ultimate size threshold for particle retention and passage to the midgut, where digestion takes place, or the effect of factors like body size or social environment. Identifying the behavior of particles at or near this size threshold will contribute to our knowledge of feeding behavior, hygiene, and pest management.

The general objectives of this study were to identify and quantify the main macromolecular components of foods collected under natural and optimal conditions, and to define the mechanical properties of those foods by establishing the physical size limits governing ingestion and the roles played by sociality and individual body size. The specific objectives were:

1) to identify the macronutrients collected by *C. pennsylvanicus* under natural conditions and to determine the changes, if any, exhibited over time in amounts collected;

2) to investigate how macronutrient collection is affected by environmental constraints on foraging, such as food availability and competition;

3) to quantify the filtering capability of the proventriculus and the effects exerted by body size and sociality.

CHAPTER 2. LITERATURE REVIEW

Ant taxonomy in North America began in 1773, when the Baron De Geer, a student of Linneaus, described *Formica pennsylvanicus* (sic), the first native ant collected in North America (De Geer 1773). Both his spelling and his taxonomy were corrected when this ant was later renamed *Camponotus pennsylvanicus* (De Geer). Twenty-five years later, Fabricius described the second endemic Nearctic ant, *Formica ferruginea*, now known as *Camponotus ferrugineus* (Fab.) (Fabricius 1798 in Creighton 1950). Three more North American species were added by Latreille four years later, among them *Formica castanea*, or *Camponotus castaneus* (L.) as we now know it (Latreille 1802 in Creighton 1950). The disproportionate representation of Camponotini among the first native ants described from America north of Mexico affirms Wheeler's (1910) description of the genus as "our largest, most conspicuous, ... and most abundant ants." With more than a thousand species worldwide, *Camponotus* is the largest and most "ecologically tolerant" of all living ant genera, occurring abundantly in both mesic and xeric habitats of all seven conventional biogeographic regions of the world (Brown 1973).

The tribe Camponotini has undergone much revision in the last hundred years (Wheeler 1910, Emery 1920, Smith 1947, Creighton 1950, Brown 1950, 1973, MacKay 1997). Subgeneric, subspecific, varietal and racial names have waxed and waned in popularity, such that at one time C. *pennsylvanicus* bore the "binomial" name *Camponotus herculeanus herculeanus herculeano-pennsylvanicus* Forel (Forel 1879 in Krombein et al. 1979). Such tortured taxonomy has been largely eliminated, with Brown (1973) even calling for the abandonment of subgeneric names entirely. Presently, the tribe comprises one genus, seven subgenera, 43 species, and 12 subspecies (Krombein et al. 1979).

The commonwealth of Virginia is home to nine species of carpenter ants, four of which are structural pests, and a fifth species occurs as a home-invading nuisance pest. North Carolina and Washington D. C., which border Virginia, harbor five additional species, whose ranges very well may be found to extend into the state. Two of those neighboring species are structural pests (Creighton 1950, Krombein et al. 1979, Smith 1965). Of the 14 species in and around Virginia, *C. pennsylvanicus*, the black carpenter ant, is by far the most common. It is the most common carpenter ant east of the Mississippi River (Mallis 1982).

2.1 Pest Status

Ants in the subgenus *Camponotus (Camponotus)* are known collectively as carpenter ants because of their habit of excavating nests in wood (Krombein et al. 1979). Structural wood and merchantable timber may be invaded, making carpenter ants pests of some economic importance. Extrapolating from survey data of pest control operators in New Jersey, Fowler (1982) estimated that $125 million (in 1982 dollars) is spent by homeowners in the northeastern U.S. annually on professional control measures. Estimates of economic damage based on professional control costs may greatly underrepresent true control costs. For example, 45% of Minnesota homeowners surveyed who suspected an infestation attempted control without the help of a professional pest control company (Hahn and Ascerno 1987).

Carpenter ants are responsible for economic losses beyond structural damage. Little information regarding economic losses to the timber industry due to carpenter ant damage exist, but records of timber loss demonstrate the severity of the problem. Attacks on balsam fir and spruce in Canada caused losses of 12% and 10%, respectively, of harvested product (Sanders 1964). In parts of Minnesota, 70% of merchantable cedar has been lost to carpenter ants (Graham 1918). Carpenter ants are known to mine many kinds of conifers and hardwoods, including cedar, Douglas fir, true firs, giant sequoia, hemlock,

and pines, apple, cherry, chestnut, elm, black locust, maple, oak, and poplar (Graham 1918, Schread 1952, Furniss and Carolin 1977, Knight and Heikennan 1980). They are also capable of killing young conifers by girdling the root collar zone (Furniss and Carolin 1977). The galleries are frequently associated with fungi, though a causal relationship has not been established (Sanders 1964). The chestnut blight pathogen has been found in the digestive tract of carpenter ant workers, though whether the ants can vector the disease is uncertain (Anagnostakis 1982 in Fowler 1982). Regardless, overall losses of merchantable timber to carpenter ants may be a "major, largely undocumented problem," particularly across the northern U. S. states and in southern Canada (Akre and Hansen 1990).

In addition to damaging timber and structural wood, carpenter ants can significantly damage utility poles, shade trees, and lawns. A survey undertaken from 1933 to 1935 revealed that carpenter ant damage necessitated the replacement of approximately 10% of telephone poles in southern New England annually at a cost of $325,000 per year in 1937 dollars (Friend and Carlson 1937). Fifty years later, a survey in rural New York found utility poles infested at a rate of 24% (Gibson 1987). Shade trees appear to be attacked at higher rates: 75% of shade trees in a suburban New Jersey town harbored carpenter ants (Fowler 1980). Extensive galleries can leave infested trees more susceptible to wind breakage and wind throw (Schread 1952, Fowler and Roberts 1982). In addition, carpenter ants tend to clear ground-level trails to their more persistent foraging sites, some of which can be maintained for several years, leading to long-term lawn damage (Smith 1965, Hansen and Akre 1985). In addition, carpenter ants can become nuisance pests when foragers invade nearby houses in search of food or nesting sites (Hansen and Akre 1985).

2.2 Colony Ontogeny and Demography

Mating is thought to occur aerially during swarming, after which males die and females found nests singly by constructing small, closed cells in the soil or in performed wood cavities (McCook 1883, Pricer 1908, Sanders 1964). Stable, polygynous colonies are rare in most species (Akre et al. 1994). Occasionally, very large colonies will contain several queens, but such colonies appear to be functionally monogynous, with discrete territories maintained by each queen (Hölldobler 1962, R. Gibson - pers. comm.). Founding queens do not feed (McCook 1883, Pricer 1908, Eidmann 1926). While rearing the first brood, they rely on reserves available from the histolysis of their large alary muscles and from fat body stores (Wheeler 1933, Cannon 1990). These metabolic reserves suffice for rearing the first workers, which are timid and unusually small (Oster and Wilson 1978). These workers, called minims or nanitics, forage shortly after emerging, providing the queen with her first food since leaving the parental nest (McCook 1883, Mintzer 1979).

After the initial founding stage, the colony begins an ergonomic stage (Oster and Wilson 1978). The queen leaves all nest maintenance duties to the brood and devotes herself entirely to egg-laying. Workers, which are sterile, stunted females, are produced such that colony growth is exponential (Oster and Wilson 1978). During this period, mean worker size increases with colony size (Pricer 1908, Gibson 1987, Fowler 1982). In carpenter ants, only small, or minor, workers are produced initially; large, or major, workers are not produced until two to three years after founding (Pricer 1908, Hansen and Akre 1985). After reaching a threshold population size, the colony enters a reproductive stage during which sexual forms are produced (Oster and Wilson 1978). This stage typically begins four or more years after founding in carpenter ants (Pricer 1908). Mature colonies produce both males and females, with the proportion of males increasing as the colony ages. Colonies in decline produce only males (Pricer 1908, Sanders 1964).

Queens oviposit in two phases each year. Eggs laid during the first phase complete development by late summer or early fall, whereas those of the second phase enter diapause in late summer and pass the winter as first instar larvae (Hölldobler 1961, Dukes 1982, Hansen and Akre 1985). In spring, larvae that have overwintered in the nest are reared serially to maturity (Eidmann 1942). This scheme produces a pattern of adult emergence that is bimodal: one peak in late spring or early summer, one in late summer or early fall (Fowler 1982). The latter peak includes both workers and alate sexual forms. Alates spend the winter in the nest, departing in spring as a swarm to mate and initiate new colonies (Pricer 1908). Carpenter ant colonies vary considerably in size, with species in the Pacific Northwest having upwards of 10,000 workers (Akre et al. 1994). In contrast, the average mature *C. pennsylvanicus* colony is estimated to contain only about 3,000 workers (Pricer 1908, Fowler 1982, Gibson 1987, Akre et al. 1994). The latter figure should be considered unreliable, however. Sanders (1964) first noted that carpenter ant colonies are polydomous, inhabiting from two to eight trees. Despite the importance of this finding for colony biology, there are no indications in the literature that authors since 1964 make any effort to ensure that they collect entire colonies rather than colony fragments, or make any distinction between the two.

2.3 Caste and Division of Labor

Caste may be defined broadly as "any set of individuals of a particular morphological type or age group, or both, that performs specialized labor in the colony" (Hölldobler and Wilson 1990). In the ants, the chief criteria for separating castes are reproductive capability, which distinguishes workers from alates (or reproductives), and sex, which distinguishes males from gynes (or females) within the reproductive caste (Wilson 1971). Within a mature colony the range of individuals of one sex varies widely in size and allometrically in proportion, a phenomenon known as polymorphism (Hölldobler and Wilson 1990). Distinctions based on such physical traits are common and lead to the designation of minor, media, and major worker subcastes (Wilson 1953).

C. pennsylvanicus is strongly and continuously polymorphic, with workers ranging in size from about 1 to almost 4 mm head capsule width (Pricer 1908, Fowler 1982, Cannon unpubl.). The worker size frequency distribution is bimodal and skewed to the left, meaning two size classes (minors and majors) exist although the smaller predominates (Smith 1942a, Sanders 1964). Some authors consider the small group of workers in a frequency trough to be medias, a third subcaste (Smith 1942a, Busher et al. 1985). Indeed, there seems to be some evidence that these workers represent a third functional caste in other species of *Camponotus* (Buckingham 1911, Gotwald 1968, Busher et al. 1985, Dejean 1988). In *C. pennsylvanicus*, the minor workers generally tend aphids, care for brood, and attend to nest maintenance, whereas the major workers act mainly as protein foragers, soldiers, and "tankers" (ferry honeydew from aphid tenders to the nest) (Pricer 1908, Fowler and Roberts 1980). Pricer (1908) made observations regarding a functional media caste in *C. pennsylvanicus*, but his remarks are difficult to interpret, as he described four size classes without defining his measurement criteria. Fowler (1982) "arbitrarily" divided workers into three size classes in order to develop a behavioral catalogue for *C. pennsylvanicus*. He found that medias participate in many low frequency nest maintenance duties, such as transporting brood, assisting with pupal emergence, carrying nestmates, removing dead, and gnawing wood.

Functional caste is determined as much by morphology as by age. Buckingham (1911) cited a number of nineteenth century authors on the temporal caste system and provided further support herself. She found that the youngest ants were nurses, slightly older ants attended the queen, middle-aged ants were foragers and "builders," and the oldest ants foraged, "built," carried nestmates during emigration, and defended the nest. In particular, foraging seems to be a strongly age-based task. In many ant species, only older workers respond to recruiters (Janzen 1967, Weir 1958a, 1958b, Wilson 1962). In *C. pennsylvanicus*, 68% of all foraging was performed by older workers, as evidenced by the condition of their ovaries, all of which had totally or partially resorbed oocytes (Traniello 1977).

2.4 Food Exchange

Division of labor is a device by which not all ants in a colony must search for food. Those ants in the colony that specialize in non-foraging activities, such as brood care, can rely on those specialized in foraging for their food. Food is freely shared between all individuals of a colony, creating what has been called the communal stomach. Food sharing is accomplished through stomodeal trophallaxis, which is the regurgitation of crop contents, or ingluvium, to another member of the colony. Within the nest, trophallaxis is stimulated in the donor through begging behavior by the potential recipient: antennation and pawing of the donor's head and mouthparts by another worker; squirming or "pouting" movements by larvae (Hölldobler and Wilson 1990). A returning forager seldom requires external stimulation; she actively seeks a recipient as a way to recruit nestmates to a profitable food source (Traniello 1977). The returning forager relieves herself of her food load fairly quickly, with distribution among nestmates occurring rather efficiently. For example, a single *F. rufa* forager is able to share her crop contents with 80 nestmates within four hours (Gößwald and Kloft 1960a). Food transmission in *Formica* is so efficient that complete colony saturation occurs within 30 hours (Wilson and Eisner 1957). Similar rapid transmission rates have been recorded for *C. pennsylvanicus* (Traniello 1977).

Few studies on food distribution in ant colonies have been completed, but work to date demonstrates that food is not distributed homogeneously among nestmates. In *Formica* spp. and *Myrmica rubra* (L.), workers freely exchange a sugar syrup, but prey juices are passed unidirectionally from foragers to nurses (Lange 1967, Brian and Abbott 1977). In advanced ants, such as *Solenopsis*, *Crematogaster*, and *Formica* spp., honey is also uniformly distributed among workers, whereas queens and larvae receive proportionately less of this food (Eisner and Wilson 1957, 1958). All segments of an *S. invicta* colony receive sugary, nitrogenous, and oily foods, but preferences are expressed: workers monopolize carbohydrates; amino acids are directed toward larvae

and protein hydrolysates toward the queen; oil is distributed among workers and larvae (Vinson 1968, Howard and Tschinkel 1981b, Sorensen and Vinson 1981, Sorensen et al. 1981). *F. polyctena* foragers rank potential recipients, sharing their crop contents with larger workers first and smaller workers last (Gößwald and Kloft 1960b). Similarly, *Iriodomyrmex humilis* nurses discriminate against small larvae, feeding large larvae preferentially (Markin 1970). It should be mentioned that food distribution is much reduced in primitive ants like *Pogonomyrmex badius* (Latr.), which may not engage in trophallaxis at all (Eisner and Wilson 1958).

2.5 Foraging Patterns

Foraging temperature ranges in ants serve to maximize activity during times of year when food is abundant (Bernstein 1979). Seasonal foraging patterns in carpenter ants show some variability. In northwestern Ontario, daily activity in *C. herculeanus*, *C. noveboracensis*, and *C. pennsylvanicus* peaks mid-afternoon in early June and gradually advances into night as the season progresses (Sanders 1972). In California, foraging activity in *C. modoc* is diurnal early in the season, shifts to become nocturnal in July and August, and returns to the diurnal pattern at the close of the season (David and Wood 1980). In New Jersey, *C. pennsylvanicus* foraged nocturnally throughout June and July, though in August this pattern was less pronounced (Fowler and Roberts 1980). Gotwald (1968) found that peak foraging in *C. noveboracensis* was substrate dependent: honeydew collection was diurnal, and sap gathering was crepuscular; peak protein collection was unspecified. In Kansas, *C. pennsylvanicus* forages consistently at night when competing with *Formica* sp. for resources, but will exploit some daylight hours when competitors are removed (Klotz 1984).

Foragers show a tendency to retrieve solid food in proportion to their size (Chauvin 1950, Lutz 1929 in Vowles 1955). When a source of food too large or numerous for a single ant is discovered, the forager returns to the nest to recruit nestmates

to the site (Dejean 1988). Recruitment repertoires are diverse in *Camponotus*. Tandem running, in which a forager physically leads one nestmate to the resource; group recruitment, in which a forager uses motor and chemical cues to guide up to 20 nestmates to the resource; and chemical mass communication, in which a forager uses pheromones alone to induce foraging behavior and to guide nestmates to the source, all have been observed in the genus (Hingston 1929, Hölldobler 1971, Hölldobler et al. 1974, Traniello 1977). *C. pennsylvanicus* demonstrates aspects of both group and mass recruitment (Traniello 1977).

The successful forager returning to the nest deposits a pheromone trail between the food source and the nest. It is this trail that recruited foragers follow. *C. pennsylvanicus* is able to follow trails laid by other *Camponotus* species, suggesting some common chemical components (Barlin et al. 1976). Trail substance in *C. pennsylvanicus* originates in the hindgut (Hartwick et al. 1977), but specific chemical components have not yet been identified. Formic acid is also incorporated into the pheromone trail (Traniello 1977). Pheromones are typically volatile, short-lived compounds, and trail pheromones are unexceptional in this regard. The trail pheromone of *C. pennsylvanicus* persists for about two hours (Hartwick et al. 1977). What is remarkable, however, is that these ants regularly reestablish trails in spring that they used the previous year (David and Wood 1980). It is possible that visual information is retained through the winter: *C. pennsylvanicus* makes use of visual cues when these are stronger than chemical signals (Hartwick et al. 1977, Traniello 1977, David and Wood 1980).

2.6 Morphology and Feeding

Ants do not build combs as do the social bees and wasps, but instead store surplus food internally in their bodies (Nonacs 1991, Wheeler 1993). The fat bodies and crops of larvae and adults serve as repositories for excess food (Wilson 1971, 1974, Rissing 1984,

Stradling 1987, Wheeler 1993, Martinez and Wheeler 1994). In times of food scarcity, those resources stored in the larvae are harvested through cannibalism (Evesham 1985, Joyner and Gould 1987, Nonacs 1991). Cannibalism of the larvae is thought to be common early in the year, when rising temperatures permit increased activity, yet temperatures are not high enough to permit profitable foraging (Eidmann 1942, Hölldobler 1961). Nutrients stored in the bodies of workers are mobilized both for personal sustenance and for larval maintenance during periods of food shortage (Sorensen et al. 1983, Wheeler 1993). Some evidence suggests that workers produce larval food in the postpharyngeal glands, but precisely how energetic reserves are translated into glandular substances is not understood (Delage-Darchen 1976, Markin 1970, Paulsen 1969 in Hölldobler and Wilson 1990).

By virtue of its cuticle lining, which prevents absorbtion of nutrients, the crop is ideally suited for food storage. The storage of surplus food in the crop may also be fairly cost-effective. It is certainly less expensive energetically than the metabolic processes of synthesis, storage, conversion and release of food-derived macromolecules (Peterson-Braun and Buschinger 1975). Long term storage in the crop in the absence of morphological adaptations would require continuous active closure of the sphincter muscles between the crop and midgut at considerable energetic expense. However, in the higher ants, particularly in the Formicinae and Dolichoderinae, the proventriculus has evolved into a passive dam to increase the capacity of the crop as a storage organ and to reduce the burden on the associated musculature (Eisner and Brown 1958). The advanced proventriculus is characterized by a narrow, rigid, cruciform slit through which fluids are transported only by active compression of the organ by circular muscles (Forbes 1938, Eisner and Wilson 1952). Elongation of the slits is an evolutionarily advanced feature of the formicine proventriculus that permits increases in the amount of material conveyed with each compression without decreasing the effectiveness with which particles are excluded (Eisner and Wilson 1952).

Obviously, if particulate matter were to be indiscriminately ingested, the crop would fill with solid material and the proventriculus could become blocked. To circumvent such problems, the higher ants have evolved a complex serial filtration system anterior to the proventriculus that involves the external mouthparts, the infrabuccal pocket, and the opening to the pharynx. The external mouthparts apparently exclude large particles in the food from the buccal cavity (Eisner and Happ 1962). Medium-sized particles may escape the actions of the mouthparts but nevertheless can proceed no further than the buccal cavity because of the small size of the slit-like opening to the pharynx (Eisner and Happ 1962). Particles small enough to enter the pharynx do reach the crop, but they also tend to collect in the infrabuccal pocket, a chamber in the floor of the buccal cavity, where they are compacted into a pellet that is actively expelled by the ant (Janet 1895 in Eisner and Happ).

Investigations are far from complete, but efficacy of filtration appear to be influenced by body size, species, and exposure to nestmates. Glancey et al. (1981) demonstrated that minor workers of the red imported fire ant, *S. invictus*, can filter most particles of 0.88 to 4.6 µm diameter from the crop contents. Unfortunately, they did not clarify whether trophallaxis was permitted and how long after feeding the ants were sacrificed. Quinlan and Cherrett (1978) found that all workers of *Acromyrmex octospinosus* (Reich), the leaf-cutting ant, are able to remove particles of 30 µm or greater from the ingluvium completely. They further found that minor workers could filter particles of 10 µm from the crop. Again, however, conditions regarding social interaction and time elapsed post-feeding were not defined. Eisner and Happ (1962) established that *C. pennsylvanicus* workers cannot ingest particles 200 µm in diameter and cannot swallow particles 150 µm in diameter. Ingested particles of 10 - 100 µm collect in the crop and the infrabuccal pocket, where they remain in the absence of social contact. Twelve hours' exposure to hungry nestmates reduces the amount of particulate matter in the crop, though the authors did not investigate how much solid material is truly removed and how much is diluted among unfed nestmates.

In contrast, ant larvae are capable of consuming solid foods under certain circumstances. Le Masne (1953) asserts that the larvae of all ants he examined received solid food in the form of grain or insect fragments by the third larval instar at the latest. *S. invictus* larvae are liquid feeders until they reach the fourth instar, when they become able to consume solids (O'Neal and Markin 1973, Petralia and Vinson 1978). Particles 45.8 µm in diameter are swallowed by fourth instar larvae, whereas 90.7 µm particles are not. The size of the smaller particle approximates the diameter of the larval esophagus at its juncture with the midgut (Glancey et al. 1981). In some ants, larvae do not consume protein solids, but digest them extraorally (Wheeler 1993).

2.7 Colony Nutrition

Carpenter ants are generally described as omnivorous. Indeed, they have been observed feeding on a broad spectrum of foods. They tend aphids and membracids for honeydew (Pricer 1908, Funkhouser 1915, Gotwald 1968), imbibe plant juices (Pricer 1908, Eidmann 1929, Ayyar 1935, Gotwald 1968), and feed on live and dead insects (Pricer 1908, Ayre 1963, Gotwald 1968, Fowler and Roberts 1980, Youngs 1983, Hansen and Akre 1985). There is some indirect evidence that fungi may be a part of the carpenter ant diet (Ayre 1967, Sanders 1964). The ants will also consume a wide variety of household foods (Smith 1965, Dukes 1982).

Honeydew is thought to be the primary food source of *Camponotus* species (Levieux 1975, Levieux and Louis 1975, Fowler and Roberts 1980, Retana et al. 1988, Rico-Gray and Sternberg 1991). There are records in the literature of carpenter ants feeding on plant sap at wounds, but this behavior appears to be restricted to limited times of day or season (Pricer 1908, Ayyar 1935, Gotwald 1968). The importance of plant sap in the diet is more of a behavioral question, as the two sources of carbohydrates are "energetically comparable" (Tobin 1993). Honeydew is a carbohydrate-rich food, consisting mainly of invert sugars, sucrose, and dextrin (Maltais and Auclair 1952,

Zoebelein 1956a). Carbohydrates supply energy quickly, and so serve the needs of the industrious worker population (Carroll and Janzen 1973). Tobin (1993) theorizes that heavy reliance by ants on plant products, such as honeydew or sap, may confer a selective advantage by supporting the higher activity levels and greater territoriality needed to monopolize resources, which in turn leads to greater access to protein and larger colony size.

Honeydew supplies not only carbohydrates, but other nutrients as well. Various levels of amino acids, amides, lipids, sterols, organic acids, alcohols, auxins, salts, minerals, and B-vitamins are found in different honeydews (Maltais and Auclair 1952, Auclair 1963, Way 1963, Strong 1965). Some authors speculate for this reason that honeydew may be a complete food (Way 1963). That is unlikely, as all ten essential amino acids are rarely present (Hagen 1987). Insects possessing gut symbiotes, on the other hand, may be able to compensate for certain nutritional deficiencies (Dadd 1985). All castes of all *Camponotus* species that have been examined harbor bacterial symbiotes in the intestinal epithelium (Steinhaus 1967, Schroder et al. 1996).

The importance of prey in the *C. pennsylvanicus* diet is unclear, but documentation to support the notion of *C. pennsylvanicus* as a strong predator is lacking. Pricer (1908) never observed his colonies taking prey. More likely, *C. pennsylvanicus* obtains its proteins through scavenging (Carroll and Janzen 1973). Several studies have found only 1% or fewer foragers return to the nest bearing solid food (Sanders 1972, Fowler and Roberts 1980). Admittedly, some prey or carrion may be transported to the nest in the crop, as has been observed in *Formica rufa* (Gößwald and Kloft 1956, Horstmann 1974). Consequently, measures based solely on arthropod reamains carried by foragers may underestimate prey/carrion collection. Nonetheless, there is little good evidence that carpenter ants in general, and *C. pennsylvanicus* in particular, are effective control agents against insect pests (Sanders and Pang 1992).

Nitrogenous nutrients must be limited to soluble proteins and amino acids, as the ants are unable to consume solid food (Eisner and Happ 1962) and proteases are lacking in the worker foregut (Ayre 1967). One interesting study raises the possibility that the ants are able to circumvent the restrictions imposed by morphology. *C. herculeanus* consumes the fluids of its prey, then sprays formic acid into the wounds and recommences feeding (Ayre 1963b). This may be an innovative way to digest macromolecules extraorally in the absence of foregut proteases. Resourcefulness in protein foraging, particularly during shortfalls, has been amply demonstrated by ants. Among the alternate sources of protein used by *S. invicta* are commensal mites, trophic eggs, larval and pupal exuvia, sick or dying workers, and injured eggs, larvae, or pupae (Le Masne 1953, O'Neil and Markin 1973). Workers of some ants, including *Camponotus*, will lick or eat the meconium immediately after it has been shed, though it is unclear what nutrients are obtained from this material (Le Masne 1953). Finally, as mentioned above, cannibalism of the brood appears to be commonplace (Wheeler and Wheeler 1988, Baroni Urbani 1991, Edwards 1991, Nonacs 1991).

The question of whether carpenter ants normally feed on fungi remains unsettled. Carpenter ants are suspected vectors of chestnut blight and have been observed feeding on the canker (Anagostakis 1982in Fowler 1982). Sanders (1964) noted that fungi frequently contaminate the galleries and wondered whether carpenter ants feed on the mycelia. Ayre (1967) found high levels of amylase activity in the salivary glands of *C. pennsylvanicus*. Amylase hydrolyzes glycogen and starch, the storage polysaccharides used by animals and plants, respectively. Predatory ants tested did not exhibit high levels of this enzyme, leading Ayre (op. cit.) to search for sources of polysaccharides unrelated to prey. In the laboratory, *C. herculeanus* workers exposed to four different fungi chewed and regurgitated onto the specimens, then consumed the regurgitate, behavior characteristic of extraoral digestion (Ayre 1967). Carbohydrate digestion certainly can begin extraorally, as the glands showing invertase and amylase activity empty onto the labium (Ayre 1963b, Hölldobler and Wilson 1990). Brand et al. (1972, 1973) discovered

mellein, a fungal metabolite, in the mandibular glands of male carpenter ants, perhaps providing further indirect evidence that fungi are eaten. The ability to digest fungi would enable the ants to exploit an alternative and convenient food source, one that may even provide an additional source of dietary nitrogen.

CHAPTER 3. AN ANALYSIS OF SEASONAL MACRONUTRIENT COLLECTION BY COLONIES OF *Camponotus pennsylvanicus* (De Geer): FORAGER CROP CONTENTS

3.1 Introduction

In a sense, division of labor in the ants extends to life stage as well as caste. The workers are responsible for care of the brood and queen, food collection and distribution, nest and trail maintenance, and colony defense. The chief task of the larvae, on the other hand, is to maximize their tissue production (Stradling 1978). These disparate energy requirements are met through very different nutritional programs. Workers rely on carbohydrates to meet their substantial energy needs, while larvae feed primarily on nitrogenous foods, though sexual larvae also require great quantities of lipid (Peakin 1972, Brian 1973, Abbott 1978, Stradling 1978, Beattie 1985, Wheeler and Buck 1992).

It follows that food collection in the ants is intimately linked with brood production (Way 1963). As brood production is cyclic, there is a seasonal component to food collection. Seasonal changes in foraging behavior have been observed by a number of authors and measured in a number of parameters: forager frequency (Fowler and Roberts 1980, Skinner 1980, Tennant and Porter 1991), prey numbers (Ayre 1959, Horstmann 1972, Fowler and Roberts 1980, Skinner 1980), prey volume (Wellenstein 1952, Horstmann 1974), crop volume (Horstmann 1972), macronutrient concentration in the crop (Horstmann 1972, Skinner 1980, Cosens and Toussiant 1986), worker energetic reserves (Ricks and Vinson 1972), energy content of food (Skinner 1980), and consumption rates of specific foods (Ayre 1959, Gotwald 1968, Kugler and Hincapié 1983, Hansen and Akre 1985).

Some of these researchers attempted to quantify the importance of nitrogen and carbohydrate in the diet, but the results have not shed much light on the issue. One problem is that the quantity of insect matter retrieved by foragers was often substituted for a more precise measure of nitrogen collection, and the quantity of honeydew gathered for carbohydrate collection. Honeydew is certainly a carbohydrate-rich food, containing from 11 to 100% of its dry weight in mono-, di-, and oligosaccharides (Auclair 1963). On the other hand, honeydew can also contain amino acids and amides, in addition to lipids, sterols, organic acids, alcohols, auxins, salts, minerals, and B-vitamins (Maltais and Auclair 1952, Auclair 1963, Way 1963, Strong 1965). In fact, the nitrogen component of some honeydews can be as high as 18.6% (Elser 1929 in Auclair 1963). The concentration of protein and amino nitrogen in insect hemolymph and tissue is quite high in comparison: as much as 636 mg / 100 ml hemolymph or 70% of tissue weight (Florkin and Jeuniaux 1974, Woodring 1985). As food items, however, insects furnish more than protein. During its lifetime, an insect is continuously engaged in the selective concentration of various nutrients from the environment (Gordon 1956), any of which may stimulate feeding in a secondary consumer.

A second problem that arises when using the weight, size, or volume of insect food items as a gauge of nitrogen collection is the lack of precision inherent in the method. On the one hand, some insects may be consumed by the forager *in situ* and be brought back to the nest hidden in the crop, resulting in underestimation of protein collection (Gößwald and Kloft 1956, Ayre 1963b, Horstmann 1974). On the other hand, some or all insect parts taken to the nest may be inedible and eventually discarded, producing an overestimate (Lange 1962).

A third problem arises when investigating nutrient collection through the manipulation of laboratory colonies. While this approach does permit the testing of finer questions than field observations, the assumption that laboratory conditions accurately recreate natural conditions may not be valid. First, there is some evidence that lab-reared

ant larvae are histologically different from field larvae (Maidof 1968 in Horstmann 1974). Second, laboratory colonies may have lower energy demands than field colonies, as they generally have sufficient quantities of food, lower nest maintenance costs, and shorter foraging distances (Horstmann 1974). Third, laboratory colonies may receive less variety in their diet and so may become less selective in choice tests (Schmidt 1938), or laboratory colonies may be overfed and may become more selective in choice tests (Schmidt-Hempel et al 1993). Finally, the use of laboratory colonies for experimentation may not reduce nest variability below that seen in field colonies (Lanza 1991).

The present study examines directly the seasonal collection of macronutrients by field nests of the black carpenter ant, *Camponotus pennsylvanicus* (De Geer). Like most carpenter ants, it is considered omnivorous, a term broad enough to conceal a great deal of ignorance concerning food preference. We know that it feeds on honeydew and insects; it also has been observed feeding on plant juices, a wide variety of household foods, and possibly fungal mycelia (Pricer 1908, Funkhouser 1915, Ayyar 1935, Ayre 1963, Sanders 1964, Smith 1965, Ayre 1967, Gotwald 1968, Fowler and Roberts 1980, Dukes 1982, Youngs 1983, Hansen and Akre 1985). However, while it may be intriguing to know that carpenter ants have been observed consuming angel food cake, hard-boiled eggs, and urine (Smith 1965, Sevastopulo 1977), the larger questions remain unanswered. Which macronutrients, in fact, are being sought in this seemingly promiscuous menu? What is their relative importance in the dietary regimen of the colony? How do these gross nutritional objectives vary with season? For most ants, these questions have never been satisfactorily addressed. Therefore, the goal of this work was to quantify the macronutrients -- carbohydrate, protein, and lipid -- collected by nests of this ant under natural conditions in the field and to examine seasonal fluctuations, if any, over the annual cycle.

3.2 Materials and Methods

3.2.1 Collection of Samples

Based on their consistently strong foraging activity, I chose four *Camponotus pennsylvanicus* nests in standing trees around the Virginia Polytechnic Institute and State University campus in early May. Foraging activity patterns of these nests were observed in May, June, and August. No two nests were closer than 208 m apart, ensuring that each belonged to a separate colony. Numbers of foragers leaving each nest were counted for ten minutes of every hour over one 24-hour period per month. Measurement was halted when precipitation curtailed activity. To control for nest size differences, hourly counts were normalized to a percentage of total activity per day per nest. The time of day at which foragers were sampled was decided based on May observations. June and August observations, used for verification, were limited to two of the four nests.

For crop content analysis, I sampled foragers during the peak daily foraging period at 20:00 hrs or later (see Section 3.3.1), in order to obtain the most representative cross-section of food collection. All four nests were sampled bimonthly, on the first and fifteenth of every month, weather permitting. I limited the number of foragers sampled as much as possible to avoid affecting foraging behavior (Horstmann 1970, Stradling in Brian 1978), instead increasing the length of the study to two years. Making the admittedly conservative assumptions that 1) the average mature nest has 1000 workers, 2) that removal of more than 5% of the population would lead to behavioral changes, and 3) that it takes 4 weeks to replace a lost forager, I allowed myself no more than 50 foragers per month from any one nest. Sampling consisted of aspirating twenty foraging workers (plus a few "spares"), ten departing the nest and ten returning to it, with a modified Car-Vac portable vacuum. Immediately upon collection, samples were placed on ice until they could be brought back to the laboratory, where they were stored at -15 ^{o}C until

dissected. Additionally, a small number of similarly-treated ants were collected on one occasion for analysis of the nitrogen content of the crop itself; five of these were analyzed.

3.2.2 Preparation of Samples

The crop of each forager collected was carefully dissected out and cleaned of any adhering body tissue. To reduce the anticipated effect of individual variability, crops were pooled in groups of five, producing a pair of pooled samples for any given date, nest, and forager direction (departing versus returning). One of the two pooled samples was analyzed for nitrogen content. The other was serially extracted and analyzed for both lipid and carbohydrate content. If the sample was intended for lipid and carbohydrate analysis, the crops were placed in capped 1.5 µl microcentrifuge tubes and placed on ice until sufficient samples had been prepared. If the sample was to be analyzed for nitrogen, the crops were placed in 1 ml distilled H_2O after dissection and kept on ice until all the samples necessary to run the extraction had been readied.

3.2.3 Quantification of Samples

3.2.3.1 Nitrogen Analysis

Nitrogen content was assayed using a micro-Kjeldahl technique (Shaw and Beadle 1949, Mullins 1971). Samples were placed in 18 mm Pyrex test tubes with one ml of a digestion mixture (313 mg anhydrous $CuSO_4$ and 129 mg selenous acid in one liter 5N H_2SO_4), and heated at 150 ^oC for about 12 hours, or until all water was driven off. The samples were then distilled at 300 ^oC for 12 hours. The test tubes were removed from heat, and three ml of distilled H_2O were added to each sample. Three ml 3.3N NaOH followed immediately by two ml Nessler's reagent (7 g K_2HgI_4 and 1.75 g gum ghatti

refluxed in one liter distilled H_2O for nine hours, then filtered) were added to all samples, which were then vortexed and allowed to stand for 10 to 15 minutes. The resultant solutions were read against a series of ammonium sulfate standards, including a digestion mixture blank, which were run through the quantification process with the samples. Absorbance was read at 490 nm in a Perkin Elmer Lambda 3B Dual Beam Spectrophotometer.

Nitrogen values as determined by the micro-Kjeldahl procedure were multiplied by a factor of 6.25. This calculation produces an estimate of crude protein. Were the source of the nitrogen to be protein monomers, the calculation would produce an overestimation of about 1% (Regenstein and Regenstein 1984). As the nitrogen-containing compounds were never identified, I will refer simply to estimates of nitrogenous material. The mean protein content of the empty crop was found to be 311 $\pm$ 39 µg (mean $\pm$ SD). All values for nitrogenous material collection given in this chapter have been adjusted accordingly by deducting the mean protein content of the empty crop.

Although spikeover samples were run through each extraction and quantification procedure to assess recovery rates, a procedural error invalidated those samples. The mean recovery rate for this procedure determined during a previous study was 106 ± 15.2% (Cannon 1990). A check of the analytical technique following the present study using paired samples spiked with 10 µg ammonium sulfate yielded a recovery rate of 102.4 ± 8.5%.

3.2.3.2 Lipid and Carbohydrate Analysis

Lipid, free sugar, and glycogen content were quantitated through a modified Van Handel (1965) serial extraction. Each pooled sample was ground in a 1.5 ml polypropylene microcentrifuge tube with a micropestle, which was then rinsed into the sample with a drop or two of distilled H_2O. Fifty µl saturated Na_2SO_4 were added to

each microcentrifuge tube to precipitate glycogen. Samples were then washed twice, once with 0.5 ml $CHCl_3$:MeOH (2:1 v/v) followed by 0.5 ml distilled H_2O, then with 0.3 ml $CHCl_3$:MeOH (2:1 v/v) followed by 0.3 ml distilled H_2O. Samples were centrifuged for four minutes at 10,000 rpm after each washing. The lipid-containing hydrophobic layer was removed to a pre-weighed microcentrifuge tube and dried under a nitrogen stream. The hydrophilic supernatant was extracted twice for free sugars, first with 1 ml, then with 0.5 ml, of Na_2SO_4-saturated 66% EtOH. Centrifugation at 10,000 rpm for four minutes followed each extraction. The supernatants were placed into test tubes, sealed with Parafilm®, and held at -15 ^oC until quantitated. The pellet remaining from the serial extraction was dried in a sand bath at 40 ^oC, then heated with 0.5 ml 30% KOH and 50 µl saturated Na_2SO_4 in a 100 ^oC water bath for 10 minutes, and finally washed with 1 ml Na_2SO_4-saturated 66% EtOH. The supernatant was discarded and the pellet was stored at -15 ^oC until it was resuspended in distilled H_2O just prior to quantification of glycogen.

Quantification of the lipid fraction was done gravimetrically. As previously stated, lipids extracted via the described technique were placed into preweighed microcentrifuge tubes, dried at ambient temperature under a stream of nitrogen for up to one hour, and then reweighed to the nearest 10^{-5} g in a Mettler AC 100 balance. The difference between initial and final weights was considered the weight of lipid extracted from the sample.

Quantification of free sugars and glycogen was achieved colorimetrically as per Van Handel (1965). Test tubes, each containing five ml of a diluted H_2SO_4 solution (5 volumes H_2SO_4 in 2 volumes distilled H_2O) into which 0.01 g anthrone reagent (Sigma Chemical Company) had been dissolved, were placed in an ice bath. One ml of the carbohydrate-containing solution from the sample was gently laid atop the anthrone solution, the test tubes were capped, the solutions were thoroughly mixed, and the closed tubes were immersed for 10 minutes in boiling water. The reaction was halted by means of an ice water bath. Absorbance was read at 620 nm in a Perkin Elmer Lambda 3B Dual

Beam Spectrophotometer. Extracted sugars were read against an array of glucose standards in 66% EtOH, extracted glycogen against an array of oyster glycogen standards in distilled H_2O. Values for carbohydrates were recorded as glucose equivalents.

Although spiked samples were run through each extraction and quantification procedure to verify recovery rates, a procedural error invalidated those samples. The recovery rates for the above-described extraction and quantification technique as determined in a previous study (Cannon 1990) were: 101.1 $\pm$ 9.9 % (lipids), 91.6 $\pm$ 12.6 % (glycogen), 92.9 $\pm$ 12.9 % (free sugars). A check of the technique after the present study using paired samples spiked with either 200 µg corn oil or 20 µg glucose yielded recoveries of 91.7 $\pm$ 8.7% for lipids and 95.1 $\pm$ 9.1% for free sugars. Glycogen recovery was not rechecked.

3.2.4 Statistical Analysis

Macronutrient determinations for the pooled samples were normalized to an average per ant measure. The difference between values obtained from the ingoing and outgoing foragers for each nutrient per nest and sampling interval was considered to be the average net weight of nutrient per returning forager. I tested the residuals of the main effects models for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively. Data were transformed where appropriate. Each nutrient data set was tested for main effects (nest, month, and year) and interaction effects using the General Linear Model ANOVA, as the data were unbalanced and the model was mixed (SAS 1990). All insignificant interactions were removed from the model and the reduced model rerun. Significant month effects were tested with contrasts. Any other significant main effects were not anticipated, thereby precluding pre-planned comparisons. In these cases, differences were explored using the Tukey-Kramer HSD multiple range test. A two-tailed paired-sample *t* test was used to determine whether

ingoing and outgoing values within a nutrient truly differed or whether the mean of the difference was equal to zero.

As only one nutrient net weight exhibited significant changes over month, I looked for differences between the four overall nutrient means. The Kruskal-Wallis Rank Sum test was used, as the combined data were non-normal in distribution even after transformation. Significant differences were explored using the Nemenyi test, a Tukey-type nonparametric multiple comparison test, substituting the modified standard error and Q statistic for use on unbalanced data as per Dunn (Zar 1996). Finally, differences between sugar and nitrogen compounds within each month were tested using the Kruskal-Wallis test.

All statistical analyses were accomplished using either SAS/STAT (SAS Institute, 1990) or JMP IN (Sall and Lehman, SAS Institute, 1996) software programs. All tests were executed with a 0.05 alpha level. Note that all data presented graphically are untransformed, though statistical results in the text refer to transformed data unless otherwise noted.

3.3 Results

3.3.1 Diel Foraging Patterns

Diel foraging patterns of the experimental nests are illustrated in Figures 3.1 - 3.3. The frequency with which foragers left the nest fluctuated at a low level during daylight hours, then escalated sharply shortly after sunset. Activity increased most sharply between 20:00 and 21:00 hours in May. Observations in June and August suggest that this increase may occur slightly later in June (between 21:00 and 22:00 hours) and slightly earlier in August (between 18:00 and 20:00 hours). Based on these results, all sampling was begun at approximately 20:00 hours.

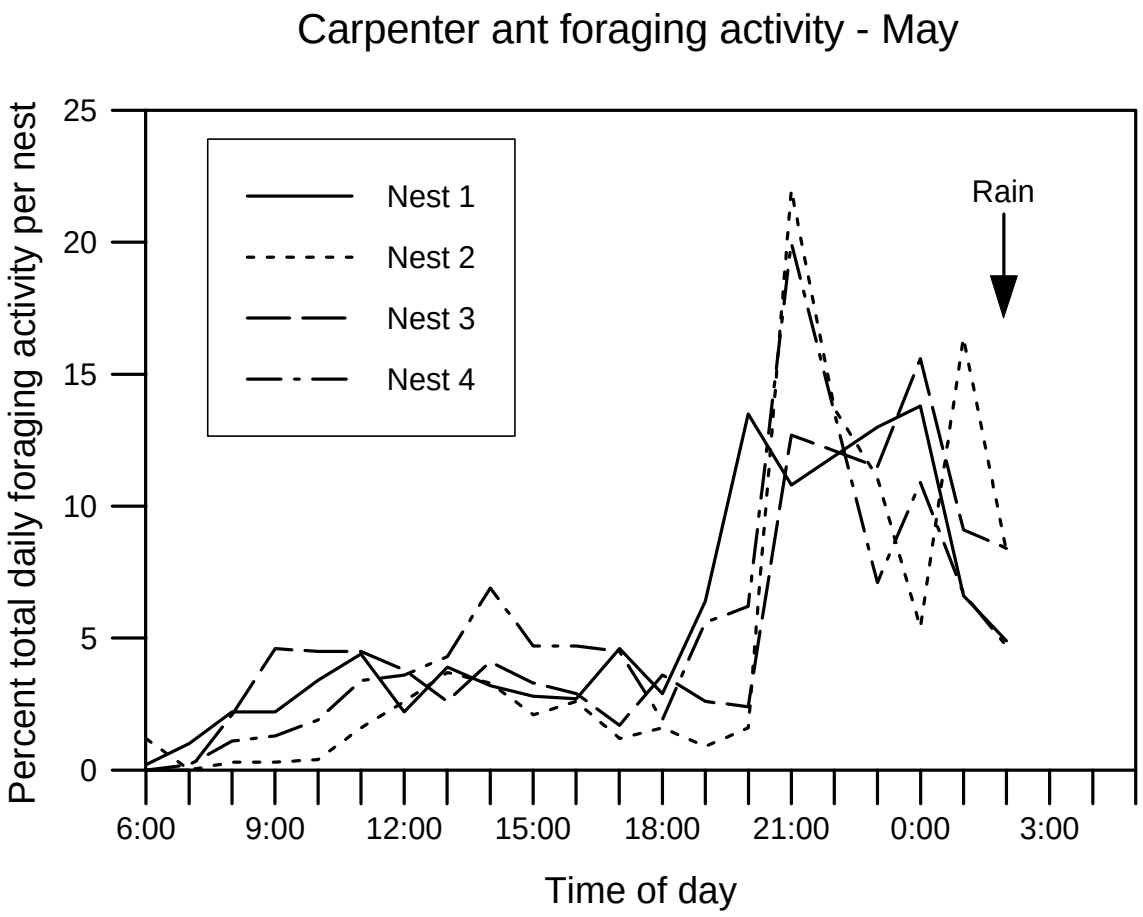

Figure 3.1 Diel foraging activity in *C. pennsylvanicus* as determined by counts of foragers over a 24-hour period in May, using trails outside of natural nests in standing trees around Blacksburg, VA. (N = 1278 for nest 1; 1158 for nest 2; 582 for nest 3; 466 for nest 4.)

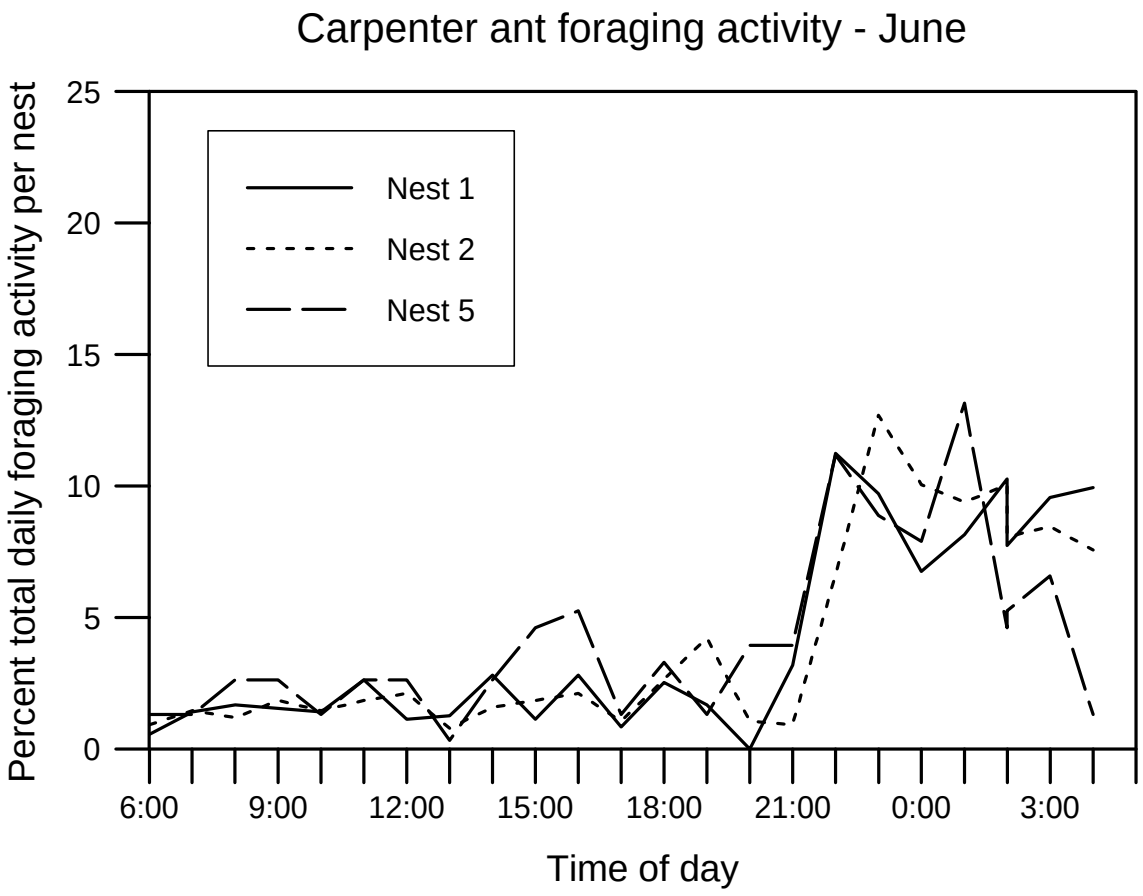

Figure 3.2 Normalized diel foraging activity in *C. pennsylvanicus* as determined by counts over a 24-hour period in June, using trails outside of natural nests in standing trees around Blacksburg, VA. (N = 1778 for nest 1; 1891 for nest 2; 760 for nest 5.)

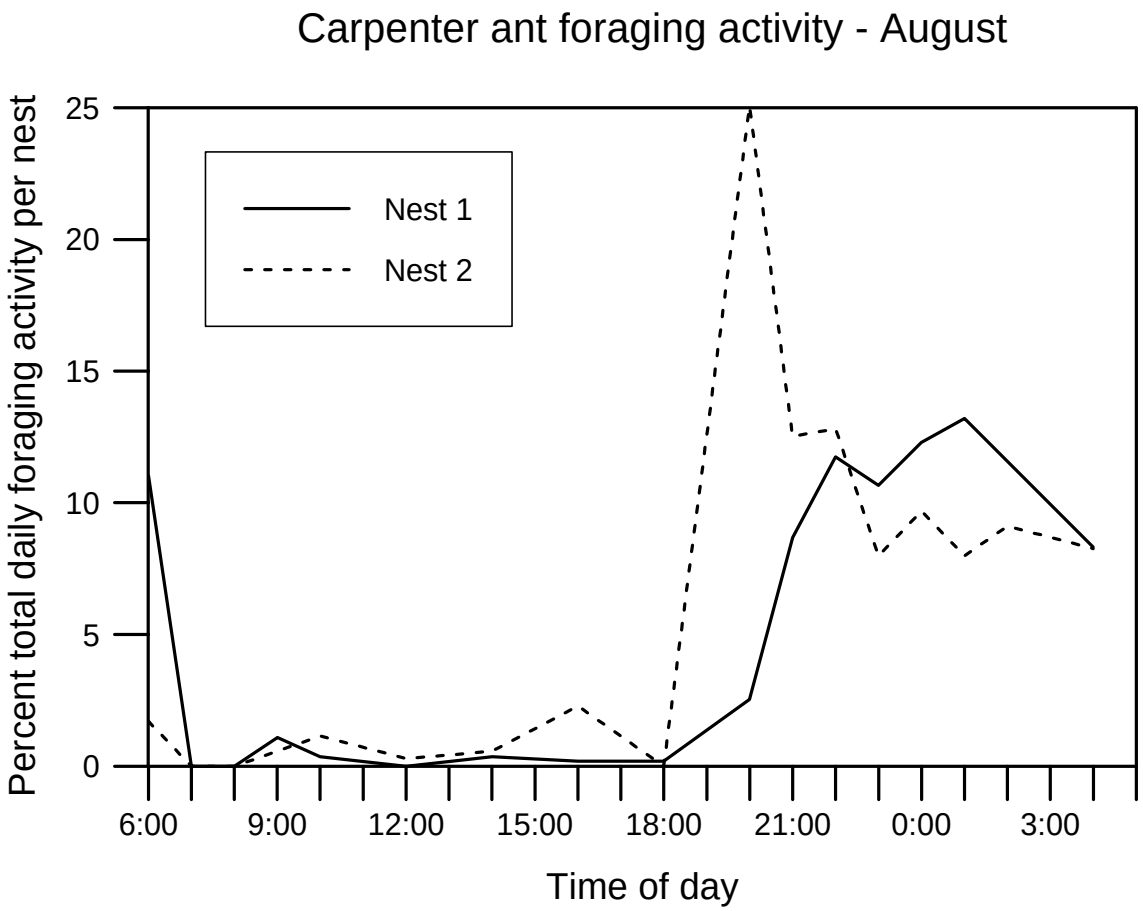

Figure 3.3 Normalized diel foraging activity in *C. pennsylvanicus* as determined by counts over a 24-hour period in August, using trails outside of natural nests in standing trees around Blacksburg, VA. (N = 2765 for nest 1; 1755 for nest 2.)

3.3.2 Solid Food Retrieval

At the onset of the study, the intention was to collect and analyze any solid food carried by an aspirated forager. However, so few foragers returned with solid food that this idea was abandoned. Fewer than 10 returning foragers of the approximately 1500 collected carried visible solid material. This amounted to less than 1% of all returning foragers.

3.3.3 Net Crop Contents of Returning Foragers

A net weight for each macronutrient was determined by deducting the mean carried by outgoing foragers from that of incoming foragers. To assess whether the resultant net amounts were significantly greater than zero, *t* tests comparing outgoing and incoming means within each macronutrient were conducted after the data were assessed for normality. All net amounts differed quite significantly from zero ($P < 0.0001$), with the exception of lipid, which exhibited a marginal difference from zero ($P = 0.0481$).

Before further analysis, the data for each net nutrient were assessed for normality by subjecting the full main effects model to the Shapiro-Wilk test. Three of the four sets were highly non-normal in distribution ($P = 0.0010$ for protein, $P = 0.0002$ for sugars, $P = 0.0078$ for glycogen, $P = 0.5489$ for lipids), but transformation in this case was successful. A square root transformation gave the most satisfactory results. Data were normally distributed ($P = 0.2359$ for protein, $P = 0.8342$ for sugars, $P = 0.8697$ for glycogen, $P = 0.6704$ for lipids) and variances within nutrient were found to be generally homogeneous across all variables. Each nutrient was then tested using a full factorial ANOVA. In no case were interaction effects significant. The significance of main effects varied by nutrient.

Figures 3.4 - 3.7 provide a picture of nutrient collection over the time of study. Figure 3.4 shows the mean net lipid taken by returning foragers. (Note that lipid data are from one year only. A technical error during the extraction procedure made the second year of data unusable.) Neither nest ($P = 0.1175$) nor month ($P = 0.7886$) significantly affected the quantity of lipid collected. The net amount of glycogen found in the crops of returning foragers is seen in Figure 3.5. As with lipid collection, no significant effect of nest ($P = 0.4154$), year ($P = 0.1051$), or month ($P = 0.5008$) on crop glycogen levels was evident. The mean of the untransformed net lipid data over all nests and months was 78 ± 38 µg per ant. (Note that all means are $\pm$ SEM unless otherwise noted.) The mean of the untransformed glycogen over nests, months, and years was 5 ± 1 µg per ant. The apparently large difference between these means was not statistically significant ($P = 0.6965$), due to the large variability.

Most carbohydrates carried in the crops of foragers were sugars (Figure 3.6). The mean net weight of crop-borne sugars over all factors was 1208 ± 169 µg per ant, roughly 250 times the weight of glycogen. While collection of this nutrient did not change significantly over month ($P = 0.1602$), nest ($P = 0.0144$) and year ($P = 0.0292$) were moderately significant factors. The year effect was due to significantly higher sugar collection in the second year of study: 870 ± 210 versus 1558 ± 256 µg per ant. The nest effect was attributed to differences between just two nests: the nest collecting the most sugar and that collecting the least. Only this pair differed significantly, according the Tukey-Kramer HSD procedure (Table 3.1).

Nitrogenous material also represented a major constituent of the crop contents (Figure 3.7). No differences among nests ($P = 0.1783$) or between years ($P = 0.3636$)

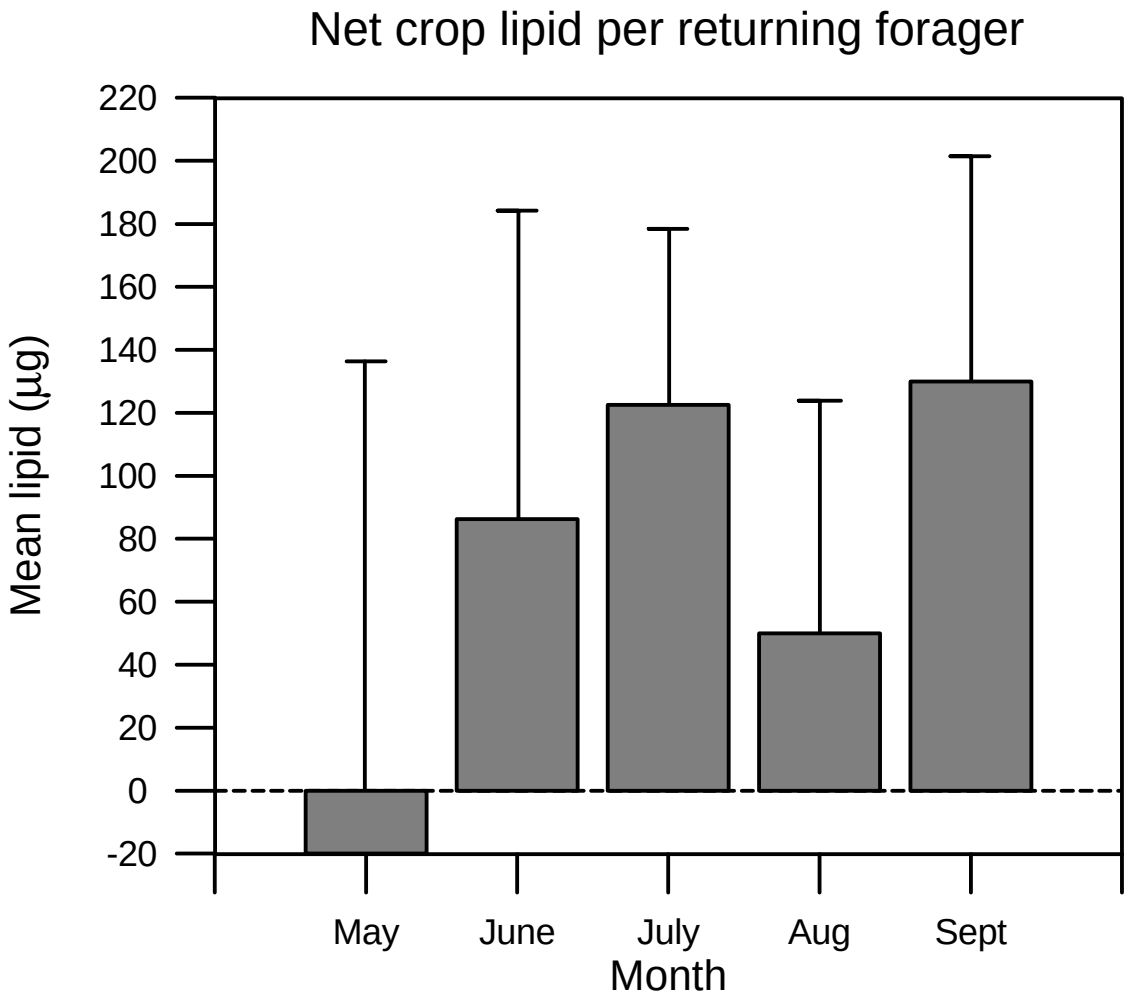

Figure 3.4 Net mean crop lipid per returning forager foraging under natural conditions in the field. (Data collected for one year. Vertical bars indicate SEM. N = 32.)

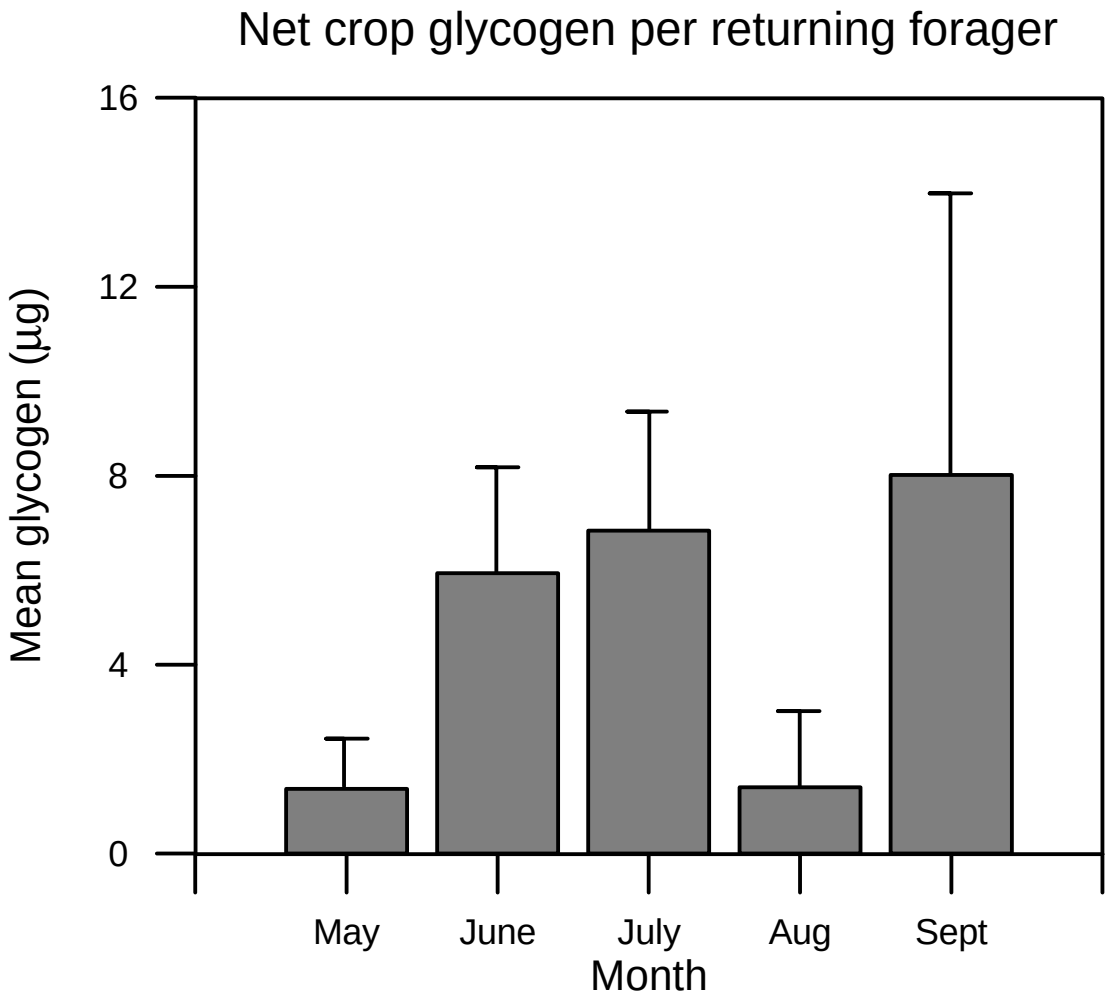

Figure 3.5 Net mean crop glycogen per returning forager foraging under natural conditions in the field. (Data collected for two years. Vertical bars indicate SEM. N = 64.)

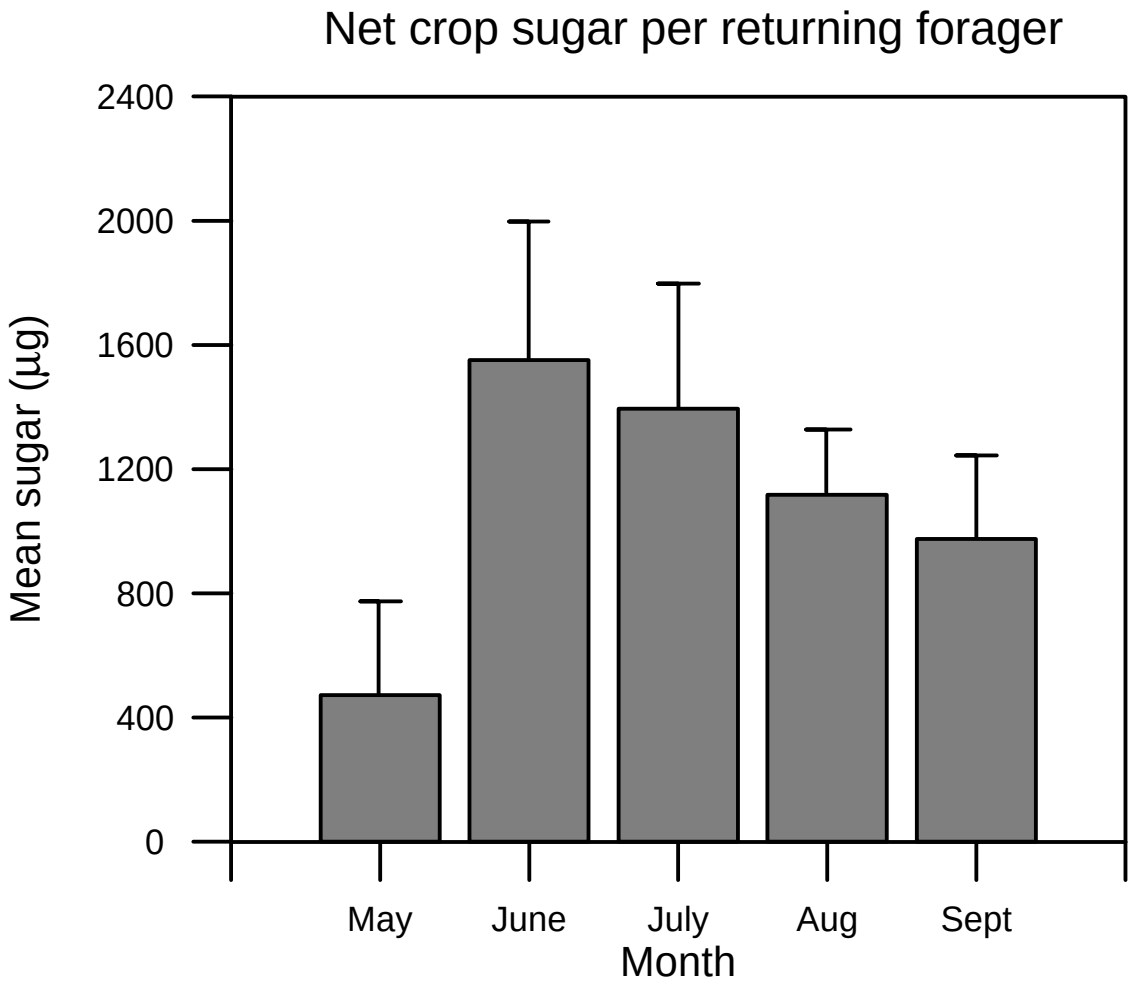

Figure 3.6 Net mean crop sugars per returning forager foraging under natural conditions in the field. (Data collected for two years. Vertical bars indicate SEM. N = 64.)

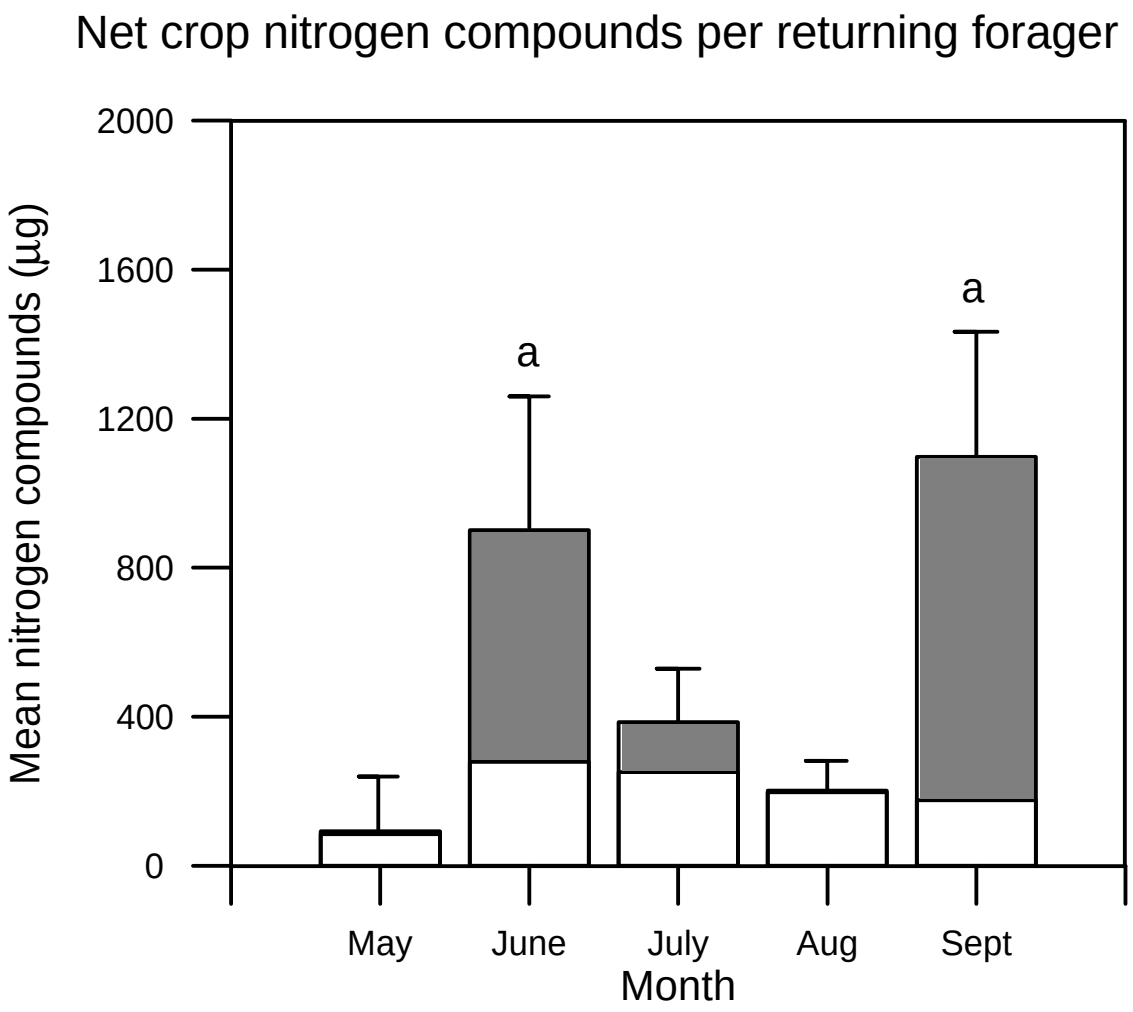

Figure 3.7 Net mean crop nitrogen compounds per returning forager foraging under natural conditions in the field. Unshaded areas represent the maximum amount of nitrogenous material attributable to honeydew nitrogen (18% of sugars collected per month, as per Auclair 1963). a = months different from all others (P = 0.0028). (Data collected for two years. Vertical bars indicate SEM. N = 64.)

Table 3.1 Tukey-Kramer Honestly Significant Difference multiple comparison test, testing for differences among nests over month and year within the nutrient sugar, controlling for an experimentwise error rate of $\alpha = 0.05$.

NEST #	4	1	3
2	-12.11	-10.83	1.81*
4	.....	-13.39	-0.75
1		.....	-1.58
3			.....

* Positive values show pairs of means that are significantly different.

were found, but changes in nitrogen collection by month were modestly significant ($P = 0.0469$). Four bimodal patterns were considered: peaks occurring in May and September; in May and August; in June and August; and in June and September. These patterns were examined via contrasts, requiring a P-value of ≤ 0.0125 as the threshold for significance. I determined that significant peaks occurred in June and September ($P = 0.0028$). The mean amounts transported were: 93 ± 147 µg in May, 901 ± 361 µg in June, 386 ± 138 µg in July, 198 ± 79 µg in August, and 1098 ± 329 µg in September. For the sake of comparison, the overall mean net weight of nitrogenous material delivered to the nest was 520 ± 115 µg per ant.

A measure of overall preference among macronutrients over the active phase of the annual cycle was achieved by combining the data for all macronutrients. Since only nitrogenous material was retrieved differentially over time, I proceeded to test for differences between the macronutrient means summed over all factors. The four data sets combined proved to be highly nonnormal when subjected to the Shapiro-Wilk test ($P < 0.0001$), and transformation did not prove helpful. Therefore, I tested the data using the nonparametric Kruskal-Wallis test. Nutrient amounts were indeed extremely significantly different ($P < 0.0001$). The Nemenyi nonparametric multiple comparison test modified as per Dunn for unequal sample sizes (Zar 1996) was used to interpret these differences (Table 3.2). Four comparisons were made, reducing the level of significance to $P = 0.0125$. Glycogen and lipid were not collected in statistically different amounts ($P > 0.5$). Significantly more nitrogenous material than glycogen was transported to the nest ($P << 0.001$), though nitrogenous material was not collected preferentially over lipid ($0.05 < P < 0.10$). Sugar was certainly retrieved in greater amounts than any of the other nutrients measured, even nitrogenous material ($0.005 < P < 0.01$). However, when sugar and nitrogen compounds were compared within months, they differed significantly only in July and August ($P = 0.0143$ and $P = 0.0053$, respectively).

Table 3.2 Nonparametric multiple comparisons between nutrient mean ranks, using the Nemenyi test with the Q statistic for unbalanced data and a comparisonwise error rate of $\alpha = 0.0125$. ($Q_{0.05, 4} = 2.639$)

NUTRIENT	Nitrogen cmpds	Lipids	Glycogen
Sugars	3.154*	.....	.....
Nitrogen cmpds	.....	2.506	5.020**
Lipids		.....	1.593
Glycogen			.....

* Significant ($0.005 > P > 0.01$).

** Highly significant ($P >> 0.001$).

3.4 Discussion

Carpenter ants are omnivores (Tobin 1993) and omnivores are opportunists (Stradling 1987). While specialist consumers operate in habitats characterized by richness, abundance, and continuous availability of food, omnivores must make the best of their limited food resources by exploiting the most nutritious sources available at any given time (Stradling 1987). In analyzing consumption of the three macronutrients under natural conditions, this study provides a picture of the nutritional needs of the colony as defined by environmental availability.

Of the three macronutrients available for consumption -- nitrogen-based compounds, carbohydrates, and lipids -- the carpenter ant colony appears to rely more or less exclusively on nitrogenous material and carbohydrate. Less lipid was collected than any of the other macronutrients measured. In fact, the amount of lipid brought back to nest was only marginally higher than the amount in the crops of departing foragers ($P = 0.0481$).

It was expected that crop lipid levels would be relatively low. Carpenter ants do not forage on seeds, an excellent source of lipid, and lipid levels in honeydew, though not well known, are thought to be low (Strong 1965a, Ehrhardt 1968). Carpenter ants are known to feed on live or dead insects, but appear to be morphologically restricted to feeding on body fluids or mascerated tissue (Eisner and Happ 1962, Wheeler 1993, see also Chapter 5). Titers of lipid in insect hemolymph are generally low (Chippendale 1972) and it remains unknown whether ants are capable of ingesting the lipid-containing fat body to any significant degree. A dietary requirement for bulk lipid in insects has apparently not yet been documented (Dadd 1985), but a crucial role in colony organization is played by lipids and lipoidal compounds, such as hormones, pheromones, antibiotic substances, and colony recognition compounds (Beattie 1985). One must assume that the dietary needs of the colony for lipid are being met. The question is how

this is done with the small amount observed in foragers' crops. Though variable in composition, plant sap and honeydew apparently provide insufficient quantities of lipids, vitamins and essential amino acids for aphids, leafhoppers, planthoppers, and for some insects feeding on honeydew. These insects typically rely on symbiotic microorganisms in the gut to provide them with needed sterols, B-vitamins, and amino acids (Dadd 1985). Carpenter ants may also resort to this strategy to remedy dietary inadequacies: all species of *Camponotus* examined harbor bacterium-like symbiotes in the epithelium of the hindgut (Steinhaus 1967, Schroder et al. 1996). It could also be possible that even the very small amounts of crop lipid observed here fulfill whatever specific dietary requirements for lipid the colony may have. Some honeydews contain a fair amount of lipid. *Myzus persicae* excretes a honeydew that is 12 - 16% lipid and contains free sterols, fatty acids, and triglycerides (Strong 1965b). In some insects, phytosterols can be used to satisfy the need for exogenous sterol (Dadd 1985).

Alternatively, it is possible that the ants are collecting more lipid than I was able to detect. The work of Peregrine and Mudd (1974) and Delange (1966) has demonstrated a capacity in ants to shunt ingested lipids directly to the postpharyngeal glands, bypassing the crop entirely. Brian (1973) has shown that the contents of the postpharyngeal glands are oily and are used in feeding brood, much as the hypopharyngeal glands of honeybees. Perhaps the amounts detected in the present study represent only the needs of the individual forager, rather than colony-level needs. The moderate activity of midgut lipase in *C. pennsylvanicus* workers would suggest a correspondingly moderate need for lipid on the part of individuals (Ayre 1967).

Also interesting is the evidence presented here that lipid collection does not appear to rise as autumn approaches. This was unanticipated. Lipid accumulation by the adult stage throughout the colony becomes quite pronounced towards the end of the active cycle, and it was expected that lipid collection would reflect this fact. First, workers appear to develop hypertrophied fat bodies (also known as adipogastry) in early

fall (Cannon unpubl., Hansen pers. comm.). These reserves, which are depleted during overwintering, are critical to surviving the period of prolonged starvation which winter represents (Cannon 1990). Second, and perhaps more important, alates are reared late in the summer and eclose early in the fall in the nest, where they remain until swarming occurs the following spring (Sanders 1964, Hansen and Akre 1985). The massive gynes in particular require high lipid input not only during larval development but also during the "maturation phase", the period of nutrient reserve accumulation extending from eclosion to swarming (Passera et al 1990, Passera and Keller 1990). It should be born in mind as well that alates are produced in extraordinary numbers to compensate for severely high mortality rates that accompany swarming (Beattie 1985).

I had expected to see at least some of the greatly increased need for lipid late in the season reflected in foraging habits. It may be that the lipids accumulated by workers for overwintering are produced largely or entirely through intermediary metabolism. The substrate required for lipid synthesis is acetyl-CoA, which results from carbohydrate, fatty acid, or amino acid oxidation (Candy 1985). Conversion of carbohydrate to lipid is common among insects (Candy 1985), and the high quantities of carbohydrate observed in the crops of foragers (see below) make this macronutrient a likely source of the acetyl-CoA necessary for fatty acid synthesis. Of course, fatty acid synthesis generally occurs in the fat body and, therefore, would not be detectable by the techniques used in this study.

Only minute amounts of glycogen were detected in the crops of returning foragers. Ayre (1967) found high amylase activity in the salivary glands of *C. pennsylvanicus*. He concluded that starch and/or glycogen, which are digested by amylase, must form a large part of the diet. Since amylase activity was not seen in *Formica integra*, a predacious species, Ayre assumed that the polysaccharides would not be derived from prey. He speculated that *Camponotus* may be feeding on fungi, and observed some indications in the lab that this is so. Fungi are often associated with the galleries excavated by carpenter ants (Sanders 1964). I weighed the possibility that ants forage for fungi (glycogen) only

within the nest, hence the low glycogen levels in the crops of external foragers. Unfortunately, a less interesting but more realistic explanation materialized upon reexamination of the literature. Tennant and Porter (1991) have demonstrated unequivocally that sucrose is rapidly converted in the crops of *Solenopsis invicta* foragers to glucose and fructose. The hydrolysis of sucrose, the most abundant sugar in honeydew, is accomplished by invertase, also known as sucrase. In the previously mentioned study, Ayre also found moderate invertase activity in the maxillary glands, where this enzyme appears to be produced or stored. Both the maxillary glands and the salivary glands empty onto the labium (Hölldobler and Wilson 1990). Consequently, simple and complex carbohydrates are probably being digested as they are consumed. In other words, the simple and complex carbohydrates in the crop cannot reliably be distinguished by my method. The very small amount of glycogen I discovered in the crops of foragers most likely represents an undigested fraction of what had been consumed. Therefore, sugar and glycogen cannot be considered separately, but the determinations for both should be pooled into a general measure of carbohydrate collection. Crop glycogen levels as determined in this study were only 1% or less those of sugars. Consequently, in order to simplify further discussion, sugar collection will be taken as representative of carbohydrate collection and will be discussed as such, though glycogen collection is included implicitly.

When examined over all variables, more carbohydrate was collected than any other macronutrient. This is not entirely surprising. It is generally accepted that workers of most ant species are primary consumers, deriving their energy for foraging from honeydew (Carroll and Janzen 1973, Stradling 1978, Tobin 1993). Preferential collection of honeydew, or liquid food, over protein, or solid food, has been demonstrated in other *Camponotus* species (Pricer 1908, Sanders 1972, Fowler and Roberts 1980, Hansen and Akre 1985), in other Camponotini (Gersani and Degen 1988), in other omnivorous Formicines (Wellenstein 1952, Horstmann 1970 and 1974), and in omnivorous Myrmicines (Brian 1973, Tennant and Porter 1991).

More interesting here is the finding that carbohydrate collection did not exhibit seasonal fluctuations. It was difficult to predict any outcome in this case based on interpretation of the literature, since few direct measures of carbohydrate gathering have been made. Skinner (1980) supposed he had observed seasonality in honeydew collection by *F. rufa*, but he never tested the crops of returning foragers for the presence of nitrogen; neither did he quantify the amount of crop sugar. His conclusions regarding sugar collection are based solely on the dry weight of ants with unidentified crop contents. The work of Edwards (1951) and Wellenstein (1952) may be similarly flawed. Both Horstmann (1972) and Tennant and Porter (1991) analyzed crop contents of *F. polyctena* and *Solenopsis* spp. foragers, respectively, and they found no association between carbohydrate collection and brood rearing. Hansen and Akre (1985), observing the behavior of laboratory colonies of *Camponotus modoc*, noted a slow decline in collection of sugars after the emergence of alates in the fall, though it was unclear whether the change was brood related or diapause related.

It would not have been surprising to find that carbohydrate gathering intensified with nitrogen collection, particularly late in the season when alates are growing and emerging. Alates that fly short distances, such as carpenter ants, accumulate carbohydrates for flight during the larval growth and adult maturation periods (Bonavita and Passera 1977, Passera et al 1990, Passera and Keller 1990). Sexual larvae also require large amounts of protein and lipid, one or both of which are obtained by consuming insects. One could easily imagine prey capture or even scavenging to be more energetically expensive than aphid tending. However, honeydew gathering may not be energetically cheap and preying on aphids may not be that costly. Besides, honeydew sugars fuel both enterprises (Carroll and Janzen 1973).

Clearly, the carpenter ant colony relies heavily and consistently on carbohydrates to meet its energetic needs. However, factors unrelated to season obviously have an impact on carbohydrate yield. Nest and year variables significantly affected carbohydrate

levels in the crop. In the present study, less carbohydrate was collected during the first year than during the second. Variability from year to year has also been observed by Horstmann (1974). Herzig (1938) found the amount of honeydew collected to be weather dependent, and speculated that temperature and precipitation play important roles in carbohydrate collection. Examination of weather data for the region showed that the months of May through August were consistently colder and wetter in the first year than in the second. Naturally, no attempts were made to collect ants during inclement weather, however the mitigating influence of bad weather on foraging may last longer than suspected. (Herzig 1938) noted that full foraging activity was sometimes not achieved for days after a heavy downpour. Regarding the observed differences between nests, one may suppose that they are the product of local habitat differences, and may provide clues to differential colony survival. It is worth mentioning that the nest recovering the least amount of carbohydrates vanished within three years of the last sampling date, though it is not known whether it had migrated or had died.

Protein may be the most limiting nutrient to colony growth (Nalepa 1993, Wheeler 1993). As the principal duty of larvae is to grow, they are the primary consumers of protein in the ant colony. Food intake is therefore related to brood production and as such is "markedly seasonal" (Stradling 1978). Such a correlation has been demonstrated experimentally in laboratory colonies of *F. polyctena* (Lange 1962), *Myrmica rubra* (Brian 1973), and *S. invicta* (Ricks and Vinson 1972). In the present observational study, the collection of nitrogen-containing compounds exhibited a clear seasonal dependence, in contrast to that of lipids and carbohydrates. The amount of nitrogenous material found in the crops of returning foragers peaked in the months of June and September. Although colony condition was not monitored, the observed peaks correspond well with the general pattern of brood production in these ants. According to Fowler (1982), oviposition frequency in *Camponotus* is typically bimodal, generating two adult cohorts. The earlier oviposition peak extends through spring and generates both workers and sexual forms in August and September. The later, smaller peak occurs in

late summer and produces a group consisting exclusively of workers the following year in June and July. Evidence from various sources supports this scenario in *C. herculeanus* (Sanders 1964), *C. pennsylvanicus* (Pricer 1908), and *C. modoc* (Hansen and Akre 1985). It is interesting that the two peaks in amount of nitrogen harvested do not differ statistically in degree. I would not have been surprised to have found the later peak somewhat larger, because the brood feeding at that time include sexual larvae and reproductive individuals are more expensive to produce for obvious reasons. It could be that the amount of nitrogenous material collected during peak intervals represents the maximum investment in foraging that the colony is able to make, and quantity and quality of brood produced are tailored accordingly.

Though the source of the nitrogen compounds collected was not determined, it can be safely deduced that honeydew collection was not escalated to provide for increased nitrogen demands. If such had been the case, we would have expected to observe the same effect of month on carbohydrate collection that was found for nitrogen. Instead, though less nitrogenous material than carbohydrate was collected overall, nitrogen compounds did not differ significantly from carbohydrate in net weight collected during months of peak nitrogen collection. Figure 3.7 demonstrates this conclusion. Auclair (1963) stated that proteins are lacking from aphid honeydew, but nitrogen compounds make up 0 - 18% of the dry matter. Horstmann (1974) found that only 1% of the carbohydrates in the crops of *F. polyctena* foragers derived from prey. Assuming conservatively, then, that all carbohydrate collected here was obtained from honeydew and that 18% of the weight of carbohydrate collected estimates the nitrogen compounds in honeydew, the hatched area of each bar represents the maximum amount of net nitrogenous material that could be attributed to honeydew collection during each month. The nitrogen unaccounted for is highest in those months during which nitrogen is collected most intensively. This would suggest another source of nitrogen is being exploited.

Insects are an excellent source of nitrogen. According to Nalepa (1993), "the best proteins tend to be of animal origin, such being most likely to provide an animal with amino nitrogen resources tailored to the production of animal protein." Were foragers in June and September augmenting their normal nitrogen loads with insects? The best evidence for scavenging or predatory success was conspicuously absent. Less than 1% of captured foragers were observed bearing insect remains. Other studies have found equally low numbers of *C. pennsylvanicus* transporting insect parts (Sanders 1972, Fowler and Roberts 1980). Pricer (1908) never encountered *C. pennsylvanicus* taking prey. The supplementary nitrogen measured during peak intervals of nitrogen collection was transported to the nest by and large in the crop. There is good evidence that some ants transport solid food internally, consuming insects in the field where they are found (Gößwald and Kloft 1956, Ayre 1963b, Horstmann 1974). The filtering action of the mouthparts and the proventriculus places strict size limitations on what can be ingested (Eisner and Happ 1962, Quinlan and Cherrett 1978, Glancey et al. 1981). For this reason, intake is restricted to dissolved proteins and amino acids (Wheeler 1993). While there are at present no indications of such behavior in *C. pennsylvanicus, C. herculeanus,* at least, does indeed get most of its dietary nitrogen from the body fluids and water-soluble proteins of prey or scavenged insects. It has been observed applying regurgitate and formic acid to prey tissues to induce liquefaction (Ayre 1963b). It is also entirely possible that solid food is of only limited use, even to larvae. Consumption of solid food could be demonstrated only in fourth-instar larvae of *S. invicta* (Petralia and Vinson 1978). Thus, it seems the method of removing soluble nitrogen from insect tissues *in situ* may be rather efficient after all, particularly when one considers that transport of insect remains may be expensive and that not all insect protein is digestible (Stradling 1978).

The object of this study was to quantify the patterns of macronutrient collection under natural conditions. All efforts were made to avoid modifying in any way the environment or colony condition of the sampled nests. The picture that emerges, then, is one of colony need within the environmentally imposed constraints on food availability.

How much these foraging patterns are based on food availability and how much on colony need is investigated in the next chapter.

CHAPTER 4. AN ANALYSIS OF SEASONAL MACRONUTRIENT COLLECTION BY COLONIES OF *C. pennsylvanicus*: RESPONSE TO CHOICE TESTS

4.1 Introduction

The results of the previous study have shown that, under natural conditions, *C. pennsylvanicus* foragers collect substantially more carbohydrate than nitrogenous food on a per-forager basis, whereas virtually no lipid food is collected. Of these three macronutrients, only nitrogen was collected in a seasonally dependent manner. During June and September, nitrogen collection peaked such that the collection rate rivaled that of carbohydrate collection. These data conform well with what we know about bimodal brood production in *Camponotus*, and suggest that the energy demands of the colony remain somewhat stable over time, not varying significantly with brood production. Of course, the picture that emerges is not that of the nutritional needs of the colony, but of the colony's efforts to fulfill its nutritional needs within the constraints imposed by its environment.

One of the principal tenets of population ecology is Liebig's law, which states that populations tend to continue to grow until one of the essential resources reaches a "critical minimum." Though the limiting resource can be a commodity of space, or habitat, the ultimate factor limiting population growth in the absence of all others is food (Price 1975). As Elton (1927) wrote, "The primary driving force of all animals is the necessity of finding the right type of food and enough of it. ...[T]he whole structure and activities of the community are dependent upon questions of food supply."

White (1978) refined the argument by hypothesizing that the "inadequate environment" in which animals compete for survival is not deficient in food in general, but rather in sufficient exploitable sources of nitrogen. He wrote, "There is a great deal of nitrogen in the world, but most of it is in a form that is not available as food for animals. And what is available tends to be thinly spread in the environment -- there is a relative rather than an absolute shortage."

By comparison, there does not appear to be any shortage of carbohydrate food sources. In fact, carbohydrates make up 75% of the earth's total organic matter, as well as 75% of the dry weight of all land plants (Whistler and Daniel 1985). Carbohydrates are the most economical and accessible form of energy for animals (McFarlane 1985).

While the requirement for proteins and amino acids continues into the adult stage, it is particularly critical in females of reproductive age and in growing young. In the ants, egg development and larval growth occur simultaneously (Wheeler 1993), so queens, gynes, and larvae are competing with each other for nitrogen. As a result, while competition within the colony for all nutrients can be expected, the fight for protein should be keen. Compounding the effects of internal competition are the effects of external, or internidal, competition for that scarce resource. In short, the nitrogen budget in an ant colony is tight and resources are limited.

Constraints imposed by limited availability of a resource can be ameliorated by synchronizing development with resource availability (Stradling 1987). We know that *Camponotus* typically produces brood cyclically (Fowler 1982), and we saw a bimodal pattern in nitrogen collection by foragers (Chapter. 3). Perhaps brood are reared in this way, early and late in the season, to accommodate seasonal fluctuations in availability of or competition for environmental nitrogen.

How would the nest or colony respond to the removal of such environmental constraints? How would foraging behavior be altered? Several predictions can be made. One would anticipate, in the absence of all environmental constraints, an increase in protein collection sufficient to satisfy all quarters of the colony competing for this resource. If availability and competition are indeed significant factors affecting foraging success, one would expect the time effect observed for nitrogen in the previous study to be dampened or even extinguished. Carbohydrate collection may remain unaffected, as the rate of carbohydrate collection did not change with the rate of nitrogen collection in the previous study. It is equally possible that if protein collection were to increase inordinately, the energy demands of the nest, and hence demand for carbohydrate, would follow of necessity. Predictions regarding lipid collection are more difficult to make, as their absence from the crop in the previous study could be due to either physiological or ecological factors.

In order to test these predictions, the ants were exposed to repeated choice tests throughout the active cycle. During each test, nutrients were available *ad libitum* and internidal competition was reduced or absent. Relative macronutrient preference over time was monitored. The question of lipid consumption was also addressed through observations of foraging behavior during the tests.

4.2 Materials and Methods

4.2.1 Preliminary Tests

Acceptability tests were done in the field and laboratory to determine preference among representative samples within each of the three macronutrient groups, with the intent of using the most phagostimulatory representative of each group in the subsequent choice test of macronutrient preference over time. A variety of laboratory chemicals and

processed foods were tested. (Since these ants are household pests, processed foods could be considered part of their natural diet.) Processed foods that consisted of 75% or more (dry weight) of one macronutrient were offered. (Although egg yolk is comprised of only 63% lipid, it was nevertheless included in choice tests because its lipid fraction includes an unusually large proportion of cholesterol, an essential component of the insect diet.) These included the following sources of lipid: coconut oil, soy oil, olive oil, shortening (Crisco®), cream cheese, lard, egg yolk, and heavy cream; and the following sources of nitrogen: strained beef, beef bouillon, canned tuna (packed in water), canned roe, egg white, and yeast. Laboratory chemicals (obtained from Sigma Chemical, unless otherwise noted) were also tested, such as: the simple sugars fructose, galactose, glucose, maltose, sucrose, and trehalose; the amino acids aspartic acid, glutamic acid, leucine, valine, and proline; the protein albumin; and the protein hydrolysates Biosate (Baltimore Biological Laboratory), Gelysate (BBL), Acidicase #2 (BBL), and Hy-Case SF (salt free). Trials were conducted on laboratory or field nests, depending on the weather. Generally, four nests of comparable strength were used per trial.

4.2.1.1 Carbohydrates

Several simple sugars were selected that the ants could be expected to encounter in their usual foods, like honeydew and insects. Galactose, glucose, fructose, maltose, sucrose and trehalose were tested in three laboratory nests for preference with respect to concentration. Sugars were diluted to 1, 5, 10, 20, and 40% (w/w) in d.H_2O. (Sugar concentrations are given as percent weight, as it is in most references concerning honeydew content. For the purposes of comparison with other references it may be noted that a 36% sucrose solution is equivalent to a 1M sucrose solution.) Each nest was offered 0.5 ml of each of the six sugar solutions of a given concentration simultaneously and allowed to feed for 30 minutes. The time required for complete consumption of 0.5 ml test solution was recorded for each nest. Solutions were tested in increasing concentration so as not to affect foraging behavior. The trial was repeated once.

Tests comparing the acceptability of fructose, glucose, sucrose, and trehalose were undertaken in the field as well. Four field nests were given a choice between 20% solutions of the four sugar solutions soaked onto dental dams cut into one cm lengths. Preference was assessed by counting the number of ants feeding on each material; counts were done every 15 to 20 minutes for a period of up to 90 minutes. The trial was repeated twice.

4.2.1.2 Nitrogen Compounds

The notion of comparing processed foods in their purchased form was abandoned after the first trial, when the difficulties of comparing forager frequencies on solid and liquid foods became apparent. These data were discarded and, in subsequent trials, solid foods were homogenized with an equal weight of water, so that all foods could be offered as liquids soaked onto cotton wicks. In two trials, beef bouillon, strained beef, tuna packed in water, and herring roe were tested simultaneously on four field nests. To measure preference, the number of ants feeding was counted every 15 to 20 minutes for 90 minutes. In two additional field trials, tuna was offered along with egg white and yeast against carbohydrate and lipid foods; in this case, however, preference among the protein sources was assessed only qualitatively.

Preference regarding water-soluble amino acids was examined in the laboratory. Solutions of aspartic acid, glutamic acid, leucine, proline, and valine were mixed in concentrations of 0.1, 0.5, 1, 2.5, 5, and 10% (w/w) in d.H_2O. Five nests were used in testing, with each nest receiving one amino acid. Again, solutions were administered in increasing concentration, and time until complete consumption of 0.5 ml test solution was recorded. All solutions were removed after 45 minutes.

The acceptability of several protein compounds was similarly compared. The protein albumin and the protein hydrolysates Hy-Case SF, Biosate, and Gelysate were

diluted with d.H_2O to concentrations of 0.5, 1, 2.5, 10, 25, and 40 % (w/w). As above, solutions were administered in increasing concentration to four laboratory nests, with each nest receiving only one protein solution. Time elapsed to total consumption of 0.5 ml test solution was noted, and solutions were removed after 45 minutes.

Two casein hydrolysates were compared for acceptability. Hy-Case SF and Acidicase #2 were each prepared as 20% solutions in 2% gelatin (w/w) and allowed to gel in 75 x 10 mm disposable polystyrene test tubes. Five laboratory nests received one pre-weighed test tube of each of the test preparations. A duplicate set of tubes, screened to prevent forager access, was also placed in each nest to monitor evaporation loss. Nests were permitted to feed for 75 minutes, then all tubes were reweighed.

4.2.1.3 Lipids

A variety of plant- and animal-derived lipids were offered in four choice tests. All liquids were presented soaked onto cotton wicks. In a choice test for preference among plant lipids, each of four field nests was offered coconut, olive, and soy oils and shortening. Feeding ants were counted every 20 minutes for a period of 80 minutes to determine preference. Response to the animal-derived lipids cream cheese, egg yolk, heavy cream, and lard was examined twice: once in a laboratory choice test, using four nests and counting ants every 15 minutes for 2 hours; and once in a field choice test, using four nests and counting ants every 15 minutes for 75 minutes. In the final choice test, foragers were allowed to choose from among plant and animal lipids. Four field nests were presented with coconut oil, soy oil, lard, and heavy cream. Again, relative acceptability was evaluated by counting the numbers of ants feeding on the materials every 15 minutes for a period of 75 minutes.

4.2.1.4 Matrix Material

Ultimately, the advantages of administering food choices in a neutral gel-type matrix became clear. Toward this end, acceptability tests were performed on agar and gelatin. A trial comparing concentration-related preference was conducted on two laboratory nests. Solutions of 1, 3, 6, and 9% (w/w) of each material were mixed and were allowed to solidify in volumes of one ml. After weighing, a complete series of gelatin samples (1% to 9%) were placed simultaneously in one nest, and an agar series in another. The materials were left for 12 hours, after which they were reweighed. The percent weight removed by foragers was the criterion used to evaluate preference.

A second trial to confirm preference tested gelatin against agar in each of four laboratory nests. A pre-weighed amount of 2% gelatin was placed along with a pre-weighed amount of 4% agar in each nest, left for three hours, and reweighed. Again, the amount removed was used to measure preference.

Finally, the effect of brand on gelatin preference was tested. One ml aliquots of supermarket gelatin (Knox brand) and laboratory-grade gelatin (Fischer Scientific) were prepared at a concentration of 2% as above, allowed to gel, and weighed. One tube of each brand was placed in each of two laboratory nests, left for 30 minutes, removed and reweighed. This procedure was repeated twice. Once again, the amount of material removed was used to evaluate preference.

4.2.2 Macronutrient Choice Tests

Based on the above-mentioned preliminary tests, a 2% preparation of gelatin (Knox brand) was chosen as the matrix. The gelatin solution was made by adding 2% gelatin by weight to boiling d.H_2O and mixing well. Before the solution congealed, 20% by weight of one of the following macronutrient representatives was added and

thoroughly mixed: shortening (lipid); sucrose (carbohydrate); and Hy-Case SF (protein). The solutions (or emulsion, in the case of lipid) were pipetted into 75 x 10 mm disposable polystyrene test tubes at 2 ml per tube, and cooled at 4ºC and allowed to gel upright. All tubes were sealed with Parafilm^{R} and held at 4ºC until they were needed.

Four field nests with strong, regular patterns of activity were selected in May and their foraging trails located. Twice a month from June through September, choice tests were carried out on dry evenings after 20:00 hours EST (8:00 p.m.). The prepared test tubes were laid beside the foraging trail within 0.5 m of the nest entrance and left for two hours. Two complete sets of four test tubes each (one of each macronutrient and one gelatin control) were used per nest and test date. To account for moisture loss, the mouths of one set of tubes were covered with a wire mesh fine enough to exclude foragers. The order in which the macronutrients were offered was rotated with night and nest to avoid any possible juxtaposition effect. All test tubes were weighed immediately before and after exposure to foraging ants. The amount of each macronutrient collected per nest was calculated as a percentage of the total weight removed from all four test tubes per nest per night. This was done to adjust for variations in foraging frequency with nest and date.

4.2.3 Statistical Analysis

4.2.3.1 Analysis of Preliminary Tests

4.2.3.1.1 Carbohydrates

A two-tailed paired-sample *t* test was used to detect a difference between consumption of specific sugar solutions at a concentration of 20%. Concentration-related differences in consumption within or between sugars were not analyzed. In the field trials

comparing feeding on four sugars at 20% concentration, a repeated measures ANOVA was used to detect any overall difference in forager frequency among sugars and differences between nests.

4.2.3.1.2 Nitrogen Compounds

In the initial field trials testing for preference among nitrogen-containing processed foods, a repeated measures ANOVA was used to detect overall differences in forager frequency among foods. However, in the preliminary field trials comparing three nitrogen-containing foods with representative carbohydrate and lipid foods, the response within a macronutrient group was noted only qualitatively, as stated above. Results from trials comparing amino acids for acceptability and protein hydrolysates for acceptability were not analyzed statistically. In the case of amino acids, there were too few data for analysis. In the case of protein hydrolysates, visualization of the data was adequate: in the interest of avoiding a type II error, apparent differences between acceptability at different concentrations were assumed to be real. The results from the trial which compared two casein hydrolysates were analyzed for differences through a two-way paired-sample *t* test.

4.2.3.1.3 Lipids

The trial comparing plant lipids for acceptability provided no data -- no foragers were observed feeding on the food choices -- and therefore could not be analyzed. All other lipid trial results were analyzed using a repeated measures ANOVA to test for differences between foods.

4.2.3.1.4 Base Materials

The initial test results for a suitable matrix material were not analyzed statistically, as differences became clear upon graphing the data. Brand effects within gelatin and differences between matrix materials at a given concentration were realized via a two-way paired-sample *t* test.

4.2.3.2 Analysis of Macronutrient Preference Tests

In order to determine whether preference among the macronutrients and the gelatin control was affected by sampling date, the data were analyzed using a multivariate ANOVA under the General Linear Models procedure. This procedure also tested for the effect of nest on preference. SAS provided probabilities for Wilks' Lambda, Pillai's Trace, and the Hotelling-Lawley Trace for the independent variables, nest and time. The question of whether the overall macronutrient means differed from one another or from the gel control was answered through application of the repeated measures ANOVA profile option which analyzed the overall macronutrient means as contrast variables.

4.3 Results

In preliminary tests, I determined preference among representative foods of the three macronutrient groups. At the same time, I conducted tests to establish the most preferred concentration of the accepted foods. The results were then used to select macronutrient representatives and concentrations for the final test of macronutrient preference over time.

4.3.1 Carbohydrates

The effect of concentration on rates of consumption of simple sugars by laboratory nests is illustrated for each sugar in Figure 4.1. Generally, acceptability increased with concentration. In some trials, however, a diminution of acceptability with concentrations above 20% was observed. Sucrose and fructose exhibited the highest overall rates of consumption and appeared to stimulate the greatest feeding rates at a concentration of 20%. The results of a two-way paired-sample *t* test indicated no differences between consumption of the two sugars at 20% concentration ($P = 0.6643$). In the field, sucrose was preferred to fructose by foragers in two of three trials, and both were consistently preferred over glucose and trehalose, just as they were in the laboratory (Figure 4.2). Nest was found to be a highly significant factor in all three field trials ($P = 0.0001$).

4.3.2 Nitrogen Compounds

Preference among the processed foods tested was consistent over both trials (Figure 4.3). Tuna and herring roe stimulated feeding most successfully in foraging ants. It was unclear whether tuna was preferred over roe, as the probabilities in both trials were marginal. When tuna was offered alongside egg white and yeast in qualitative field tests, tuna was clearly preferred over both. Use of these heterogeneous, processed foods in the macronutrient test over time was rejected in order to exclude the possibility that some uncontrolled factor was responsible for the acceptability pattern observed, such as differences in salt or vitamin content, or a compound associated only with fish or with beef. Nonetheless, these proteineaceous foods elicited foraging behavior strikingly different from that observed during choice tests of sugar solutions. Whereas ants quickly settled and slowly imbibed sugar solutions soaked onto cotton wicks, ants encountering the tuna homogenate became quite active, pulling and biting the cotton, even cooperating

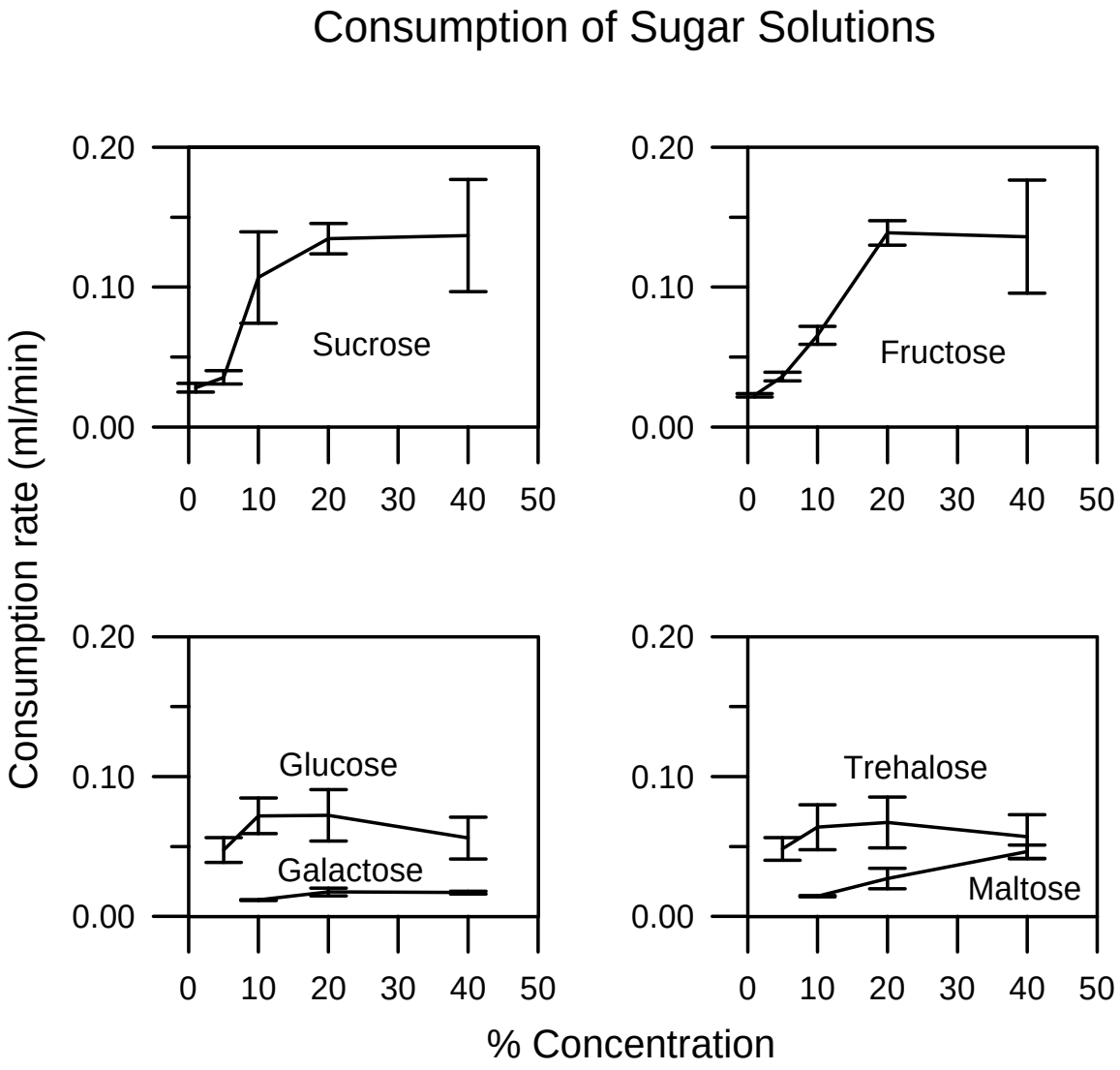

Figure 4.1 Mean acceptability of simple sugars to three laboratory nests during two trials. Consumption rates measured as time to complete consumption of 0.5 ml of test solution. (Vertical bars = ± SEM.)

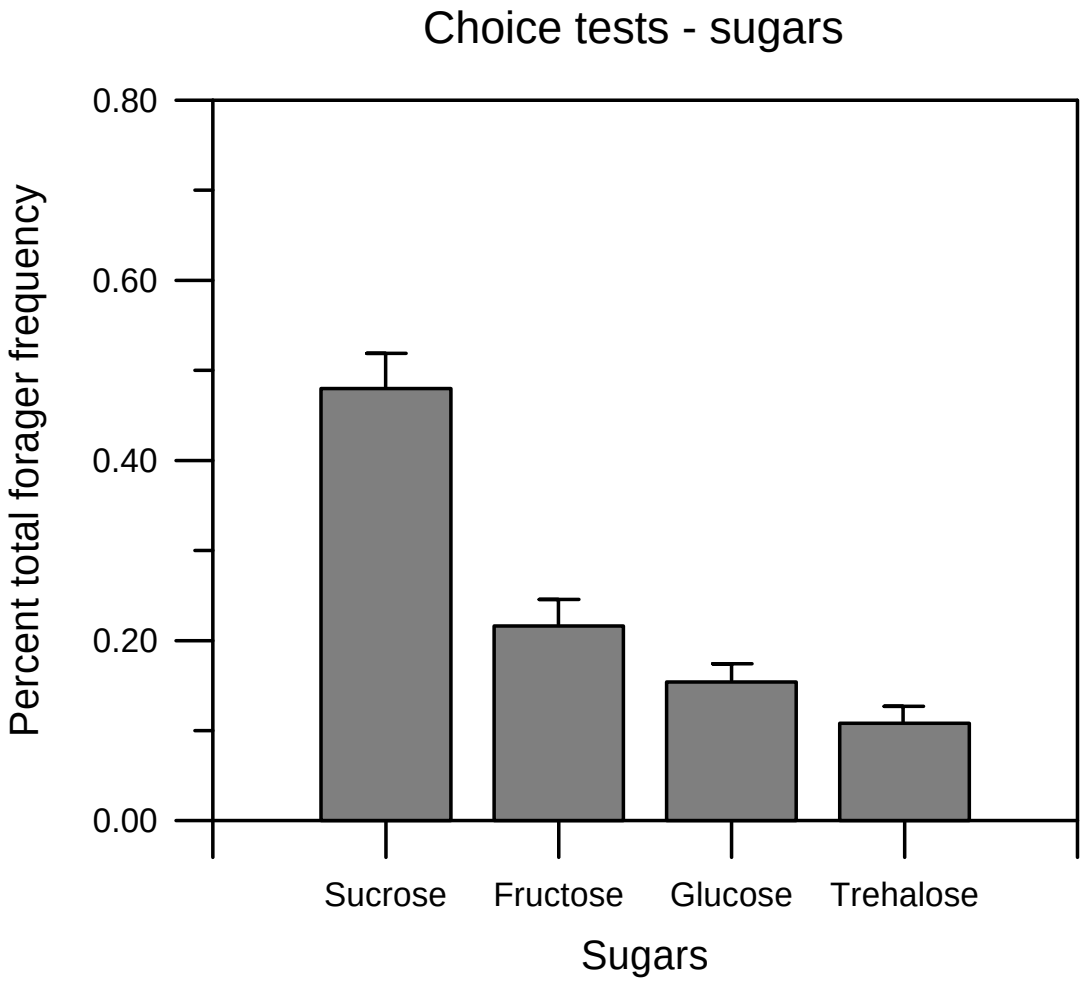

Figure 4.2 Mean acceptability of 20% solutions of simple sugars to four field nests as measured by forager counts at 15-20 minute intervals during three trials. All adjacent means significantly different at P = 0.0001, except P = 0.0229 for fructose - glucose. (Vertical bars = ± SEM.)

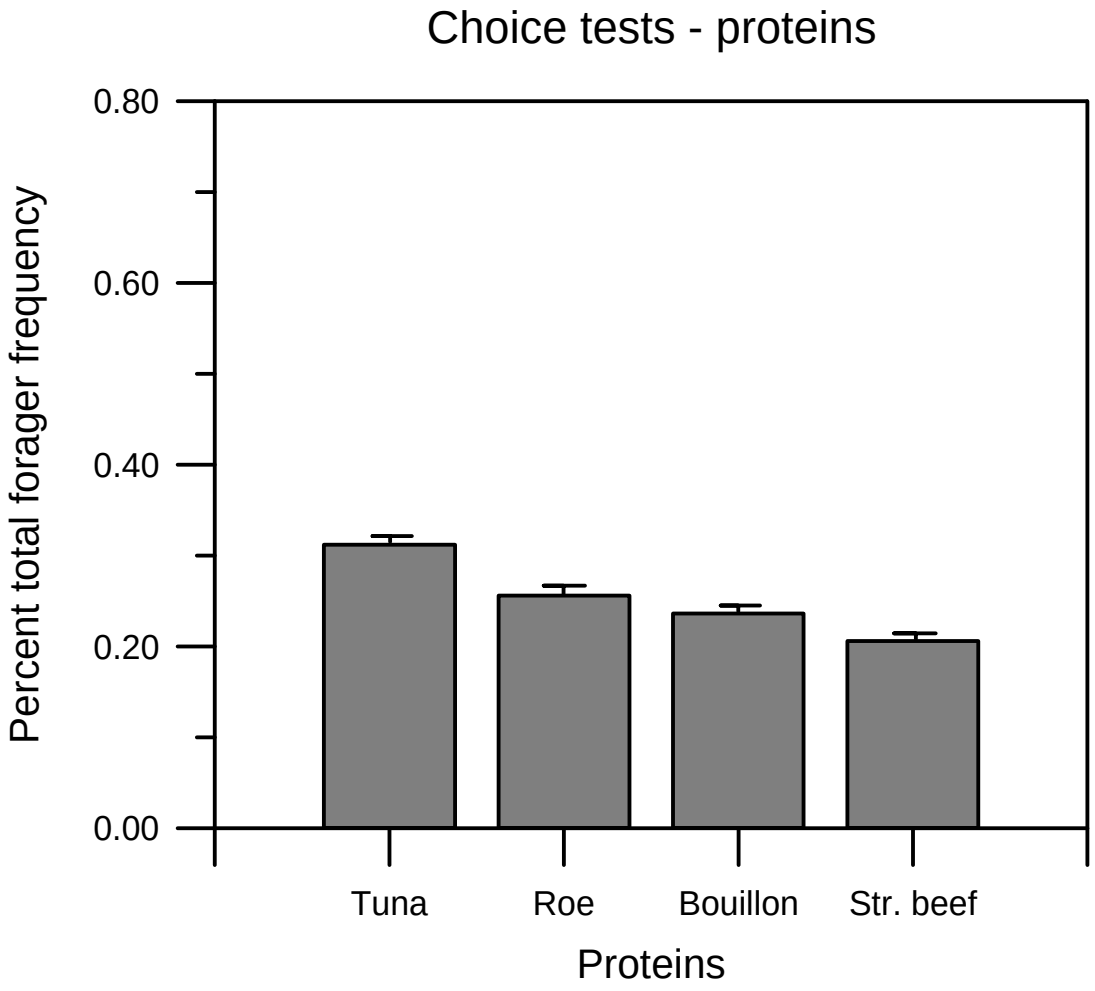

Figure 4.3 Mean acceptability of aqueous homogenates of processed proteinaceous foods to four field nests as measured by forager frequency rates during two trials. All adjacent means significantly different at P = 0.0001, except P = 0.0067 for tuna - roe. (Bouillon = beef bouillon; str. beef = strained beef; tuna = canned tuna packed in water; roe = herring roe. Vertical bars = ± SEM.)

in their attempts to carry the wick off whole. One group of foragers was eventually successful in moving the cotton ball out of the tray in which it had been offered. This behavior was not observed during later trials in which substances in a gelatin matrix were offered.

The comparative test on amino acids did not produce useful results. In order to avoid introducing solvents into the analysis, only water-soluble amino acids were used. Solubility was limited in some cases, restricting the range of concentrations tested. Despite precautions, some precipitation of 2.5% leucine occurred during the trial, precluding use of higher concentrations of that amino acid. I observed virtually no feeding on aspartic acid, glutamic acid, and valine. Consequently, there are no data for these amino acids. Measurable results were obtained only with leucine and proline. Ants were able to detect leucine at lower concentrations: 0.5, 1, and 2.5% solutions were all completely consumed in 20 to 30 minutes. Higher concentrations were not tested for reasons already given. Proline was acceptable only at higher concentrations: the 5% solution was consumed in 38 minutes, the 10% in 19 minutes.

In a test for concentration effects on consumption rates, protein hydrolysates appeared to be favored over albumin, which was consumed too slowly to produce quantitative results (Figure 4.4). Hy-Case SF showed the greatest acceptability at all concentrations, and did not appear to decrease in acceptability at higher concentrations, as the other hydrolysates did. In a separate trial measuring preference between casein hydrolysates, I found no difference between consumption of Hy-Case SF and Acidicase #2 ($P = 0.3511$). Hy-Case was selected nevertheless for use in the macronutrient test over time because more of it was consumed, even if that difference was not significant.

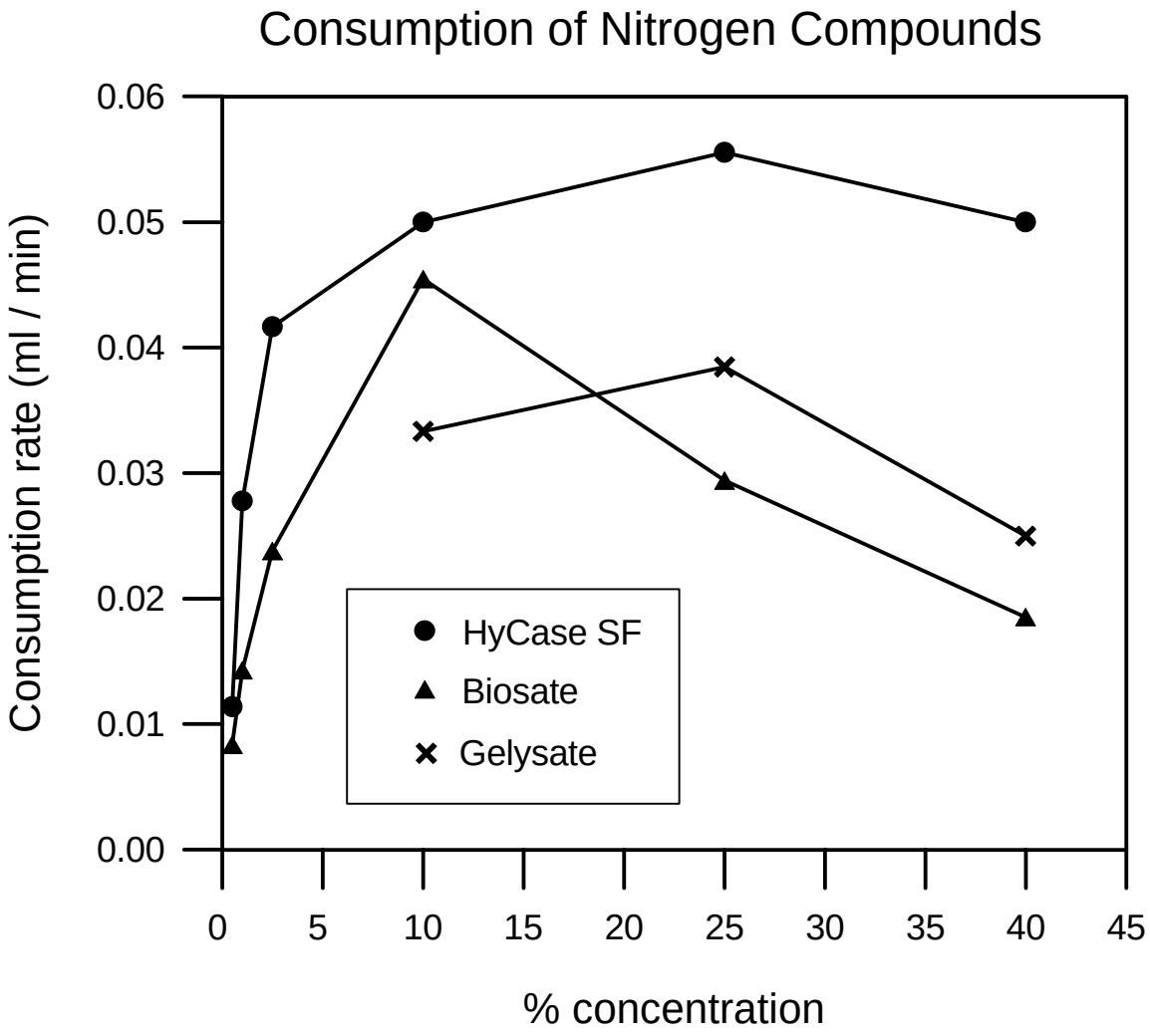

Figure 4.4 Mean acceptability of aqueous solutions of laboratory grade hydrolyzed protein sources to four laboratory nests as measured by consumption rates during one trial.

4.3.3 Lipids

I conducted four trials examining preference among various lipids. There is no data for the first trial, in which only plant-derived lipids were offered, as no foragers were observed feeding during the 80-minute test interval, though several ants were seen exploring the foods and activity along the foraging trail appeared normal. Preference was established among animal-derived lipids, with egg yolk the most and lard the least acceptable foods (Figure 4.5). When the two least-preferred animal lipids (lard and heavy cream) were compared with two plant lipids (soy and coconut oils), no differences in acceptability were seen ($0.3370 \geq P \geq 0.1000$). For reasons already given above (see Section 4.3.2), I decided against including any substance not 100% lipid as the representative in the macronutrient choice test. Shortening was chosen for reasons of practicality (it is solid at room temperature), and because it consists of several types of lipids, one of which might ultimately prove acceptable during the choice test.

4.3.4 Matrix Material

As noted above, the acceptability test comparing agar and gelatin was not analyzed statistically, though the data suggest that gelatin stimulated less feeding than agar. Foragers consumed 16% of the 1% gelatin, 30% of the 3% gelatin, 38% of the 6% gelatin, and 28% of the 9% gelatin. The 1% and 3% agar solutions did not gel; however, foragers removed 74% of the 6% agar and 47% of the 9% agar. When 2% gelatin was tested against 4% agar, the results suggested a difference may exist, though it was not statistically significant ($P = 0.0971$). Means (± SEM), however, were quite different: $1.72 \pm 1.01\%$ of the gelatin was consumed, versus $16.81 \pm 6.11\%$ consumption of agar. As sample size was rather small and the P-value was marginal, it seemed likely that a difference existed but was undetectable under the conditions of the test. Consequently, gelatin, which stimulated less feeding than agar without deterring feeding, was chosen as the matrix material for the macronutrient choice test. Tests comparing laboratory-grade

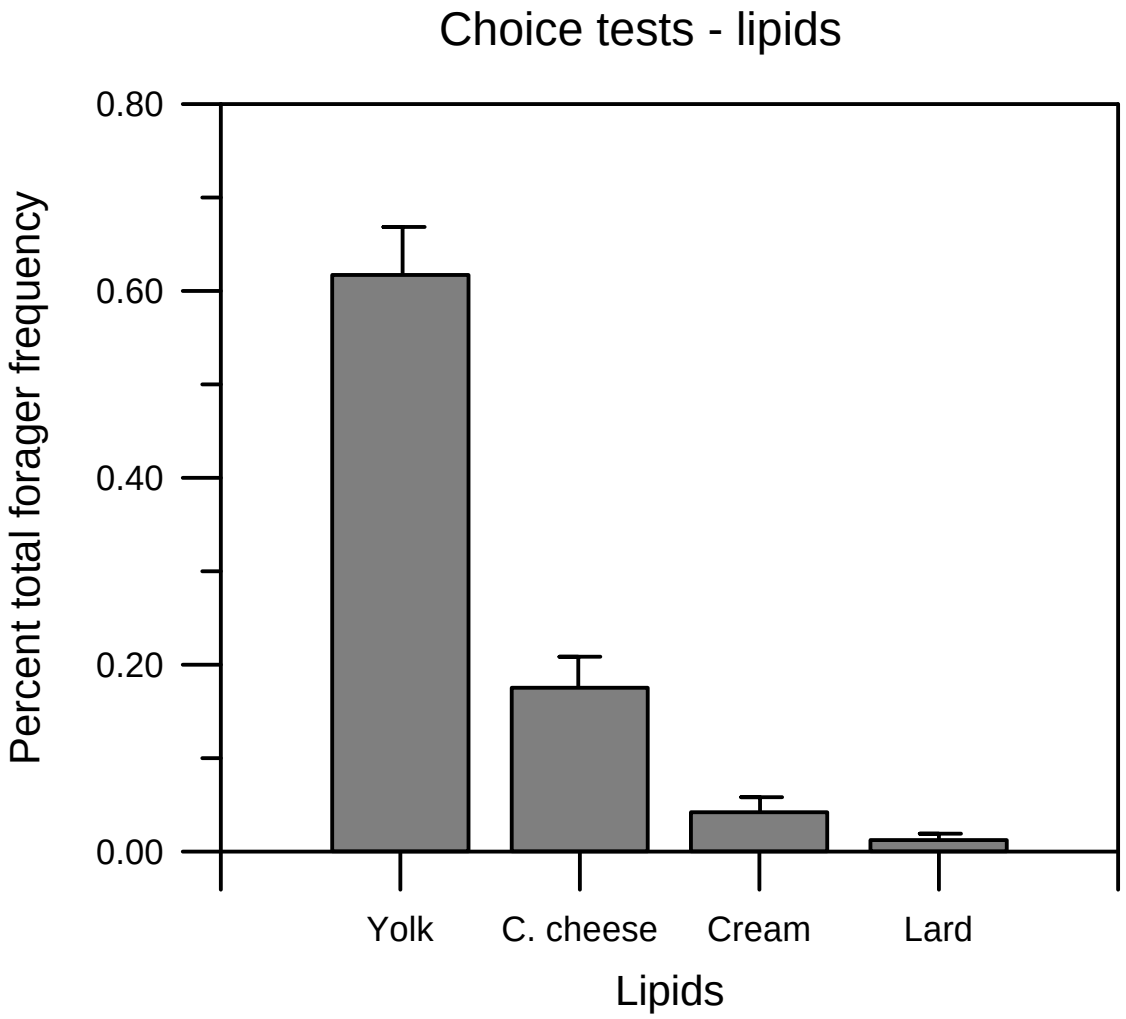

Figure 4.5 Mean acceptability of processed lipoidal foods to four field nests and four laboratory nests as measured by forager frequency rates of solutions during one trial each. All adjacent means significantly different at P = 0.0001. (Vertical bars = ± SEM.)

gelatin with a supermarket brand showed a slight preference for the lab-grade chemical ($P = 0.0323$). As the supermarket brand gelatin had been used in the preceding matrix tests, those test results remained valid and the supermarket gelatin was chosen for use in the macronutrient choice test.

4.3.2 Macronutrient Preference Test Over Time

The pattern of macronutrient preference over the interval from May to September is shown in Figure 4.6. Casein hydrolysate was clearly preferred over all other foods offered in this choice test. The overall mean amount of casein hydrolysate removed relative to the amount of all food material removed (78.60% by weight) was approximately four times greater than the amount of sucrose taken (19.65%). A repeated measures ANOVA contrasting macronutrient means proved that this difference was highly significant ($P = 0.0001$). Sucrose was still a very acceptable food source, highly preferred over shortening ($P = 0.0001$), of which only 0.83% had been taken. The amounts of shortening and plain gelatin removed were not statistically different ($P = 0.7092$). As the weight change of the gelatin control was less than 1% (0.95%), I thought it unnecessary to apply a correction factor to the data to account for evaporation. When the data from all three macronutrients and the gelatin control (Figure 4.6) were tested together for overall time and nest effects in a multivariate ANOVA, neither factor was found to significantly affect macronutrient consumption (Tables 4.1 and 4.2). The independent variables also had no significant effect on the dependent variables when each macronutrient was considered separately under a multivariate ANOVA (Table 4.3).

4.4 Discussion

The previous study (Chapter 3) described the nutritional needs of the carpenter ant nest under the constraints imposed by environment. The purpose of the present study was

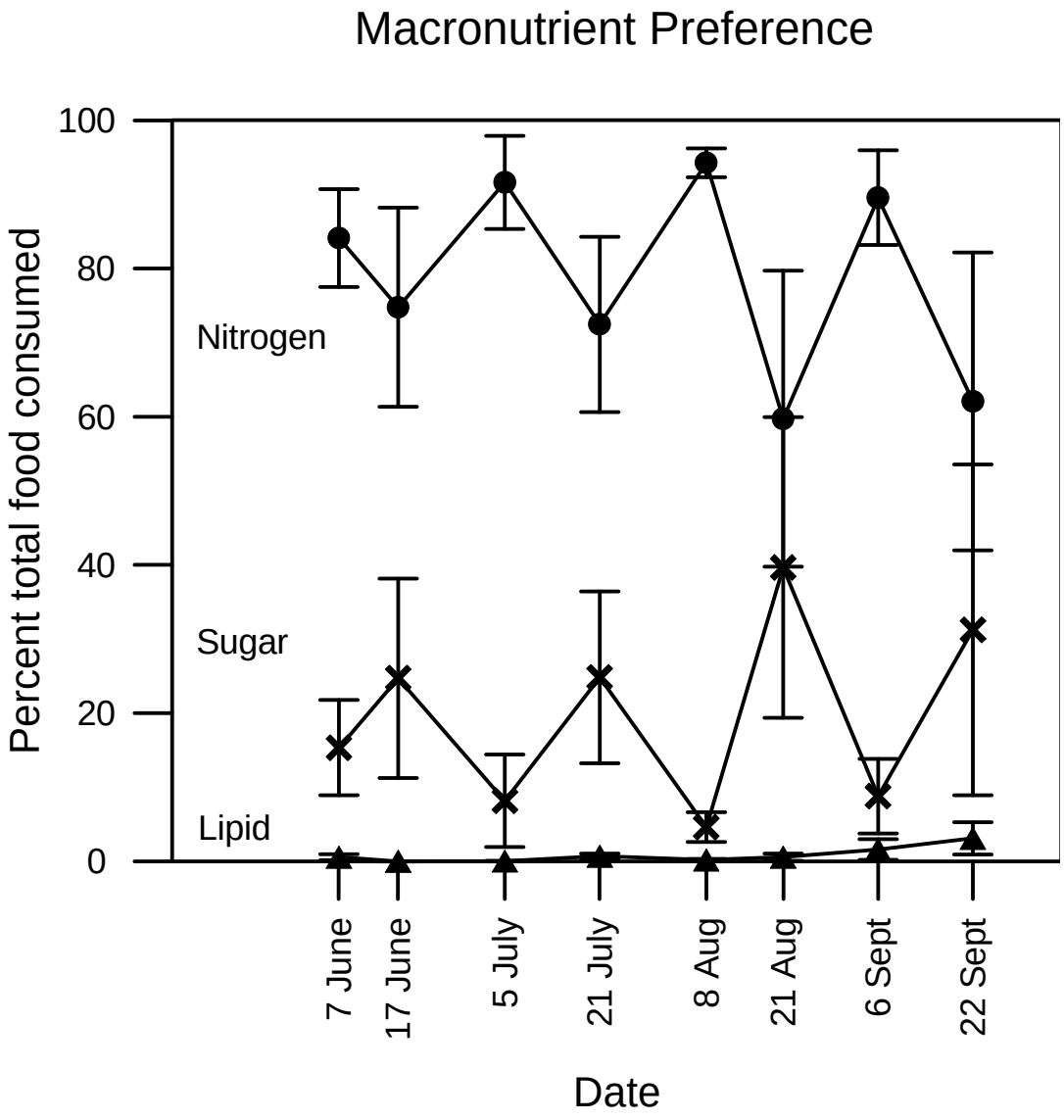

Figure 4.6 Macronutrient preference over time as measured by weight of material removed during choice tests on four field nests. (Macronutrients = 20% sucrose, casein hydrolysate, and shortening in a 2% gelatin matrix. Vertical bars = ± SEM.)

Table 4.1 MANOVA test criteria and F approximations for the hypothesis of no overall effect of time on macronutrient preference (H = type III SS&CP matrix for time, E = error SS&CP matrix).

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilk's Lambda	0.2336	1.1126	28	62.7166	0.3545
Pillai's Trace	1.1426	1.1424	28	80	0.3152
Hotelling-Lawley Trace	1.9383	1.0730	28	62	0.3978

Table 4.2 MANOVA test criteria and F approximations for the hypothesis of no overall effect of nest on macronutrient preference (H = type III SS&CP matrix for time, E = error SS&CP matrix).

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilk's Lambda	0.6120	0.7693	12	45.2693	0.6777
Pillai's Trace	0.4357	0.8071	12	57	0.6418
Hotelling-Lawley Trace	0.5561	0.7260	12	47	0.7189

Table 4.3 General Linear Models MANOVA testing for time and nest effects within the dependent variables of the macronutrient choice test.

Sugar:

Source	DF	Type III SS	MS	F	Pr > F
Time	7	4295.3395	613.6199	0.93	0.5053
Nest	3	1196.6528	398.8843	0.60	0.6198

Nitrogen:

Source	DF	Type III SS	MS	F	Pr > F
Time	7	5026.7156	718.1022	1.18	0.3581
Nest	3	1218.9716	406.3239	0.67	0.5821

Lipid:

Source	DF	Type III SS	MS	F	Pr > F
Time	7	28.6557	4.0937	1.04	0.4342
Nest	3	7.9568	2.6523	0.67	0.5775

Matrix:

Source	DF	Type III SS	MS	F	Pr > F
Time	7	45.1602	6.4515	2.09	0.0924
Nest	3	11.5860	3.8620	1.25	0.3172

to illustrate how these needs change when the environmental constraints of food availability, competition, and predation are removed even temporarily. Food availability was unrestricted in that the experimental time interval of two hours was not long enough for test foods to be completely consumed. Intraspecific competition was thwarted by placing the foods near the nest entrance of these ants, which are very territorial. Interspecific formicine competitors were observed on only two occasions. A small number of thief ants (*Solenopsis molesta* (Say)) were found in the test tube containing the shortening. As they appeared only twice, only in the lipid food, and are quite small insects, their effect was considered negligible. During trials in which yeast was used, snails were a problem, and a skunk disrupted another protein trial, though it may have been preying on the ants rather than competing for food. Regardless, once foods were administered in gels from test tubes, no further interference was observed.

When released from these environmental constraints, carpenter ant foragers collected mainly carbohydrate and nitrogen compounds. As in the previous chapter, there had been some expectation that lipid would become acceptable to foragers late in the season, due to the emergence of alates into the nest and the approach of winter. Nothing of that nature was seen here. Pure lipids, whether of plant or animal origin, were consistently unacceptable, though they were consumed when combined with other nutrients, such as sugars or proteins. For example, egg yolk, which is also high in protein, vitamins, and minerals, stimulated substantial feeding. Other workers have shown that carpenter ants are indifferent to a wide range of oils and fatty acids, the latter eliciting repellency behavior in some cases (Fell and Mollet, unpubl.). One factor which I had not taken into account was presence of hydrogenated fats. Hydrogenation of oils converts liquid oils to fats that are solid at room temperature (Nawar 1985), a characteristic I found desirable for the conditions of this test. Unfortunately, hydrogenation of oils may make them less acceptable, according to trials on the grease-loving imported fire ant (Lofgren et al. 1964).

The relative acceptability of sugars tested corresponded fairly well with their prevalence in honeydew. The three sugars most favored by the ants -- fructose, glucose, and sucrose -- are the three most common honeydew sugars (Auclair 1963). Sucrose was preferred both by laboratory and field nests. A preference for sucrose seems to be widespread among the ants (Schmidt 1938, Ricks and Vinson 1970). The adaptiveness of this becomes clear when one considers that sucrose is probably the predominant sugar in honeydew (Tennant and Porter 1991).

Acceptability of sugars increased with concentration only to a point. The two most preferred sugars, sucrose and fructose, elicited the greatest feeding rates at a 20% concentration. At 40%, the highest concentration tested, some sugars showed evidence of reduced acceptability. Feeding response to richer solutions may be affected by factors such as receptor adaptation. The sugar concentration provoking maximal phagostimulatory response in insects normally lies between 0.1 and 1.0 molar concentration (Hsiao 1985). Twenty percent solutions of mono- and disaccharides lie in the range of 0.5 - 0.9 molar concentration. Selection may have favored chemoreceptor response in this range. The water content of freshly excreted honeydew is 80%. The remaining 20% consists mainly of sugars, though as the water evaporates this fraction will rise, even to the point of crystallization (Zoebelein 1956). If "fresher" honeydews are somehow nutritionally superior to older excretions, it would be adaptive for foragers to prefer sugar solutions of about 20%.

An interesting, though incidental, finding of this study was that the fish products were much preferred by foragers to beef and other products. It is difficult to explain why this should be, without conducting a host of additional tests. As I did not measure nutritional content of these foods directly and homogenized most with water, conclusions based on nutritional content must be drawn cautiously. If product label information is considered accurate and a dilution factor of two (for solid foods) is accepted, the cause of preferential feeding may simply have been protein content. Accordingly, the protein

contents of foods as administered would be as follows: tuna (13%), roe (12%), strained beef (8%), and beef bouillon (3%). Recall that this was also the order of preference. It is equally possible that some specific component of fish served as a phagostimulant. In comparison to beef, fish is high in low molecular weight organic nitrogen compounds, including free amino acids, peptides, nucleotides, and urea (Finne 1985). The latter is consumed at low concentrations by carpenter ants (Shetty 1982). Tuna fish in particular has an especially high concentration of free histidine (Finne 1985). Fell and Mollet (unpubl.) found histidine to be one of the most, perhaps the most, preferred amino acids to foraging carpenter ants. *Leptothorax* sp. and *Monomorium* sp. are not attracted to histidine (Lanza and Krauss 1984), whereas *Solenopsis saevissima saevissima* Smith and *S. s. richteri* Forel feed minimally on solutions containing histidine but much prefer leucine and valine (Ricks and Vinson 1970). Histidine, an essential amino acid, is found in honeydews but not in plant phloem fluids (Ehrhardt 1962).

One of the ingredients normally added during processing to tuna canned in water is hydrolyzed casein. Laboratory-grade casein hydrolysates tested in this study were extremely effective at stimulating feeding in ants from both laboratory and field nests. There is some evidence that insects choose food based on nutritional content (Zucoloto 1991, Lanza 1991), and casein, derived from bovine milk, is one of the most nutritious proteins. It not only contains all of the common amino acids, but is especially rich in those considered essential (Windholtz 1983). At least 50% of the total nitrogen in protein hydrolysates comprises amino nitrogen, providing a nutritive boost since digestion of protein is sometimes incomplete (Cheftel et al. 1985).

Hydrocolloids are hydrophilic substances that disperse in solution as colloids (Lindsey 1985). They are natural emulsifiers (Nawar 1985), and, as such, capable of stabilizing the oil/water suspension of shortening in a water-based matrix. Two hydrocolloids were tested for their suitability as neutral (i.e., neither stimulating nor deterring feeding) matrices: agar and gelatin. Agar, a plant gum derived from seaweed, is

a polysaccharide consisting principally of D- and L-galactose units (Whistler and Daniel 1985). Gelatin, a protein derived from collagen, is one of the few noncarbohydrate hydrocolloids (Lindsay 1985). Gelatin was less acceptable than agar. Collagen is unaffected by most proteases, but gelatin represents a partly denatured form of collagen that is generally subject to proteolytic action (Hultin 1985). Proteases are not present in the foregut of *C. pennsylvanicus*, and ant larvae may not be able to digest gelatin in its crystalline form (Petralia and Vinson 1978). It is thus unlikely that the gelatin represented a source of protein in this study.

In virtually every preliminary trial, preference within a macronutrient group differed from nest to nest, this difference often being highly significant ($0.0033 \geq P \geq 0.0001$). During the macronutrient choice test, in contrast, preference between macronutrients did not differ from nest to nest (Table 4.2). Although I suspect the shorter testing intervals of the preliminary trials were at least partly responsible, heterogeneity among nests may be commonplace. Lanza (1991) found that colony and day affected food preference in *S. invicta* and that "day to day variability" was substantial, even when laboratory nests were exposed to the same diet and environmental conditions. She also showed that measuring the amount of food removed during a trial rather than forager frequency reduced this variability somewhat (Lanza et al. 1993). Weight removed was indeed the quantity I measured during the macronutrient choice test over time, whereas forager frequency was measured in nearly all preliminary tests. This difference in methodology may explain the different influence of nest that I observed.

The main findings of this study are those from the macronutrient choice test. First, under the conditions of the test, macronutrient preference patterns remained stable over time. Second, carpenter ants exhibited a decided preference for nitrogenous food over sugary food, and ignored lipid food. In considering these results, one should bear in mind that preference as measured by the test was relative, not absolute, and that some fluctuation in relative preference was evident but did not signify a detectable trend. It is

perhaps more important to remember that normal foraging proceeded alongside the choice test, and consequently the true picture of overall macronutrient retrieval may be altogether different. What is demonstrated by the test results, then, is not naked nutritional need, but need coupled with opportunism.

Opportunism can be defined as the ability of a colony to assess its nutritional needs and to exploit the foods that best meet those needs when the appropriate food sources are unpredictable. The feedback mechanisms governing this colony-level phenomenon are not well understood in the ants. In general, insects are able to evaluate the nutritional content of different foods and to choose the more nutritious food based on information obtained through their chemosensory receptors (Chapman 1995, Zucoloto 1991). In the leaf-cutting ants, successful foragers are apparently able to transmit information regarding food quality upon their return, which elicits a recruitment response among nestmates that is proportional to the food's nutritional content (Roces and Núñez 1993). Individual foragers must be able to evaluate not only food quality but colony need as well. The feedback mechanisms governing such decisions have been examined in the honeybee. Pollen collection by foraging bees is regulated through inhibitory cues whereby non-foraging nurse bees distribute proteinaceous secretions to foragers when pollen levels in the nest are adequate (Camazine 1993). Similarly, a water forager is stimulated to collect more water by a short delivery time, which is the time necessary for her to be relieved of her water load by non-foraging nestmates (Lindauer 1954). It would be reasonable to posit a similar feedback mechanism in ants, whereby a returning forager unable to find a recipient for its crop contents would not be stimulated to continue foraging for that combination of nutrients.

Taken together with those from Chapter 3, the results here suggest that the ants are obtaining insufficient quantities of nitrogen from their environment either because the environment provides inadequate amounts of nitrogen or because the ants are inefficient at harvesting nitrogen. A case for environmental deficiency is presented convincingly by

White (1978). He claims that food itself is not limiting, as there is apparently no shortage of carbohydrate food in the world. He hypothesizes instead that, "for many if not most animals, the single most important factor limiting their abundance is a relative shortage of *nitrogenous* food for the very young." The adverse effects of such shortage on the young may even precede birth if reproductive females cannot obtain enough nitrogen for the maturation of eggs or embryos. All animals, whether herbivorous or carnivorous, are affected. As food, plants are abundant but inherently low in nitrogen, averaging 2.1% nitrogen by weight according to Scribner (1984). Prey and carrion, on the other hand, are high in nitrogen -- 7 - 14% by weight (Scribner 1984) -- but generally scarce, even in the tropics (Carroll and Janzen 1973). In summary, "most, if not all, species of animals during most, if not all, generations" struggle for survival is a "passively hostile, inadequate environment" in which "the right type of food", that is nitrogen, is the universal limiting resource (White 1978).

There is some evidence that ants encounter nitrogen shortages in the environment. Traniello et al. (1992) report a decrease in the availability of proteinaceous food after early July as estimated by sweep net samples of arthropods. Seasonal variation in density and variety of the prey of steppe ants was documented by Reznikova and Kulikov (1978 in Tarbinskii 1991). Even in tropical regions, insect prey availability varies with season (Janzen 1968, 1973). Carpenter ants are known to rear their brood serially (Eidmann 1926), which is suggestive of a chronic shortage of protein, although other explanations are possible. The proficiency ants demonstrate in dealing with nitrogen shortfalls further suggests that nitrogen shortage is a phenomenon that occurs regularly enough to have exerted selective force favoring this behavior. *S. invicta* larvae, for example, are fed a host of unusual nitrogen-containing items during shortages, including cast larval skins, pupal exuvia, commensal mites, and sick or dying workers, in addition to more typical sources of protein such as trophic eggs. At such times, workers could be seen eating injured eggs, larvae, and pupae (O'Neal and Markin 1973).

Alternatively, insufficient nitrogen may be collected simply because the ants are poor predators. In fact, there is little evidence that *C. pennsylvanicus* is a predator at all. Pricer (1908) never saw them prey on living insects, though they were observed imbibing the juices of dead insects they encountered. Ayre (1967) was puzzled by their "unexplainably" weak reaction for protease in the midgut, in contrast to *C. herculeanus*, which has demonstrated predatory skills (Ayre 1963). He assumed the two species were similar in feeding habits. A number of authors have shown that fewer than 1% of returning *C. pennsylvanicus* foragers carried arthropod remains (Sanders 1972, Fowler and Roberts 1980, Chapter 3). Unfortunately, there is often no way of knowing whether arthropods carried in the mandibles were captured and killed or were scavenged. Furthermore, solid food retrieval is not a reliable indication of how much protein is actually brought to the nest. Gößwald and Kloft (1956) have shown that arthropods may be ingested by foragers *en route* to the nest, and results of the previous study (Chapter 3) have shown that a substantial amount of nitrogen, whatever its origin, is transported to the nest in the crop.

Perhaps *C. pennsylvanicus* aren't predators. Tobin (1993), who questions the long-held belief that the ants as a group are essentially carnivorous, concludes from the available literature that the genus *Camponotus* is omnivorous and herbivorous. The study he cites as evidence of herbivory in *C. sylvaticus* (Olivier) is, unfortunately, seriously flawed: crop contents were never chemically analyzed, and the choice test employed only protein foods high in salt and fat, both of which I have observed to be unacceptable in high concentrations to foragers. It is disappointing that Tobin (*op. cit*.) neglects the issue of omnivory and its importance, since he himself designates as omnivores more than half of the genera for which he claims we have reliable data. A more precise, quantitative definition of omnivory is clearly needed. For example, do carnivores, omnivores, and scavengers exhibit consistent differences in the proportion of protein they consume? If behavior changes substantially when release from a regulating factor occurs, how should that affect the consumer level designation? There is also the problem of defining social

insects, whose colonies consist of two types of consumers: workers are technically herbivorous, whereas larvae are primarily carnivores (Stradling 1978, New 1991).

As omnivores, carpenter ants would likely include scavenging in their foraging repertoire. The extent to which this occurs may be underestimated. Carroll and Janzen (1973) claim that most ants will scavenge animal matter opportunistically, and that many of the larger genera rely almost entirely on scavenged material, though their paper lacks supporting documentation for these points. White (1978) does not explicitly include scavengers in his argument, but it appears that they, too, must compete for limited sources of nitrogen. Scavenged food items are unpredictably dispersed in time and space and are highly variable in their nutritional content (Carroll and Janzen 1973). Competition for scavenged food items is keen, and is most intense in habitats that are unpredictable (Carroll and Janzen 1973). Even in the tropics, a dead insect will remain available as a free food item only for a few minutes (Carroll and Janzen 1973). Thus, if scavenging plays an important role in colony nutrition, it is possible that both of the explanations given above are correct: that the environment provides inadequate amounts of nitrogen and *C. pennsylvanicus* fares poorly against competitors for this precious resource.

One last remark is warranted regarding the significance of the relative preferences observed in this study. Although four times as much nitrogenous food as sugary food was collected, it seems unlikely that this accurately reflects the extent of unmet nutritional need by the nest or colony at the time of the test. I suspect that the unexpected surfeit of nitrogen was being hoarded. According to population ecology theory, organisms will produce more offspring than are likely to survive because only then will enough survive for the population to persist (White 1978). White (1978) has addressed both the importance of nitrogen to young animals and the ephemeral nature of nitrogen as a food source. Ants have evolved storage behavior in order to better reconcile food supply with colony demand (Stradling 1978). As a rule, protein does not keep very well, unless stored as living tissue by workers or by larvae (Stradling 1978, Nonacs 1991, Wheeler 1993).

Larvae make particularly handy storage vessels in that they "have low overhead costs, put little resource into inedible cuticular structure, can contain large fat bodies, and have long starvation times" (Carroll and Janzen 1973). There is good evidence that larvae are overproduced during times of protein surplus when supply has been erratic, and that cannibalism of larvae in times of protein scarcity is a widespread adaptation to fluctuations in protein availability (Dlussky and Kupianskaya 1972, Alexander 1974, Cannon unpubl.). In the end, this faculty may give social insects an additional adaptive edge, since they "produce higher numbers of offspring than related non-social taxa" (Hunt and Nalepa 1993).

CHAPTER 5. MICROPARTICLE INGESTION IN THE CARPENTER ANT, *C. pennsylvanicus*

5.1 Introduction

In order for a food to be suitable for consumption, its chemical composition must suit the needs of the consumer. The ancestral food source of the Hymenoptera was nectar. Consequently, foods acceptable to extant hymenopterans must offer the same fundamental nutrients as nectar (Hunt 1991). Just as important as chemical composition are the mechanical characteristics of a food, which must meet the restrictions imposed by the animal's feeding apparatus (Schmidt-Nielsen 1983). Although all adult aculeate Hymenoptera are mandibulate insects and many are predaceous, as a group they are anatomically restricted to feeding on liquids (Hunt 1991). Predaceous adults feed captured prey to the young, while the adults themselves feed on larval saliva, conspecific ingluvial fluids, prey hemolymph, honeydew, honey, plant sap, or nectar (Hunt 1982).

The mechanism restricting the intake of solid food varies within the social Hymenoptera. The bees are constrained behaviorally rather than anatomically in that they do not provision their young with prey, but instead with nectar and pollen, both of which are also consumed by the adults (Winston 1987). In the wasps, particles taken in during feeding and grooming gather in an invagination of the mouth cavity below the pharynx known as the infrabuccal pocket (Janet 1895b in Eisner and Happ 1962). Several anatomical factors limit the ants to liquid foods. Physical exclusion begins with the actions of the external mouthparts and the diameter of the pharynx, both of which prevent larger solids from being ingested (Eisner and Happ 1962). The infrabuccal pocket serves as a receptacle for smaller ingested solids, which are collected there into a compact mass and ejected as a pellet (Janet 1895a, 1905 in Eisner and Happ 1962). Finally, in the

Formicinae and Dolichoderinae, the proventricular valve has been modified into a specialized structure that excludes particulate matter from the midgut (Eisner and Wilson 1952). Thus contained in the crop, particulate matter is subject to repeated filtration during the frequent trophallactic exchanges in which most ants engage (Eisner and Happ 1962).

The principle that adult aculeate Hymenoptera consume no solid food has become so axiomatic that statements in the literature to that effect are sometimes unsupported by references. Clearly, some very small solids can enter the midgut, however. Pollen, for example, is collected and consumed by foraging honey bees and by workers of the turtle ant, *Zacryptocerus rowheri* (Creighton and Nutting 1965, Winston 1987). Workers of the leaf-cutting ants, *Atta* and *Acromyrmex* spp., feed on gongylidia from their cultured fungus, though possibly they digest only the gongylidial juices (Hölldobler and Wilson 1990). Fungal gongylidia are 30 - 50 µm in diameter (Weber 1966), whereas pollen grains vary in size from 5 to 200 µm in diameter (Stanley and Linskens 1974). Do these food sources represent the maximum size at which solids are no longer detectable as such by the insect's filtering mechanism?

The size threshold at which particles are detected and filtered out of the communal food supply has been investigated in four ant species: *Acromyrmex octospinosus* (Reich), *Solenopsis invicta* Buren, and *Zacryptocerus rowheri* (Wheeler) (all myrmicines) and *Camponotus pennsylvanicus* (De Geer) (a formicine). *A. octospinosus* workers of all sizes are able to remove particles as small as 30 µm diameter from their crop fluids completely, whereas only minor workers can completely filter 10 µm particles from the crop fluids (Quinlan and Cherrett 1978). Particles 125 and 180 µm are collected by the infrabuccal pockets of major and media workers, never by minor workers, all of which suggests caste differences in filtration efficacy (Quinlan and Cherrett 1978). Glancey et al (1981), who examined minor workers of *S. invicta*, did not observe complete filtration of particles 1 µm or greater from the ingluvium within one

hour of feeding, but far fewer particles of all sizes appeared in the crop than in the infrabuccal pocket. In the only study to examine proventricular filtration, Roche and Wheeler (1991) discovered that *Z. rowheri*, an ant having the unusual habit of including pollen in its diet (Creighton and Nutting 1965), is able to exclude particles 5 µm in diameter from its midgut. The fate of 10 - 300 µm particles fed to medium-sized *C. pennsylvanicus* workers has been explored by Eisner and Happ (1962). Their results show that particles 200 µm or greater are not consumed by these ants; that particles of 150 µm are consumed but are completely sequestered in the infrabuccal pocket; and that particles 10 - 100 µm are captured by the infrabuccal pocket with varying efficiency. Not surprisingly, the presence of nestmates increased filtration efficacy, though even under social conditions complete elimination of particles from the crops of all individuals was not achieved within 12 hours (Eisner and Happ 1962).

With one exception, these studies focus entirely on the filtering action of the mouthparts and infrabuccal pocket, and do not pursue the fate of particles able to reach the crop. Only Roche and Wheeler (1991) directly address the role of the proventriculus in barring solids from the midgut. Their results show that *Z. rowheri* possesses a fine filtering mechanism not at all typical of the myrmicines. The Myrmicinae are characterized by a highly muscularized, lightly sclerotized proventriculus and by a peritrophic membrane, both of which are found in insects consuming solid food (Waterhouse 1953, Eisner and Happ 1962). In contrast, the Formicinae, which lack a peritrophic membrane, have evolved a proventriculus that functions as a passive damn (Eisner and Wilson 1952, Eisner and Brown 1958, Eisner and Happ 1962). By eliminating much of the attendant musculature and reducing the portal valve to a rigid cruciform slit, liquids are transported past the portal valve only through the active pumping motions of the proventriculus (Eisner and Happ 1962).

How effective is the specialized formicine proventriculus at excluding particles from the midgut? In this study, I investigate the fate of particles of various sizes ingested

by *C. pennsylvanicus* workers, the effect of sociality on particle transmission to the midgut, and the effect of worker size on particle transport beyond the foregut. I hypothesize that: 1) the proventriculus of *C. pennsylvanicus*, as the final element in the serial filtration system, should function as a very fine filter, excluding from the midgut particles small enough to escape the filters anterior to it; 2) in the absence of trophallaxis, less efficient filtration should take place; in other words, larger particles will reach the crop and, consequently, the midgut of ants held in isolation in comparison to ants maintained in social groups; and 3) a caste effect exists whereby larger individuals pass larger particles to the midgut than smaller individuals.

5.2 Materials and Methods

5.2.1 Test for Ingestibility of Fluorescent Microparticles

A study to test the microparticles for acceptability and ingestibility was conducted using fluorescent polymer beads of 43.7 ± 3.17, 9.18 ± 3.09, and 5.99 ± 0.096 µm diameter (Fluoresbrite™ microspheres, Polysciences, Inc.). Twenty workers were removed from laboratory nests and isolated singly in 10 x 75 mm disposable polystyrene test tubes (Fisher Scientific) plugged with moistened cotton wicks. The ants were maintained for 24 hours without food, in darkness, and under ambient laboratory temperature and humidity. I then placed one drop from a stock solution (composed of 27 aliquots 20% sucrose solution plus one aliquot from each of the three concentrated suspensions, making a 30-fold dilution of a given particle concentration) on the wall of each test tube, and verified complete consumption. Twelve hours after imbibing the droplet, all ants were CO_2-killed and dissected. The mouthparts and alimentary tracts from 10 samples were qualitatively observed under fluorescence microscopy (Zeiss Photomicroscope III with Neofluor objective). By applying pressure to the genae, it was possible to eject the infrabuccal pellets, when present, for examination as well.

5.2.2 Transit of Fluorescein through the Gut

In order to identify the post-feeding interval in which the maximum amount of ingested material is present in the mid- and hindgut, I executed a timed feed-through trial using sodium fluorescein. Three groups of 15 to 60 workers were collected from laboratory colonies and held in plastic nest boxes at ambient laboratory temperature and humidity. Access to water and a 10% sucrose solution was provided. After two weeks, all groups were given access to a 20% sucrose solution containing 1.5×10^{-4} mM sodium fluorescein such that each group received sufficient solution for 10 µl per ant. The solutions were offered on a glass slide within a ring drawn with wax pencil. After one hour, the sodium fluorescein solutions were removed. The ants were then given continuous access to 20% sucrose solutions. From three to five workers were randomly sampled from each group every four hours post-feeding.

Sampled workers were held at -15 ^{o}C until they were dissected. A group of 5 workers, taken from a fourth nest and fed only 20% sucrose solution, was also frozen and dissected. Though not a true control, this group provided a qualitative comparison. All workers were dissected within 24 hours of sampling. The mid- and hindgut of each worker was removed, placed into a 1.5 ml polypropylene microcentrifuge tube, and ground in 1 ml 5 mM HEPES buffer (pH 7.5) with a micropestle.

Samples were read in a Perkin Elmer LS 50 spectrofluorometer against a standard curve of sodium fluorescein in HEPES buffer. The data were plotted against time and used to establish the incubation time for subsequent experiments. The extent to which tissue in the samples may have contributed to fluorescence quenching was not explored; however, a certain degree of homogeneity in gut contents among individuals was assumed due to the pre-trial feeding regimen.

5.2.3 Fate of Ingested 3, 6, and 10 µm Microparticles Under Conditions of Sociality

I removed five groups of 100 - 125 workers of all sizes from five laboratory nests that had been collected from the field not more than three months previously. Each group was housed in a 15 x 30 x 10 cm plastic nest box and maintained at ambient laboratory temperature and humidity. All were starved for six days, but provided with abundant water. The intention had been to draw one block of four experimental units of 10 -20 ants from each of the three strongest groups. Only two groups, however, remained strong enough to provide a block of experimental units of sufficient size. The last block needed had to be drawn from the remaining three nests, and consequently was not truly a block. The effect this had on data analysis will be addressed below (Section 5.2.7).

Twelve experimental units were used, three for each of the three treatments and three control groups. The experimental units, consisting of 12 ants each, were housed in 125 x 65 mm covered glass crystallizing dishes. Water was provided through the dish entry port. All experimental units were allowed to acclimate for 24 hours in darkness at ambient laboratory temperature and humidity.

Test solutions were made by mixing one aliquot of a 40% sucrose solution and one aliquot of a concentrated suspension of fluorescent polymer beads of a given diameter (Fluoresbrite^{TM} microspheres). The diameters and concentrations of the microparticles as purchased were: 2.762 ± 0.057 µm at 2.16 x 10^9 particles/ml; 6.112 ± 0.421 µm at 1.99 x 10^8 particles/ml; 10.10 ± 1.45 µm at 4.59 x 10^7 particles/ml. Each experimental unit was given a volume of test solution equal to 5 µl per ant. Solutions were administered on a glass slide within a ring drawn by wax pencil. Control units were given 20% sucrose solutions in the same manner. After one hour, all solutions were removed from the dishes and replaced with a 20% sucrose solution. After 20 hours, all ants were freeze-killed, and held at -15 ^oC until they could be dissected.

Prior to dissection, ants were decapitated with iris dissecting scissors. A subsample of heads were checked for the presence of infrabuccal pellets by pressing on the genae. All head capsules were then mounted with the mouthparts exposed. Mouthparts could then be examined for fluorescent particles. Body size was determined by taking the broadest measurement across the eyes to the nearest 10 µm using a compound microscope fitted with a Zeiss Boeckler filar eyepiece.

I dissected out the alimentary tracts of ten ants, or all survivors if less than ten, from each experimental unit. Each crop was placed into a 1.5 ml polypropylene microcentrifuge tube containing 0.5 ml distilled H_2O. Mid- and hindguts from the same specimen were placed into a second 1.5 ml microcentrifuge tube with 0.5 ml distilled H_2O. I ground the gut material with a micropestle (Fischer Scientific) to release all gut contents. The microcentrifuge tubes were closed, sealed with Parafilm^R, and held at -15 ^oC until they could be analyzed quantitatively.

Fluorescence in the gut was quantified using a Perkin Elmer LS 50 spectrofluorometer. According to the manufacturer, the proprietary dye used in the yellow-green fluorescent microspheres is a fluorescein equivalent characterized by an excitation maximum at 458 nm and emission maximum at 540 nm. Samples were excited and read at these wavelengths against a standard curve made from the appropriate microparticle suspension diluted in distilled H_2O. In order to accommodate all samples, concentrations in each standard curve covered up to three orders of magnitude, but remained linear over the full range ($0.9996 \geq R^2 \geq 0.9744$). Just before reading, particles were resuspended by vortexing. Statistical analyses were performed on the natural log of the emission intensity rather than on concentration estimates. Possible fluorescence quenching due to the presence of tissue in the samples was not explored; however, the pre-test starvation interval was thought to ensure a certain degree of homogeneity in gut contents among individuals.

5.2.4 Fate of Ingested 0.05, 0.1, and 1 µm Microparticles Under Conditions of Sociality

Four laboratory nests that had been collected from the field less than three months earlier were selected. A group of 125 workers of all sizes was taken from each nest. Groups were housed and maintained as described above (Section 5.2.3). All were starved for eight days while abundant water was provided. One group suffered mortality losses of about 90% and had to be excluded from the experiment. The three remaining groups were each divided into four experimental units consisting of 15 - 20 ants each, one unit for each treatment and one control unit, for a total of 12 units. Experimental units were housed and provided with water as described above, and allowed to acclimate overnight in darkness before beginning treatment.

Three test solutions were made as above by mixing one part 40% sucrose solution to one part concentrated suspension of fluorescent microparticles of a given diameter. The diameters and concentrations of the microparticles used were: 0.045 ± 0.003 µm at 4.49×10^{14} particles/ml; 0.09 ± 0.005 µm at 6.24×10^{13} particles/ml; 0.93 ± 0.01 µm at 5.88×10^{10} particles/ml. Each experimental unit was given a volume of test solution equal to 5 µl per ant. Test and control solutions were administered as in the previous study. Solutions were removed from the dishes after one hour and replaced with a 20% sucrose solution. Twenty hours after feeding, the ants were freeze-killed, and held at -15 ^{o}C until dissected. Head capsule measurement, infrabuccal pellet removal, dissection of the gut, and quantification of gut fluorescence proceeded as described above.

5.2.5 Fate of Ingested 0.05, 0.1, and 1 µm Microparticles Under Solitary Conditions

Groups of ants were drawn from laboratory nests, but were immediately divided into experimental units, in contrast to the previous studies (Sections 5.2.3 and 5.2.4). Experimental units consisted of ten workers of all sizes, given the range of the sample removed from the nest. Ants were placed individually into 10 x 75 mm disposable polystyrene test tubes (Fisher Scientific). In the closed end of each tube, a small water reservoir was dammed with a cotton wick. The open end of each tube was stoppered with the tapered end of an intact, empty 1.5 ml polypropylene microcentrifuge tube. Test tubes were placed in racks and the racks were inclined at a slight angle to provide room for movement. The ants were held in darkness at ambient laboratory temperature and humidity but, to minimize trauma, were starved for only 24 hours.

Test and control solutions were formulated as described in Section 5.2.4 above. After the starvation period, each ant was fed 5 µl of a solution containing either fluorescent spheres in 20% sucrose solution or, in the case of the control group, simply a 20% sucrose solution. Using a 5 µl microcapillary tube (Drummond), solutions were applied as a droplet either to the mouthparts directly or to the test tube wall. Consumption was monitored and any ant not completely ingesting the droplet was excluded from the experiment. Once all ants had been fed, the microcentrifuge tubes used to stopper the housing tubes were withdrawn. A small portion of the tip of each was removed with a razor blade. The microcentrifuge tubes were then filled with a 20% sucrose solution, recapped, and returned to the mouths of the housing tubes. Ants were observed feeding on this solution.

Twenty hours after feeding, all surviving ants were freeze-killed. They were held at -15 ^{o}C until they were dissected. Head capsules were mounted and dissection proceeded as described in previous protocols for trials under social conditions. Because

mortality greatly reduced the size of each experimental unit and because pressing the genae sometimes resulted in deformation of the head, infrabuccal pellets were not extracted in this experiment.

5.2.6 Statistical Analysis

The results of the test of microparticle ingestibility were qualitative, and not intended for statistical analysis. Similarly, the data from the fluorescein trial were assessed visually rather than statistically. The three experimental procedures investigating particle transit through the gut were subject to statistical testing. Both experiments involving the 0.05, 0.1, and 1 µm particles were randomized complete block designs (RCBD) with subsampling, as each cell contained more than one datum. Nest was the random blocking factor. In both cases, differential mortality left the data unbalanced. Mortality also affected the experiment investigating 3, 6, and 10 µm particles, leaving it both unbalanced and incomplete. Since the third "block" was an amalgamation of three different nests, the experiment could not be analyzed as a randomized complete block design. Rather than discard the data from the third "block," I decided to treat the experiment as a completely randomized design (CRD) with subsampling. In this design, all experimental units are considered independent and randomly assigned, and the "blocks" are considered replicates. The completely randomized design is less powerful than the randomized complete block design, as it cannot account for any variation due to nest.

In both RCBD and CRD experiments, three continuous and two categorical variables were recorded: mid/hindgut fluorescence, crop fluorescence, head capsule size, treatment, and either nest or replicate. The initial model incorporated all five variables, treating crop fluorescence and head capsule size as covariates. I explored the extent to which the covariates explained variance by running the model with and without the covariates. The relationship of each of the covariates to the dependent variable was

explored by examining the correlation between them while holding treatment fixed. As a result of these investigations, I removed the covariate "crop fluorescence" from the model, though this data was used in other analyses to verify consumption; I also replaced the continuous variable "head capsule size" with the classification variable "caste." The overall effect of caste on treatment was tested on the data set without the control group data, as caste differences in natural fluorescence could bias the results. To explore effects within caste, the full data set was run, including control data, but castes were analyzed separately. Within the latter framework, contrasts comparing treatments with the control were very useful. Finally, I checked to ensure that body size did not vary significantly among nests or treatments by testing the continuous variable "head capsule size" as the dependent variable in a factorial ANOVA.

Consumption was verified in three ways. First, feeding was observed during the experiments. During the experiment on solitary ants, I watched all ants and removed any that did not feed to completion. Observations were also made during the experiments under social conditions, though for obvious reasons it was more difficult to be sure that all ants had fed. Second, feeding could often be verified during dissection because a high concentration of particles in the crop produced a bright yellow color that was easily seen through the crop wall. Third, the crops of all experimental animals were analyzed for fluorescence content. These data were transformed to the natural logarithm and subjected to analyses and contrasts with the control as described above for crop fluorescence.

5.3 Results

These experiments were devised to discover the fate of different-sized particles ingested by carpenter ant workers, and to determine how particle size, body size, and social interaction may affect particle fate. I first tested particles for acceptability and ingestibility. Then I established the post-feeding interval at which ingested material

reaches a maximum concentration in the mid/hindgut. Finally, the fate of six sizes of particles in the gut was examined under social and solitary conditions.

A schematic diagram of the carpenter ant digestive tract is presented for reference in Figure 5.1.

5.3.1 Fluorescent Microparticle Ingestibility

Seventeen of 20 ants completely consumed the droplet as soon as it was antennated. I observed no behavior that could be interpreted as aversive, such as aggression, avoidance, or interrupted feeding. Examination of the ventral surface of the head under fluorescence microscopy showed that the mouthparts, particularly the palpi, of most individuals were lightly to heavily dusted with microparticles of all three sizes. About half of the head capsules examined produced an infrabuccal pellet when pressure was applied to the genae. Perhaps the intervening 12 hours between feeding and dissection provided sufficient time for some ants to eject a pellet naturally before they were killed. The infrabuccal pellets comprised a substantial number of particles (Figure 5.2). All three sizes were present, though the 10 and 6 µm particles predominated. Particles were easily observed through the walls of the esophagus (Figure 5.3), crop (Figure 5.4), and intersepalary intima of the proventriculus (Figure 5.5). 45 µm particles were not seen in these regions of the foregut. Observation of the contents of the mid- and hind gut was more difficult, since the walls of these organs were fairly opaque and their contents more heterogeneous. The mid- and hindguts were prepared as squashes on a glass slide, with a small amount of distilled H_2O added as necessary, and examined under fluorescence microscopy. No particles were detected in the digestive tract posterior to the proventriculus.

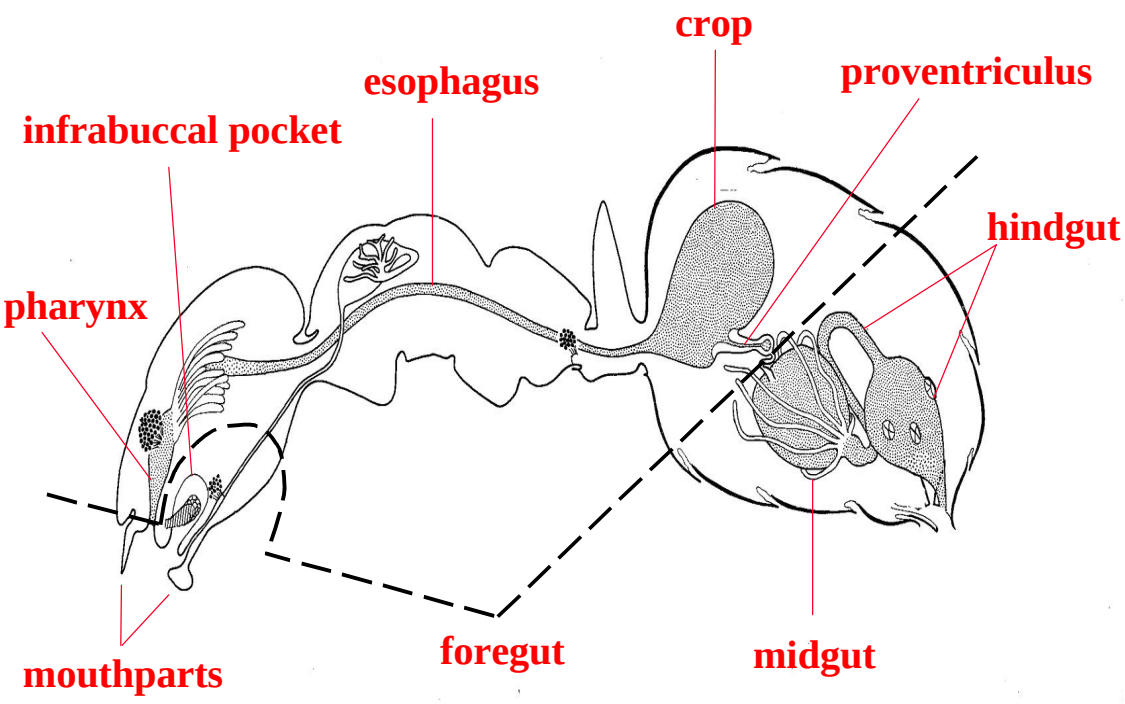

Figure 5.1 Schematic diagram of the alimentary tract of a formicine ant. (Modified from Hölldobler and Wilson 1990. Used with permission).

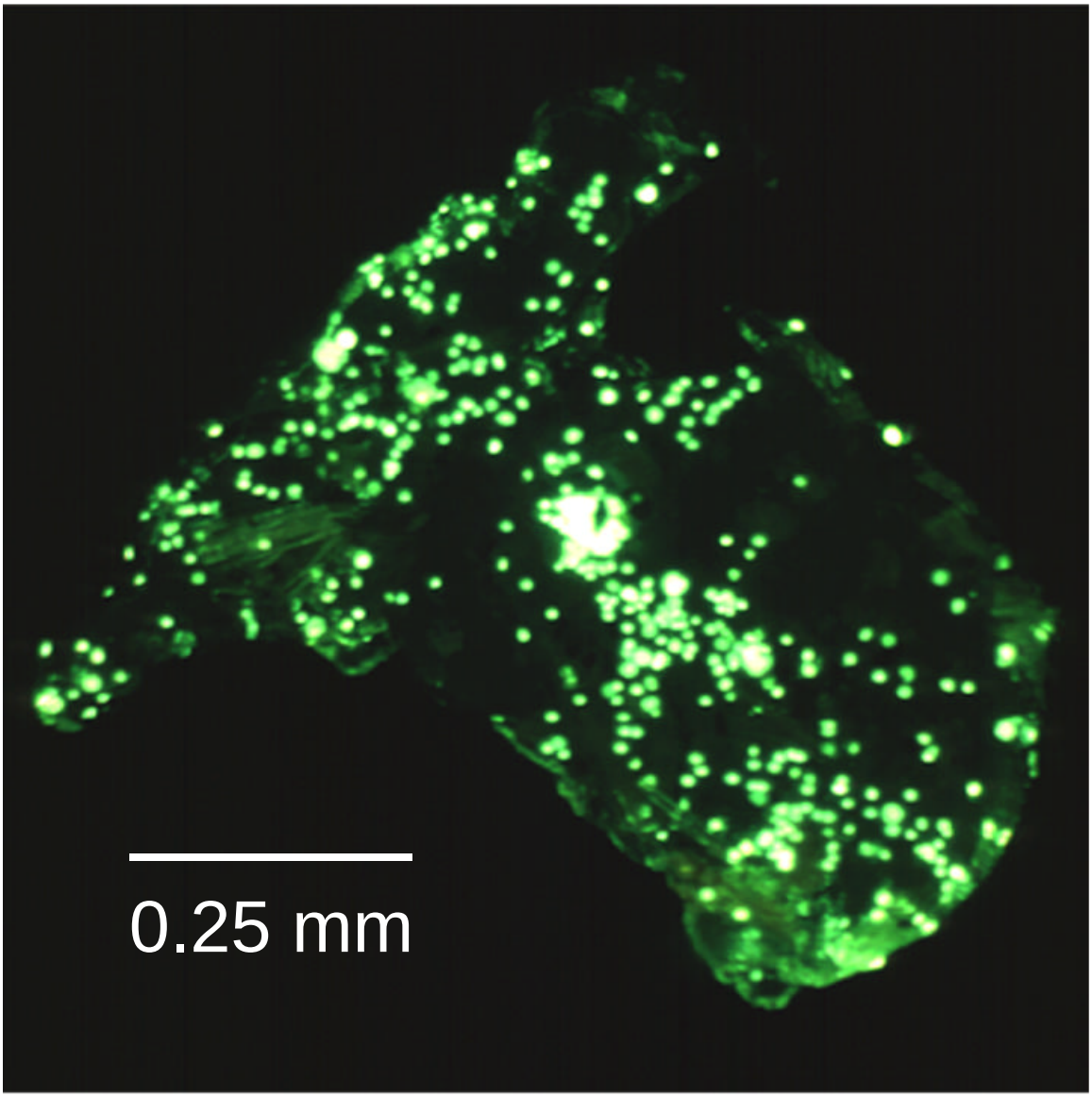

Figure 5.2 Infrabuccal pellet from worker fed a solution containing fluorescent microspheres 6, 10, and 45 µm diam.

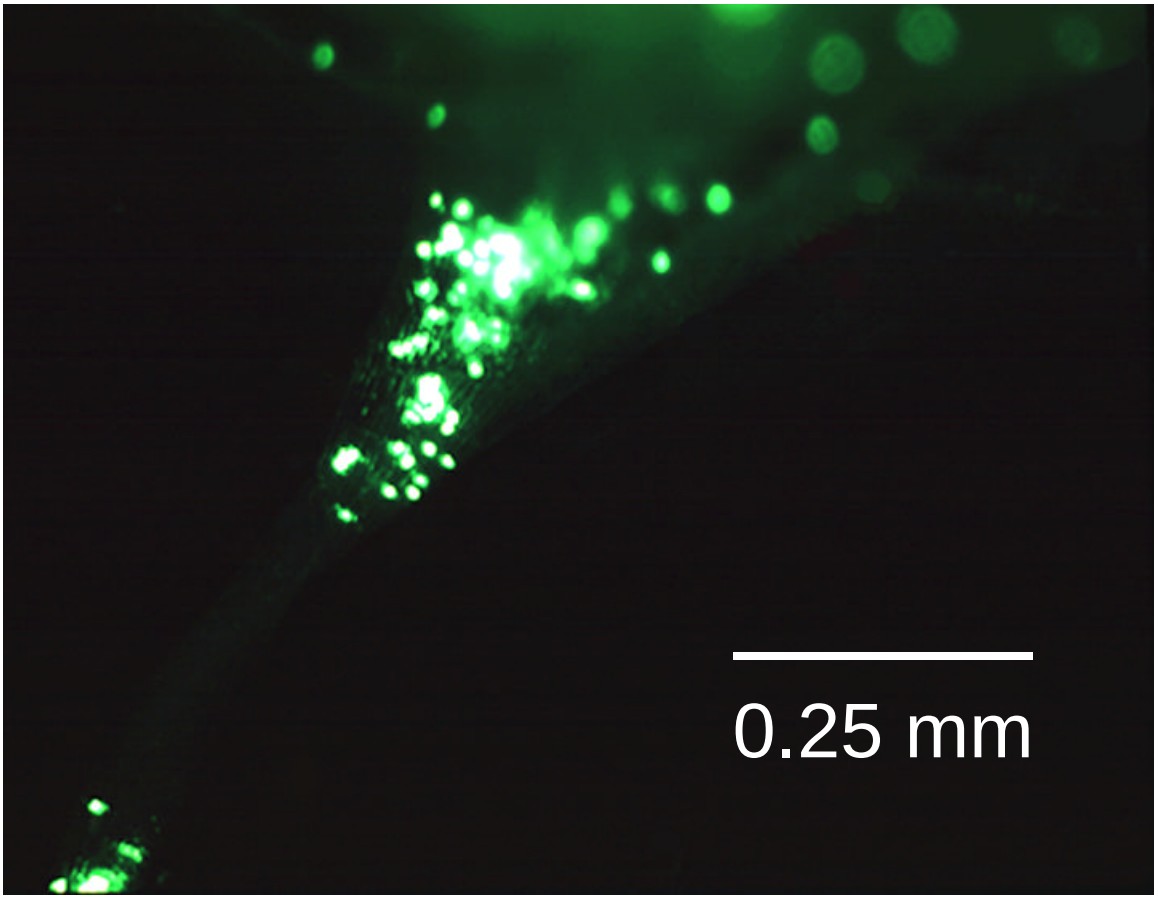

Figure 5.3 Juncture of esophagus and crop containing fluorescent microspheres 6 and 10 µm diam.

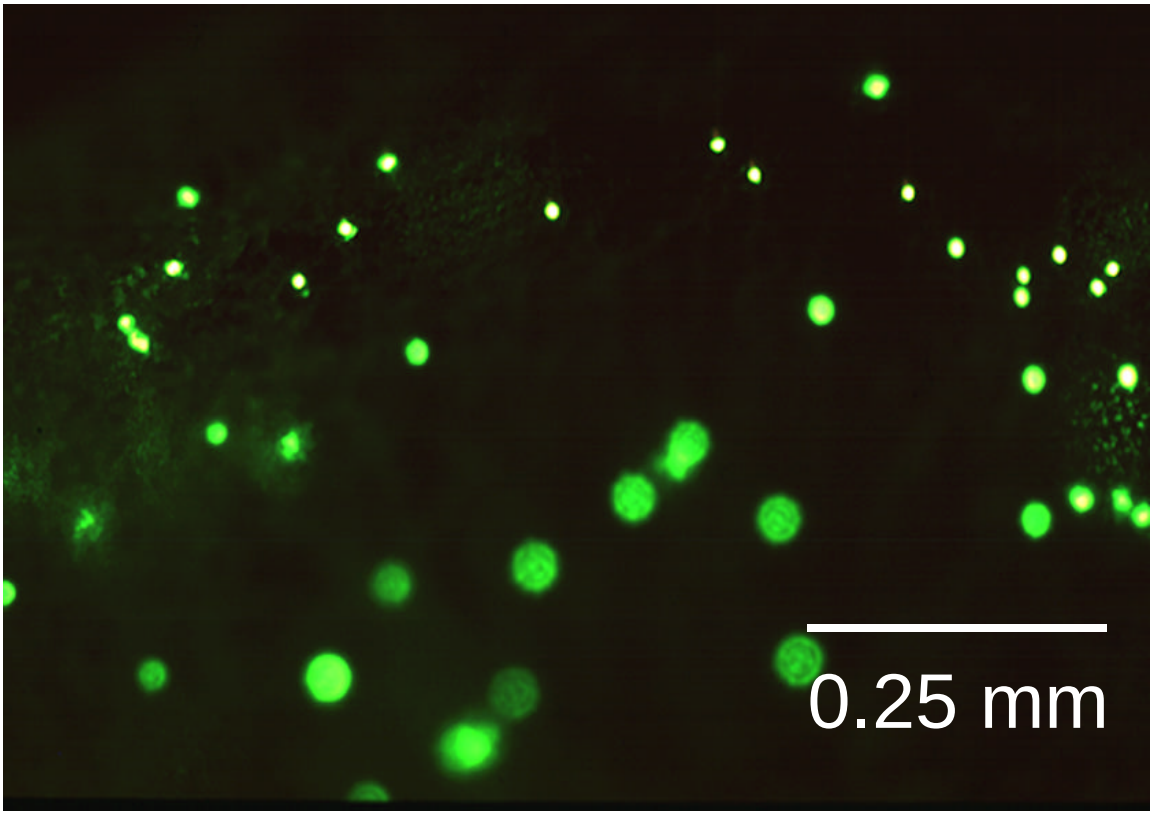

Figure 5.4 View through the crop wall of fluorescent microspheres 6 and 10 µm diam.

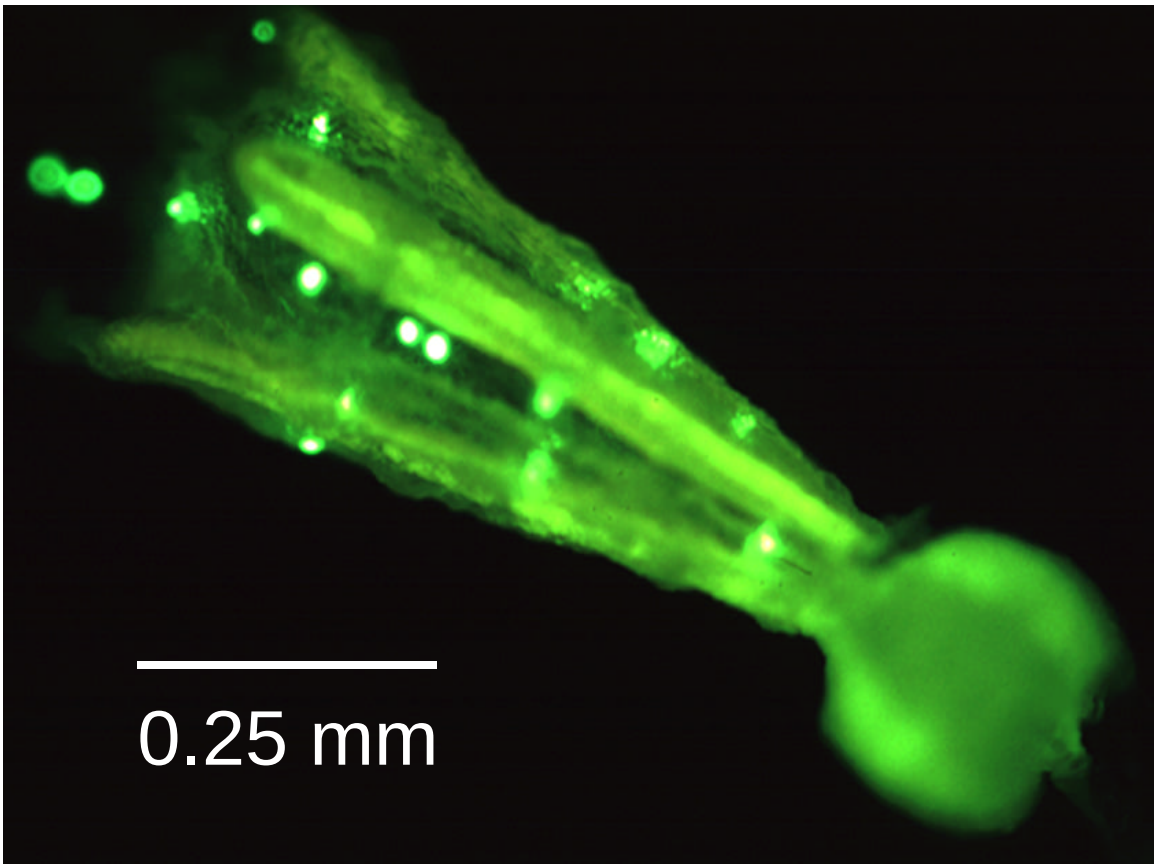

Figure 5.5 Fluorescent microspheres of 6 and 10 µm diam between the intersepalary arms of the proventriculus.

5.3.2 Transit of Fluorescein Through the Gut

Mean fluorescence detected in the mid/hindgut remained fairly stable between four and 16 hours post-feeding, but a small peak could be seen at 20 hours post-feeding (Figure 5.6). Fluorescence declined somewhat by 24 hours post-feeding. Although sample sizes were small (ten ants per test interval) and the change at 20 hours was not dramatic, the data suggest that, if a peak in the amount of food being shunted through the proventriculus indeed exists, it occurs around 20 hours post-feeding. For this reason, ants fed fluorescent microparticles in the subsequent experiments were incubated 20 hours before they were killed.

It is also worth noting that, in contrast to subsequent experiments, ants in this trial were given access to a 10% sucrose solution for two weeks in order to stimulate hunger and to cleanse the gut in preparation for the test solutions. The volume of test solution provided each group allowed for consumption of 10 µl per individual. I found that the test solutions were neither quickly nor completely consumed by the ants upon presentation. I concluded that either 10% sucrose was energetically adequate, under the pre-test conditions, or a mean of 10 µl test solution per individual was too great a volume. Consequently, in the experiments that followed, test groups were starved for about one week prior to testing and were permitted only 5 µl test solution per individual.

5.3.3 Fate of 3, 6, and 10 µm Fluorescent Microparticles Following Ingestion

In general, the antennae and visible mouthparts of the experimental animals were fairly free of fluorescence at 20 hours post-feeding (Figure 5.7). Most infrabuccal pellets that could be obtained from the experimental animals, however, consisted largely of tightly packed fluorescent microspheres (Figure 5.8). There may be a causal relationship between these findings.

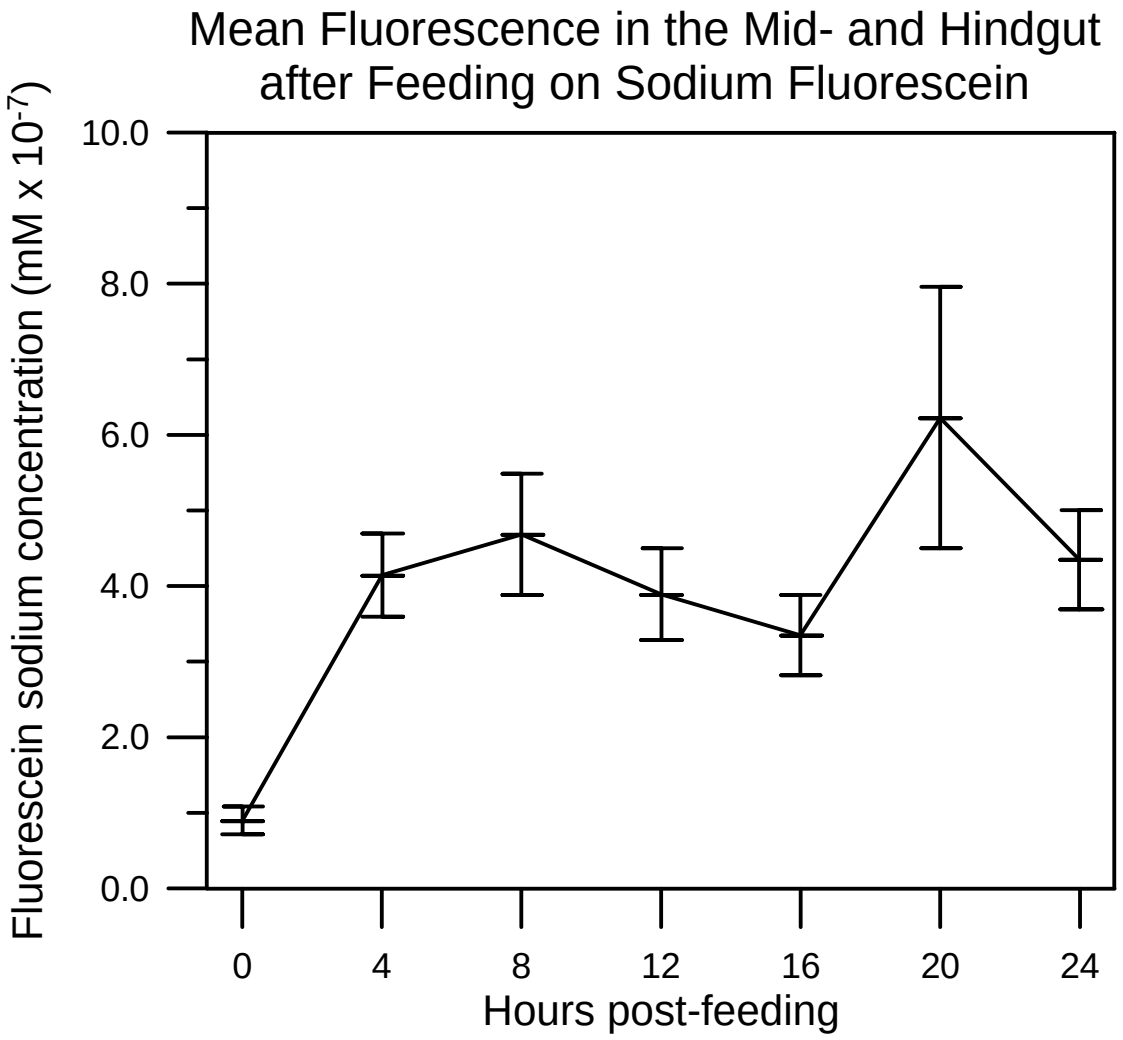

Figure 5.6 Mean fluorescence detected at four-hour time intervals in the mid/hindgut of workers fed a solution of 1.5 x 10^{-4} mM sodium fluorescein in 20% sucrose (w/w) at time 0. (N=10 for each time interval. Vertical bars = $\pm$ SEM).

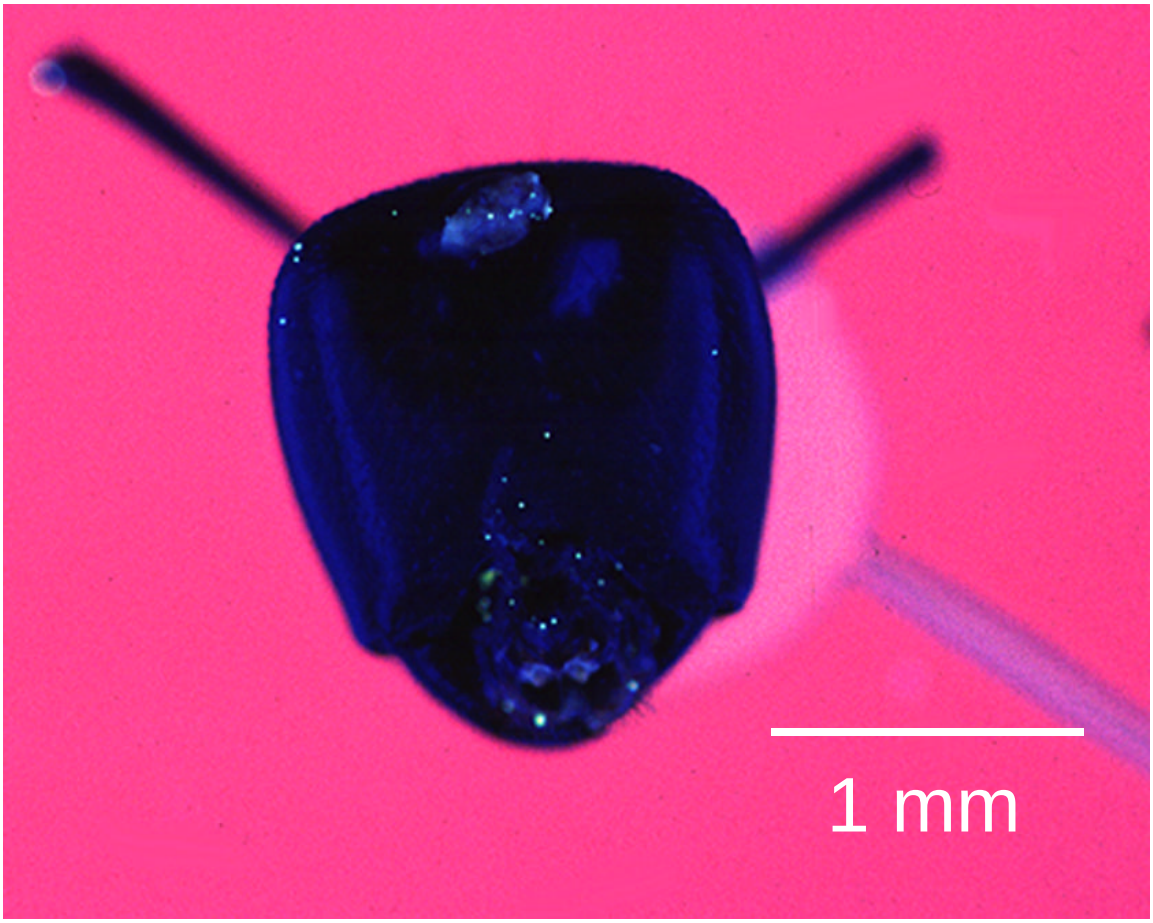

Figure 5.7 Ventral surface of the head of a major worker after feeding on a solution containing 10 µm fluorescent microspheres.

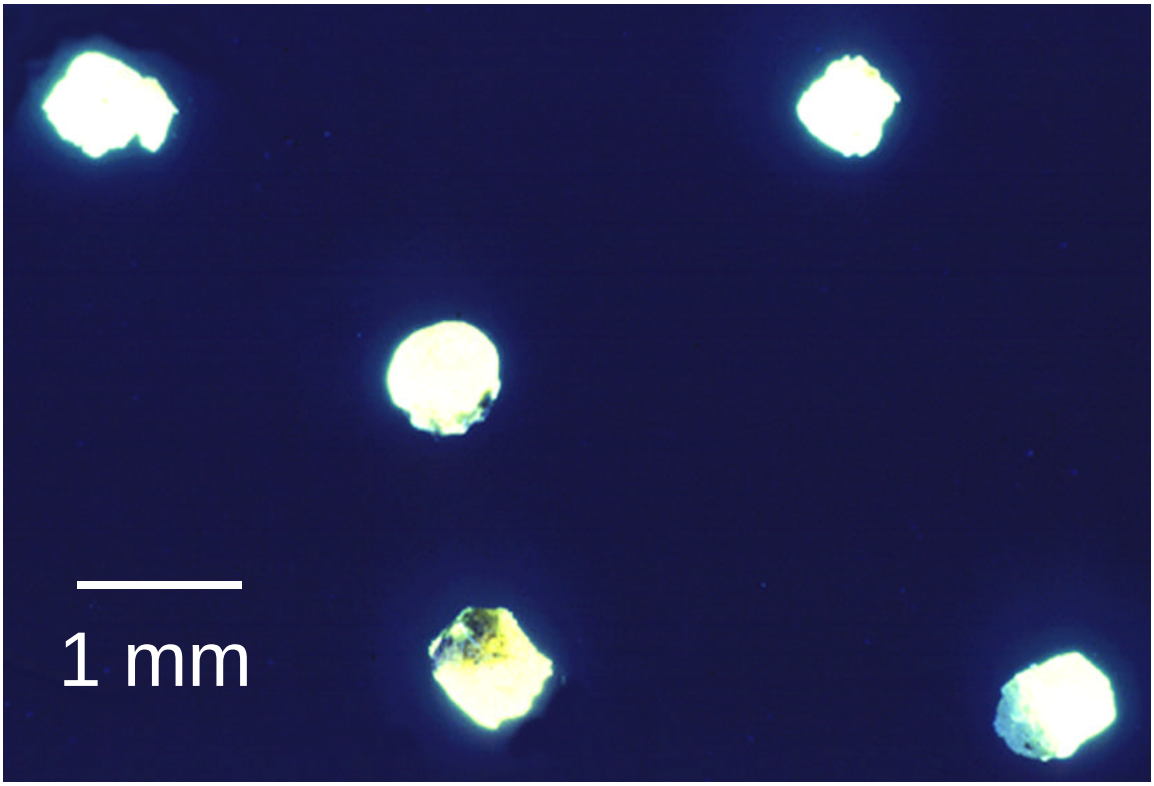

Figure 5.8 Infrabuccal pellets from workers fed a solution containing fluorescent microspheres either 3 or 6 μm diam.

Mean crop fluorescence by caste and treatment is shown in Figures 5.9 and 5.10. The effect of treatment on crop fluorescence was extremely significant (P = 0.0001), indicating the presence of a high concentration of fluorescent particles in the crop when the experiment was ended. Contrasts comparing the treatment groups with the control showed that all treatments differed from the control to a highly significant degree (P = 0.0001).

Figures 5.9 and 5.10 also display mean fluorescence in the mid/hindgut by caste and treatment. The caste by treatmentinteraction was not a significant factor (P = 0.2269) and could be removed from the model. Treatment did not significantly affect mid/hindgut fluorescence (P = 0.1693). The effect of caste on mid/hindgut fluorescence was significant (P = 0.0246), although including control data and analyzing castes separately showed that treatment was not a significant factor either for minors (P = 0.2113) or for majors (P = 0.7740). Contrasts within caste comparing each treatment group with the control were also not significant (Table 5.1). No differences in body size among replicates (P = 0.6958) or treatment groups (P = 0.2248) were seen.

5.3.4 Fate of Ingested 0.05, 0.1, and 1 µm Fluorescent Microparticles Under Conditions of Sociality

With these particles, the limits of light microscopy were reached and individual particles could no longer be seen, though accumulations of particles on the external mouthparts were visible under fluorescent illumination. Most ants examined had mouthparts apparently free of fluorescence. A small number bore mouthparts well-dusted with fluorescent particles (Figure 5.11). Infrabuccal pellets were difficult to extract from this group and some specimens were destroyed during attempts at removal. Therefore, efforts to examine infrabuccal pellets in this group were abandoned.

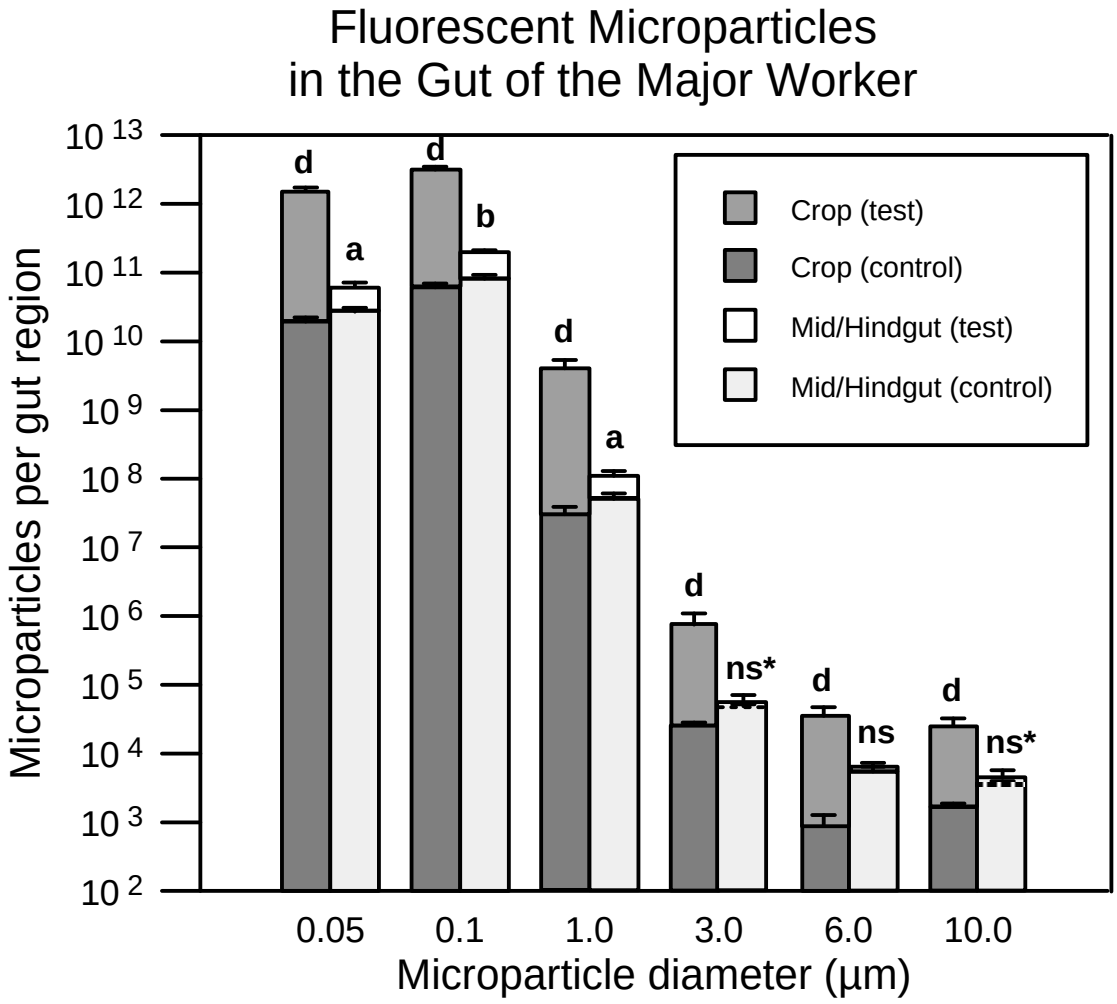

Figure 5.9 Mean microsphere concentration in the crops and mid/hindguts of major workers fed a solution containing fluorescent microspheres 0.05, 0.1, 1, 3, 6, or 10 µm diam under social conditions. Control values are dummy concentrations obtained by applying the natural fluorescence of the gut region to the appropriate standard curve. a= $0.05 \geq P > 0.01$; b = $0.01 \geq P > 0.001$; c = $0.001 \geq P > 0.0001$; d = $P \geq 0.0001$; ns = not significant; * dotted line represents a test mean arithmetically smaller than the control. (N = 18 for control group; N = 14 for 0.05 and 0.1 µm treatment groups; N = 22 for 1 µm treatment group).

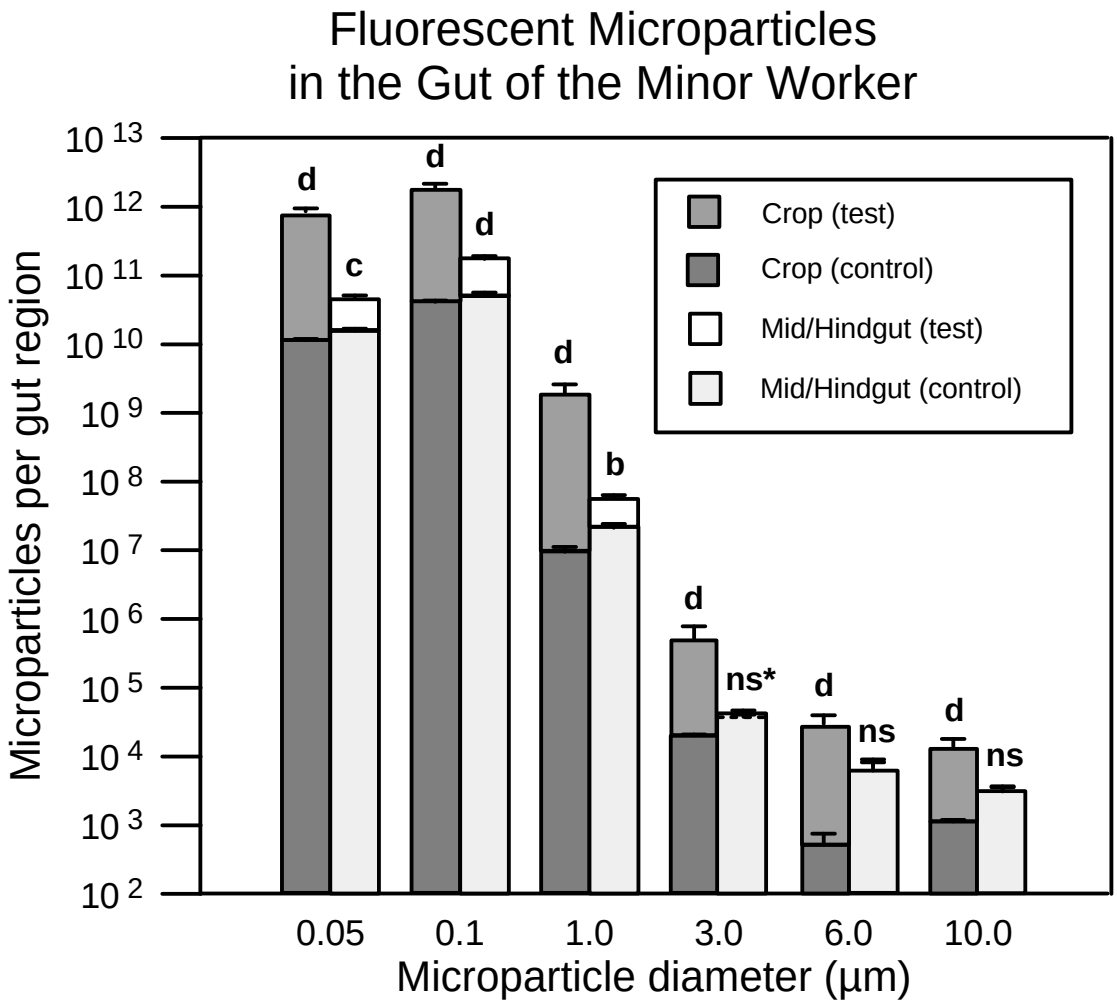

Figure 5.10 Mean microsphere concentration in the crops and mid/hindguts of minor workers fed a solution containing fluorescent microspheres 0.05, 0.1, 1, 3, 6, or 10 µm diam under social conditions. Control values are dummy concentrations obtained by applying the natural fluorescence of the gut region to the appropriate standard curve. a= $0.05 \geq P > 0.01$; b = $0.01 \geq P > 0.001$; c = $0.001 \geq P > 0.0001$; d = $P \geq 0.0001$; ns = not significant; * dotted line represents a test mean arithmetically smaller than the control. (N = 18 for control group; N = 14 for 0.05 and 0.1 µm treatment groups; N = 22 for 1 µm treatment group).

Table 5.1 Orthogonal contrasts of mid/hindgut fluorescence within caste between treatments (3, 6, and 10 µm particles) and the control group ($\alpha = 0.05$).

Control vs. Treatment (µm)	Probability > F	
	Minors	Majors
3	0.9538	0.5462
6	0.0816	0.7590
10	0.4159	0.7405

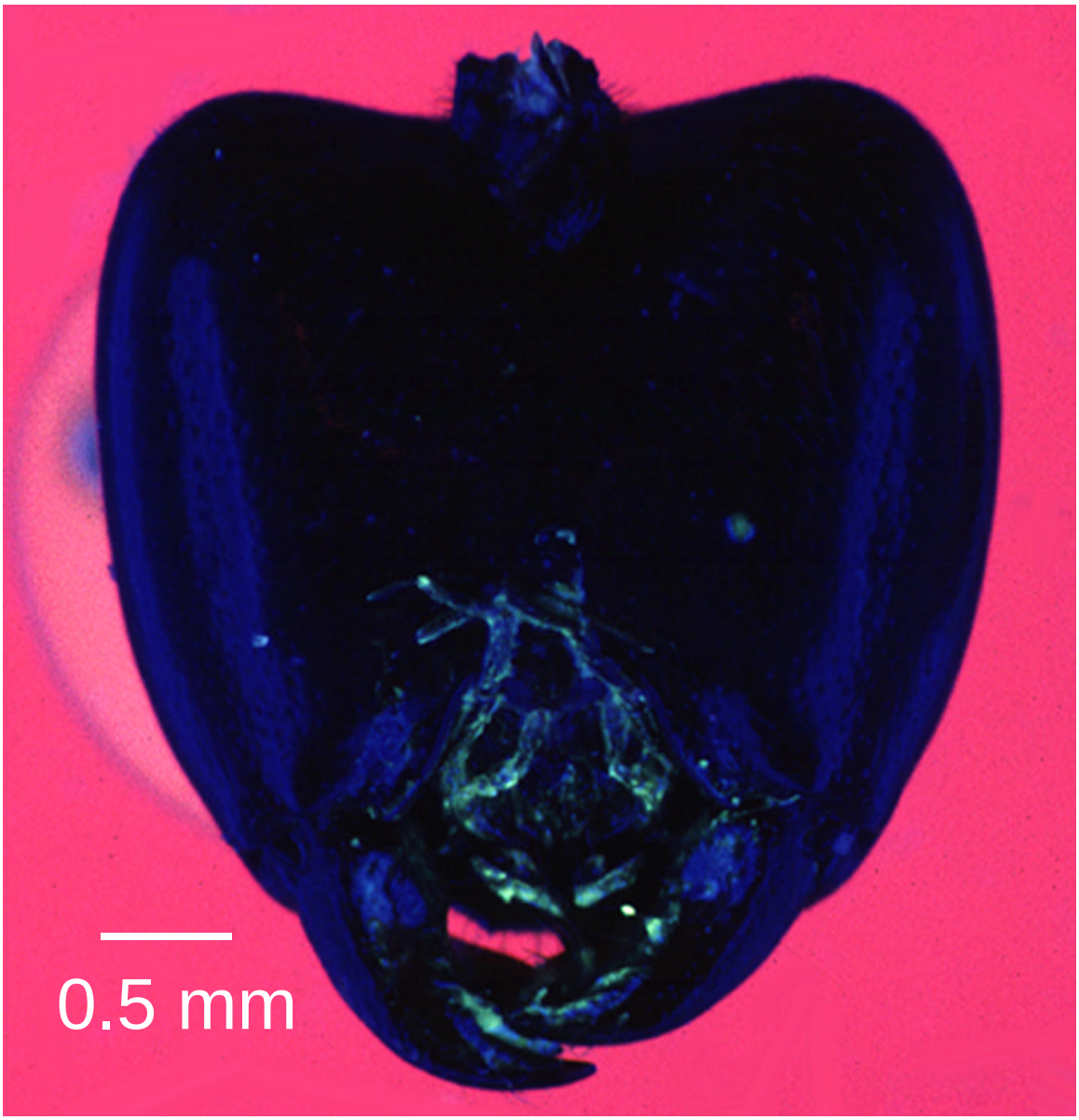

Figure 5.11 Ventral surface of the head of a major worker after feeding on a solution containing 1 µm fluorescent microspheres.

Mean crop fluorescence by caste and treatment is presented in Figures 5.9 and 5.10. Treatment exerted an effect on crop fluorescence that was highly significant (P = 0.0001). All contrasts of treatment groups with the control were equally significant (P = 0.0001). As in the previous study (Section 5.3.3), this indicates that the crops of test animals from all treatment groups contained high concentrations of fluorescent particles at the time they were killed.

As noted previously, I examined the contributions of crop fluorescence and head capsule size as covariates in a RCBD model. The effect of treatment on mid/hindgut fluorescence was substantially smaller when both covariates were included in the model (P = 0.0477) than when both covariates were removed from the model (P = 0.0007). I concluded that the covariates were not helping to explain any variance in the data beyond that explained by the classification variables (treatment and block) and may in fact have contributed additional noise. These concerns were confirmed when the relationships between each covariate and the dependent variable were examined (Tables 5.2, 5.3). Neither covariate exhibited a consistent or strong linear relationship to mid/hindgut fluorescence. Consequently, both were removed from the model, though head capsule size data proved useful once it was transformed to the classification variable "caste".

Mean mid/hindgut fluorescence is also given by caste and treatment in Figures 5.9 and 5.10. The interaction between caste and treatment had no significant influence (P = 0.5561), and so was removed from the model. The effect of treatment on mid/hindgut fluorescence was highly significant (P = 0.0006), but caste did not make a significant contribution to explaining variance (P = 0.2281). Examining the treatment effect within castes produced slightly different results. The effect of treatment on mid/hindgut fluorescence in the minor caste was very strong (P = 0.0004). In the major caste, the effect of treatment was significant (P = 0.0263). Contrasts conducted within caste proved interesting. While all comparisons within the minor caste were quite significant, contrasts within the major caste demonstrated only modest to marginal

Table 5.2 Relationship between the dependent variable "mid/hindgut fluorescence" and covariate "crop fluorescence " across nest by treatment combinations as measured by slope (m) and adjusted coefficient of determination (R^2_a)

Nest	Treatment							
	Control		0.05 µm		0.1 µm		1 µm	
	m	R^2_a	m	R^2_a	m	R^2_a	m	R^2_a
1	0.9497	0.6819	1.0123	0.7615	0.5560	0.6436	0.6418	0.4482
2	0.3472	0.2051	-0.7675	0.4627	0.1181	-0.1111	0.5313	0.0103
3	0.7912	0.2210	0.2803	-0.0734	0.3571	0.0882	0.5974	0.6735

Table 5.3 Relationship between the dependent variable "mid/hindgut fluorescence" and covariate "body size" across nest by treatment combinations as measured by slope (m) and adjusted coefficient of determination (R^2_a).

Nest	Treatment							
	Control		0.05 µm		0.1 µm		1 µm	
	m	R^2_a	m	R^2_a	m	R^2_a	m	R^2_a
1	0.8153	0.7314	0.5529	0.7144	0.3086	0.2538	0.2072	0.3887
2	-0.9400	0.1731	0.5460	-0.0851	0.5895	-0.0385	-0.0519	-0.1159
3	0.8803	0.4977	-0.3764	0.1622	0.2328	0.0775	0.1441	0.1387

significance (Table 5.4). I verified that differences in mean body size by treatment and nest both within and across caste were negligible and therefore did not influence the effects observed. Across caste, that is comparing experimental unit means by treatment and nest, body size did not differ with treatment ($P = 0.6981$) or nest ($P = 0.5937$). Within caste, minor worker means were comparable across treatment ($P = 0.7578$) and nest ($P = 0.5394$). Major castes did not show differences across treatment ($P = 0.9358$), but nest had a weak effect ($P = 0.0218$). Exploring nest means through least squares means, I found that the majors of one nest were slightly smaller than those of the other nests ($P = 0.0133$), though this probability was only just significant, as the significance threshold for comparing three means is $P = 0.0167$. The effect of this difference is therefore likely to be inconsequential.

5.3.5 Fate of Ingested 0.05, 0.1, and 1 µm Fluorescent Microparticles Under Solitary Conditions

Two behaviors exhibited by ants during this experiment were unusual enough to warrant comment. First, isolated ants suffered from especially high mortality rates. Fifty-seven percent of those ants that fed at the start of the experiment died during the 20-hour *post*-feeding interval. By comparison, the post-feeding mortality rate in the experiment examining particles from 3 to 10 µm diam was only 7.5%. When considered in light of the great disparity in pre-treatment starvation intervals for the two experiments (24 hours for the present experiment, seven days for the experiment involving 3 to 10 µm particles), the observed mortality rates are even more surprising. Mortality in the experiment examining smallest particles under social conditions was not recorded, but is estimated to be 25%.

Second, only nine test tubes out of 68 appeared to be free of fluorescent staining by the experiment's end. In the other 59 test tubes, moderate to strong fluorescence was visible in droplets on the inner wall, on the cotton plug damming the water source, or in

Table 5.4 Orthogonal contrasts of mid/hindgut fluorescence within caste between social treatment (0.05, 0.1, and 1 μm particles) and control groups ($\alpha = 0.05$).

Control vs. Treatment (μm)	Probability > F	
	Minors	Majors
0.05	0.0003	0.0190
0.1	0.0001	0.0058
1	0.0051	0.0531

the sucrose solution held by the microcentrifuge tube. With two exceptions, the fluorescence in the crops of those ants in the "tidy" test tubes was 1.2 to 48.3 times greater than that of any other ant in the same experimental unit. All ants in the "tidy" test tubes belonged to the minor caste.

A comparison of mean crop fluorescence by caste and treatment is presented in Figures 5.12 and 5.13. As in the two previous experiments, the effect of treatment on crop fluorescence was very significant ($P = 0.0017$). All treatments differed from the control in contrasts: control vs. 0.05 µm ($P = 0.0031$), control vs. 0.1 µm ($P = 0.0011$), control vs. 1 µm ($P = 0.0006$). Again, these values offer evidence that microspheres were consumed and retained in the crop until the test animals were killed.

Figures 5.12 and 5.13 also shows mean mid/hindgut fluorescence for solitary workers fed particles 0.05 to 1 µm. Once again, the caste by treatmenteffect was insignificant ($P = 0.9911$) and was removed from the model. Both caste ($P = 0.0018$) and treatment ($P = 0.0045$) exerted very significant effects on mid/hindgut fluorescence. Within the minor caste, contrasts comparing treatments with the control were marginally to very significant: $P = 0.0590$ (0.05 µm particles), $P = 0.0023$ (0.1 µm particles), $P = 0.3243$ (1 µm particles). Contrasts within the major caste could not be tested because so few survived, but neither the treatment effect within the caste ($P = 0.4693$) nor the overall F test for the model ($P = 0.3439$) were significant. Overall body size did not differ significantly by treatment ($P = 0.8505$) or nest ($P = 0.6449$). Likewise, body size was not a significant factor within castes: treatment was unaffected in minors ($P = 0.1791$) and majors ($P = 0.8662$), and nest was unaffected by minors ($P = 0.8812$) and majors ($P = 0.7202$).

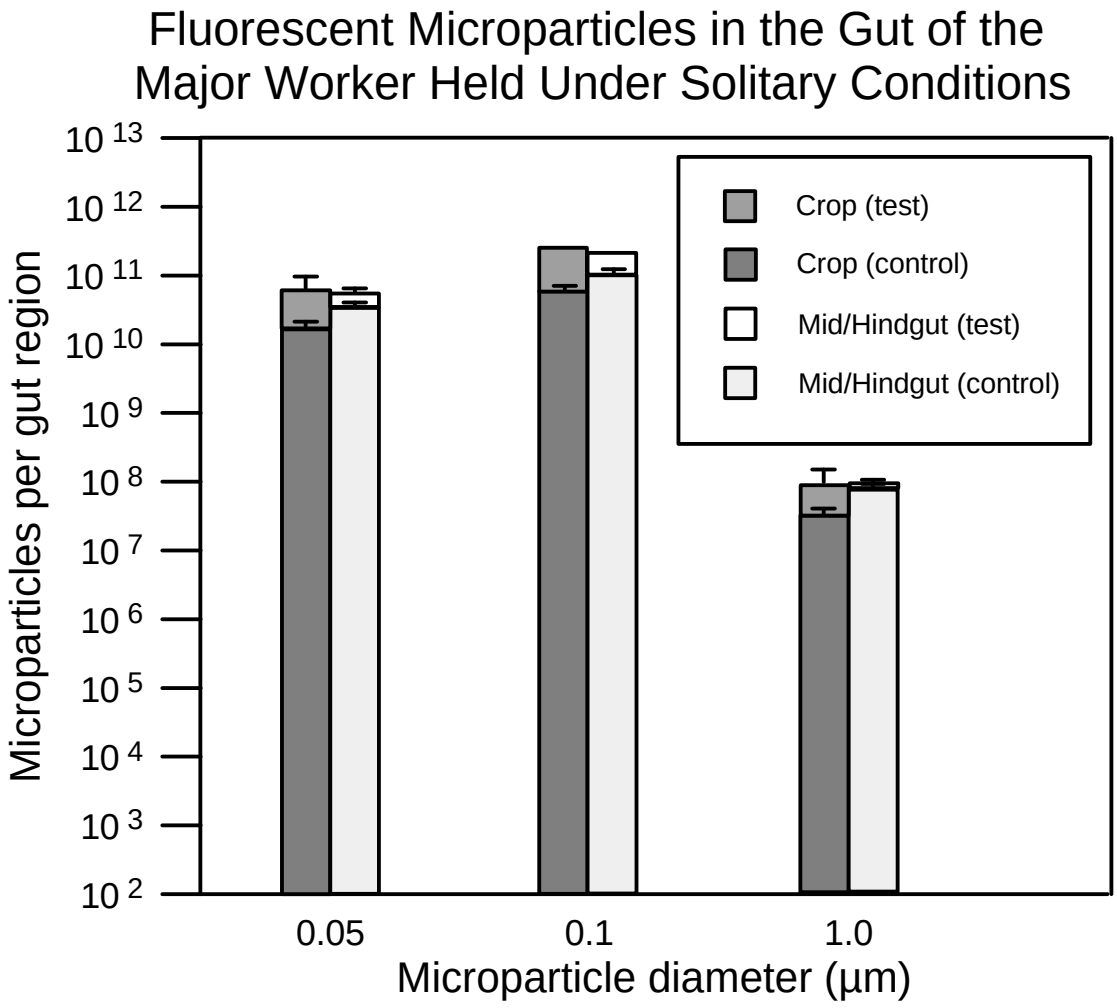

Figure 5.12 Mean microsphere concentration in the crops and mid/hindguts of major workers fed a solution containing fluorescent microspheres 0.05, 0.1 or 1 µm diam under solitary conditions. Control values are dummy concentrations obtained by applying the natural fluorescence of the gut region to the appropriate standard curve. High mortality produced groups too small to be statistically examined for differences. (N = 18 for control group; N = 14 for 0.05 and 0.1 µm treatment groups; N = 22 for 1 µm treatment group).

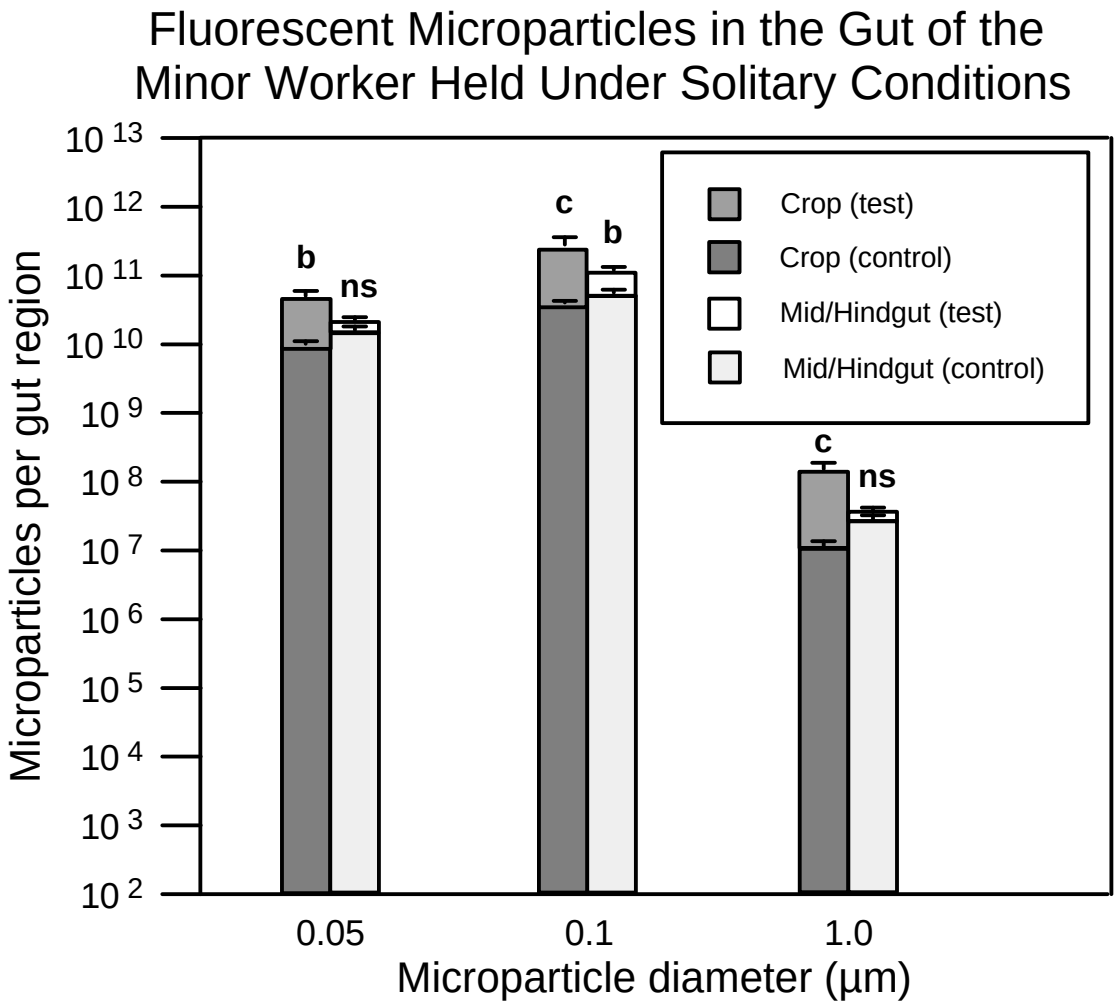

Figure 5.13 Mean microsphere concentration in the crops and mid/hindguts of minor workers fed a solution containing fluorescent microspheres 0.05, 0.1 or 1 µm diam under solitary conditions. Control values are dummy concentrations obtained by applying the natural fluorescence of the gut region to the appropriate standard curve. a= $0.05 \geq P > 0.01$; b = $0.01 \geq P > 0.001$; c = $0.001 \geq P > 0.0001$; d = $P \geq 0.0001$; ns = not significant. (N = 18 for control group; N = 14 for 0.05 and 0.1 µm treatment groups; N = 22 for 1 µm treatment group).

5.4 Discussion

Microspheres of the sizes used in this study were unproblematic in terms of acceptability, ingestibility, and visibility under fluorescence illumination. Consumption occurred readily, and the presence of particles in the gut could be verified through fluorescence microscopy or spectrofluorometry.

Solitary workers fed particles of 6, 10, and 45 µm diameter were able to filter out the largest of these: 45 µm particles were found on the external mouthparts and in infrabuccal pellets, but not in the crop. The two smaller-sized particles were found on the external mouthparts, in infrabuccal pellets, and in crops. This suggests that the infrabuccal pocket and the external mouthparts aid in removing larger particles from the ingesta. Forward-projecting hairs lining the buccal tube (Forbes 1938) may also play a role in particle exclusion. Spine-like projections occur in the buccal tube of *S. invicta*, which is capable of filtering particles of 1 µm from its food (Glancey et al. 1981).

Work by Eisner and Happ (1962) on "medium-sized" *C. pennsylvanicus* workers produced results comparable to those here. When Eisner and Happ (1962) fed ants carbarundum particles of 10 and 100 µm diameter, the larger particles tended to accumulate in the infrabuccal pocket, whereas the smaller particles tended to predominate in the crop. However, when they fed their subjects a range of particle sizes, they found a "substantial amount" of particles of 10, 20, 30, 80, and 100 µm in the ants' crops, which conflicts somewhat with their previous results and with my own. Differences in methodology may partly explain the discrepancy. Eisner and Happ (1962) killed and examined their subjects immediately after the ants had fed, whereas in the present study the test animals were killed 12 hours after they had fed. The isolated ants may have repeatedly regurgitated and consumed the test solution during the prolonged post-feeding interval, in effect filtering it multiple times, thereby removing particles from the ingluvium. Replete workers of *S. invicta* and *Iridomyrmex humilis* will regurgitate onto

the cage floor if prevented from engaging in trophallaxis for several hours after feeding (Howard and Tschinkel 1981a, Markin 1970).

The concentration of sodium fluorescein in the mid/hindgut appeared to peak at 20 hours after feeding, though the increase was not statistically validated. There is little quantitative data in the literature with which to compare this observation. The rate of internal food transport has been examined in only two ants, *Formica polyctena* and *S. invicta* (Gößwald and Kloft 1960a, 1960b, Howard and Tschinkel 1981a). Isolated workers of the fire ant, *S. invicta*, will pass some of a 5% sucrose solution to the midgut within seconds of ingestion, but levels in the midgut are highest between six and 24 hours after consumption (Howard and Tschinkel 1981a). The wood ant, *F. polyctena*, does not pass honey water to the midgut until "several" hours after feeding, and the process is slower in social groups than in isolated workers (Gößwald and Kloft 1960a, Gößwald and Kloft 1960b). In *F. polyctena*, the ultimate rate of transport through the proventriculus also depends on whether food is obtained directly (feeding at the source) or indirectly (via trophallaxis). Only 20% of honey water consumed directly is seen in the midgut 4.5 hours after feeding, whereas 77% of the radioactive honey water received during trophallaxis reaches the midgut 2.5 hours after feeding (Gößwald and Kloft 1960b). My fluorescein testing protocol involved ants in small social groups, rather than isolated individuals, and many ants were observed feeding directly on the test solutions. This may explain the long time interval between feeding and peak mid/hindgut concentration.

While the fluorescein trial was intended only to maximize the ability to detect fluorescent particles in the gut, the possibility that solids (i.e. microparticles) move through the gut at a different rate than liquids (i.e. fluorescein) must be recognized. In honey bees, for example, the transport of pollen through the alimentary canal is begun immediately after consumption and occurs in a steady stream, in contrast to fluids consumed (Schreiner 1952). At this point, such a possibility can be little more than acknowledged, as potential differences in transport rates cannot be explored before first

verifying that particles are indeed able to enter the carpenter ant midgut, which was the purpose of the present experiment.

Infrabuccal pellets from ants fed particles from 3 to 45 µm diameter contained densely packed microparticles, suggesting that the mouthparts play an important role in filtering particles in that size range from the ingestate. Infrabuccal pellets were quite difficult to extract from ants fed particles from 0.05 to 1 µm diameter, presumably because their infrabuccal pockets were not full. Perhaps most pellets were ejected prior to examination, however few fluorescent pellets were detected on the container floors after the experiment. Alternatively, the mouthparts may be less effective at filtering particles of such small size, though this inference would need to be confirmed though further work.

High levels of fluorescence were present in the crops of ants of all treatment groups at 20 hours post-feeding, thus proving that all test solutions were freely consumed. The persistence of measurable fluorescence in the crop for 20 hours is interesting. It demonstrates that, in small social groups at least, high concentrations of particles 0.05 to 10 µm diameter cannot be completely extracted from the crop fluids within that period of time. Eisner and Happ (1962) found that 12 hours was insufficient for *C. pennsylvanicus* workers in small social groups (55 - 80 ants) to filter particles of 10 µm from the ingluvium. Though particles had filled the infrabuccal pockets of some ants, 50% of the experimental animals still had crops up to 1/4 full of particles after 12 hours. Similarly, *Acromyrmex octospinosus* (Reich) and *S. invicta* are capable of some filtration of 10 and 1 µm particles, respectively, from the crop contents shortly after ingestion, though some particles were still detected in the crops (Quinlan and Cherrett 1978, Glancey et al 1981).

Isolated major workers fed 0.05 - 1 µm particles had substantially less fluorescence in their crops than those in social groups. Isolated workers displayed a general tendency to regurgitate the test solutions, as fluorescent staining in most

containers made clear. These results suggest that social conditions encourage the retention of food in the crop. Under natural conditions, foragers that have fed to repletion probably require positive feedback from nestmates to retain the crop contents for a period of time, as discussed in Chapter 4 (see Section 4.4). The absence of feedback under natural conditions may cause foragers to discard the undesirable material by disgorging it, freeing them to forage for a more acceptable food source. I suspect that conditions of isolation mimic the absence of nestmate receptivity, thereby provoking the disgorgement response.

Although particles remained in the crop for at least 20 hours, the 3 to 10 µm diameter particles were not found in the mid- and hindguts of either major or minor workers. It could perhaps be argued that the particles had passed through and out of the digestive system within 20 hours, but it is unlikely to be the case. Previous testing indicated that the highest concentration of food could be found in the mid/hindgut at 20 hours post-feeding. Furthermore, particles smaller in size were detectable in the mid/hindgut at 20 hours, and liquid fluorescein persisted in the mid/hindgut for at least 24 hours. It is more likely that 3 to 10 µm particles were prevented by the proventriculus from entering the midgut. Particles of 0.05 to 1 µm diameter, on the other hand, were clearly present in the mid- and hindguts of both major or minor workers. These results strongly suggest that the efficiency of the proventriculus as a filtering mechanism breaks down between 1 and 3 µm. No morphological examinations were undertaken in the present study, but Eisner and Wilson (1952) prepared diagrams to scale from morphological and histological preparations of the proventriculus in *Camponotus americanus* Mayr, which is comparable in size to *C. pennsylvanicus* (Wheeler 1910). In those diagrams, the filtering slit of the sepal is quite narrow and the sepal canals themselves, which act as viaducts to the midgut, appear to range from only 5 to 10 µm diameter (Eisner and Wilson 1952). If this estimate is accurate, such dimensions would be sufficient to physically exclude 6 and 10 µm particles, while

movement of 3 µm particles through the canal could be hindered. Naturally, further work would be needed to support this hypothesis.

The amount of fluorescent particulate material beyond the proventriculus was influenced by both caste and grouping regimen (social/solitary conditions). When maintained in social groups, ants contained significantly more fluorescence in the mid/hindgut than the controls; the difference for minor workers was extremely significant. When maintained in isolation, major workers did not differ from the control group at all, and minors differed only modestly. Here I suspect that behavior, rather than morphology, is the factor governing transport of food within the gut. Running in ants is seven times more energetically expensive than resting (Jensen and Holm-Jensen 1980), and activity levels in *C. pennsylvanicus* are known to vary inversely with body size (Fowler 1982). Despite their smaller size, therefore, minor workers may tend to consume proportionately more of their crop fluids. The higher numbers of particles observed in the mid- and hindguts of minor workers may simply be evidence of their higher energetic demands. The lower numbers of particles observed among isolated ants in comparison with those in social groups is surely a result of the observed tendency of ants in isolation, especially major workers, to disgorge the crop contents, as discussed above.

The influence of caste was also apparent in social groups when 1 µm particles were consumed. Under these conditions, the mid- and hindguts of major workers did not contain measurable fluorescence, whereas fluorescence in the mid- and hindguts of minor workers was significant. It is tempting to conclude that proventricular function is in some way associated with body size. A correlation between caste and filtering ability anterior to the proventriculus has been demonstrated in *A. octospinosis*, in which smaller workers filtered smaller particles from their crops (Quinlan and Cherrett 1978). The results of the present work suggest an inverse correlation between body size and filtration, an arrangement for which there is no ready explanation. Perhaps worker size exerts an effect not on filtering ability but on food transport. Major workers, being more sedentary than

minors, may pass to the midgut quantities of food that are so small as to be undetectable by the methods of this work.

These results demonstrate the efficiency with which particulate matter is filtered from the communal food supply: particles 45 µm or greater are not ingested, particles 10 µm or greater are sequestered in the infrabuccal pocket, and particles 1 µm or greater are excluded from the midgut. The implications are clear for control strategies that may need to reach the midgut for maximum effect, such as bait and microencapsulation technologies or microbial control agents. The implications for colony nutrition and the possible adaptive significance of the serial filtration system are both less clear and more interesting. It seems reasonable to conclude that particles smaller than 1 µm are too small to abrade the midgut wall, which in this group is unprotected by a peritrophic membrane. Perhaps by protecting itself from potentially damaging elements entering the communal food supply, however, the colony is simultaneously collecting and concentrating a useful food source. Pollen grains can range in size from 5 to 200 µm (Stanley and Linskens 1974). Fungal spores can vary from 2 to 200 µm (Weber and Hess 1976). Hemocytes and protein granules from arthropod hemolymph are at least 1 µm, and tissue fragments from prey or carrion are certain to be larger (Woodring 1985). Such "debris" may represent a valuable source of nutritious food, if *C. pennsylvanicus* larvae are able to consume solids, as *S. invicta* larvae are known to do (Petralia and Vinson 1978, Glancey et al. 1981).

A final point of interest concerns the mortality observed in the experimental groups. Ants fed 3, 6, or 10 µm particles suffered the least mortality, while those fed 0.05, 0.1, or 1 µm particles in isolation suffered the highest rate. Certainly, the stress of isolation can account in large part for the very high mortality seen in the latter group (Grassé and Chauvin 1944), but the mortality rate of ants fed 0.05, 0.1, or 1 µm particles in social groups was intermediate. The disparity between mortality rates in the two groups maintained under social conditions can be attributed to one of two factors in

which the two experiments differed: starvation interval and particle size. The length of the pre-test starvation period was two days shorter for those ants fed the larger particles. This adjustment was made precisely because of the high pre-test mortality observed for the longer interval. Perhaps the difference between six days and eight days of starvation is critical. Because the first phase of digestion produces a net loss in energy (Chapman 1985), an insect truly at the end of its energetic reserves may not be saved by a sudden feeding. Alternatively, the particles in the two experiments differed in size, raising the possibility that the particles themselves contributed to mortality. Three micrometer diameter particles were not able to reach the midgut, whereas 1 µm diameter particles were. Obviously, particles of 1 µm diameter are close to the upper size limit, and may not pass as freely through the proventriculus as smaller particles. My results have shown that mid/hindgut fluorescence means of both castes and both groups (social and isolated) fed 1 µm diameter particles were lower than those fed 0.1 or 0.05 µm particles. Could particles of 1 µm diameter collect in the sepal canals in such a way that flow of materials to the midgut is obstructed? Disruption of food flow to the midgut is less likely to explain mortality, as death due to starvation tends to be prolonged in insects. Conversely, personal observation has shown that mortality in carpenter ants can occur very quickly in the absence of sufficient quantities of water. This possibility is worthy of further research, as it represents a potential avenue for non-toxic control of these pests.

CHAPTER 6. SUMMARY

The ants are a large and diverse group of insects contained in the single family Formicidae. It is thought that most ants are omnivorous scavengers, but data on the nutritional ecology of some of the largest ant genera are incomplete (Carroll and Janzen 1973, Tobin 1993). *Camponotus* is one of the largest and most widespread formicid genera, ranging from southernmost Chile to the Arctic Circle and occupying all seven zoogeographic regions worldwide (Brown 1973). *C. pennsylvanicus*, the most common North American carpenter ant east of the Mississippi, forages in homes and nests in wood. As such, it is a pest of some economic importance. This study investigated the nutritional ecology of *C. pennsylvanicus* by examining: macronutrient collection under natural conditions in the field; macronutrient preference in field-based choice tests; and the fate of microparticles in the gut of the worker.

Under natural conditions, foragers collected more carbohydrate than any other macronutrient. The carbohydrate consisted almost entirely of simple sugars, although the results are likely to have been affected by carbohydrate digestion in the foregut. Both invertase and amylase, which hydrolyze sugars and complex carbohydrates respectively, are formed in glands that empty onto the labium (Ayre 1967). Carbohydrate digestion is thus likely to occur either extraorally or in the foregut. Rapid hydrolysis of sucrose to glucose and fructose in the crop has been demonstrated in *C. herculeanus* and *S. invicta* (Ayre 1963b, Tennant and Porter 1991). Further study is consequently needed to determine what dietary contribution, if any, starch or glycogen make to carpenter ant nutrition.

Carbohydrate collection did not show seasonality, but the amount collected differed among nests and between years. Such effects have been observed in other feeding studies and are probably commonplace. Laboratory colonies of *S. invicta*, for

example, exhibit "day-to-day" variability in food preference, despite exposure to identical diets and environmental conditions. Yearly fluctuations in food collection have been recorded for *F. polyctena,* leading Horstmann (1974) to conclude that temperature and precipitation can strongly affect foraging. Others have also suggested that the effects of weather on food quality and, therefore, on foraging behavior are of primary importance (Herzig 1938, White 1978).

In choice tests, *C. pennsylvanicus* showed a preference for those sugars most commonly occurring in honeydew: fructose, glucose, and sucrose (Auclair 1963). Sucrose, the predominant honeydew sugar (Tennant and Porter 1991), was the most preferred of all simple sugars tested. Consumption rates of preferred sugars increased with concentration to 20% (wt/wt); more concentrated solutions were not consumed faster. Insect neurophysiology supports these findings. Specialized chemoreceptors receive maximum stimulation from sugar solutions having concentrations between 0.1 and 1.0 molar (Hsiao 1985). Twenty percent solutions of mono- and disaccharides are equivalent to 0.5 - 0.9 molar concentrations. Evidence for similar preferences in other ants can be found in Horstmann (1970), who found that returning *F. polyctena* foragers bore 20% (wt/vol) sugar solutions in their crops. It is intriguing that freshly excreted honeydew has a sugar concentration of 20% (Zoebelein 1956).

The crops of workers foraging under natural conditions were found to contain very little lipid. Foods high in plant- or animal-derived lipid were largely ignored by foragers during choice tests. Lipids are not only important structurally and energetically, but, as hormones, pheromones, antibiotics, and colony recognition compounds, they are crucial to colony organization. In *C. pennsylvanicus*, the biological need for lipid does not appear to be met through exogenous sources of lipid. Perhaps the ants rely on intermediary metabolism to transform some of the carbohydrate they avidly collect into lipid (Candy 1985). Alternatively, the endosymbiotic bacteria harbored by carpenter ants in the midgut may be providing their hosts with nutrients missing from their diet, such as

sterols (Steinhaus 1967, Dadd 1985, Schroder et al. 1996). Finally, there remains the possibility that some lipid truly was consumed but remained undetected by the methods of this study. In some ants, lipid is shunted directly to the post-pharyngeal glands, bypassing the crop entirely (Delage-Darchen 1976). Moderate levels of lipase in the midgut of *C. pennsylvanicus* suggest that lipid is at least occasionally consumed (Ayre 1967). Further research is needed to determine the origin of lipid reserves.

After carbohydrate, the most heavily collected macronutrient was nitrogenous material. In contrast to carbohydrate collection, retrieval of nitrogenous material varied not with nest or year, but with season. Collection was bimodal, with peaks in amount collected per forager seen in June and September. These peaks correspond well with the generalized pattern of brood production in *Camponotus*, which is also bimodal. Adults begin emerging in June and September, with peak emergence occurring toward the end of those months (Fowler 1982). The largest larvae, that is the last instar(s), surely require the greatest quantity of protein. As the pupal stage lasts about three weeks, a peak in late-instar larvae can be calculated at about three to four weeks before peak adult emergence (Cannon, unpubl.). Correlations between protein collection and brood production have been demonstrated in laboratory studies of *F. polyctena*, *M. rubra*, and *S. invicta* (Lange 1962, Ricks and Vinson 1972, Brian 1973).

The source of the nitrogen-based compounds was not investigated; it could have been derived from honeydew in non-peak months. Auclair (1963) cites references describing honeydews containing up to 18% nitrogen compounds by weight. The amount of nitrogenous material collected by *C. pennsylvanicus* in May, July, and August was about 18% of the amount of carbohydrate collected in those months. In peak months, however, nitrogen compounds were collected at a far greater rate approaching that of carbohydrates. Clearly, better nitrogen sources than honeydew were being exploited. Were the ants collecting prey or carrion? Hagen (1987) claims that, in the few terrestrial insect predators studied, dietary protein and carbohydrate are required in a 1:1 ratio. An

approximately 1:1 ratio of nitrogenous material to carbohydrate was observed during months of peak nitrogen collection. This ratio does not prove predation, but rather demonstrates that, in those months when they are rearing brood, foragers collect foods suited to the needs of a predator. The nitrogenous food collected by foragers to meet those needs may be prey or scavenged material.

In choice tests of high-nitrogen laboratory chemicals, casein hydrolysate proved more acceptable to foragers than other protein hydrolysates. A 20% casein hydrolysate solution elicited the strongest feeding response. In tests using processed nitrogenous foods, fish products were consumed at higher rates than beef products. Differences in consumption rates may have been due to differences in protein content, as solids were homogenized with water. Conversely, there may be an intrinsic compound in fish that acts as a phagostimulant. For example, fish is higher in low molecular weight organic nitrogen compounds than beef (Finne 1985). Canned tuna, the most preferred processed food tested, normally contains hydrolyzed casein as an additive, which has been shown to stimulate feeding in these ants, as mentioned above. Tuna is also especially rich in histidine (Finne 1985). Histidine is an essential amino acid that is found in honeydews (Ehrhardt 1962). In amino acid preference tests, Fell and Mollet (unpubl.) found histidine was preferred by foraging carpenter ants to many other amino acids.

Choice tests comparing the relative attractancies of carbohydrate, lipid, and nitrogenous material over time revealed a preference for sugars and nitrogenous material. These two "indispensable energy-producing nutrients" (Dadd 1985) comprised nearly all of the crop contents of foragers examined in the previous study. Taken together, the results from both studies emphasize the importance of proteins and carbohydrates in the carpenter ant diet. Interestingly, though, the choice tests provided results that differed from those of the crop contents study in two ways. First, macronutrient preference did not exhibit seasonal shifts but remained stable over time. The amount of nitrogen carried in the crops of workers foraging under natural conditions, on the other hand, varied with

month. Second, nitrogenous material was collected more intensively than carbohydrate. About four times as much nitrogenous material as carbohydrate was removed during choice tests, whereas only one half to one third as much nitrogenous material as carbohydrate was observed on average in the crops of returning foragers.

The contrasting results, particularly with respect to nitrogen, lend support to White's (1978) hypothesis that the environment provides insufficient quantities of nitrogen. Prey, the best source of protein, are generally scarce and may show seasonal changes in density and variety (Carroll and Janzen 1973, White 1978, Traniello et al. 1992). Conversely, the ants may be inefficient harvesters of nitrogen. There is little evidence for predatory behavior in *C. pennsylvanicus*, and competition for scavenged food items is also intense (Pricer 1908, Sanders 1972, Carroll and Janzen 1973, Fowler and Roberts 1980, Chapter 3). Because foods high in nitrogen are unpredictably dispersed in time and space, (Carroll and Janzen 1973), protein is hoarded in times of plenty. Excess protein is commonly stored as living tissue in larvae, which can be cannibalized in times of shortage (Dlussky and Kupianskaya 1972, Alexander 1974, Nonacs 1991)

In examinations of particle consumption, fluorescent microspheres of various sizes were fed to ants under social and solitary conditions. Ants were able to exclude 45 µm diameter microspheres from the crop through the filtering action of the external mouthparts and the infrabuccal pocket. Particles of 6 and 10 µm diameter were found in the crop, as well as on the mouthparts and in the infrabuccal pocket, indicating that filtering by those mechanisms is incomplete in that size range. When ants were fed microspheres 1 µm diameter or less, infrabuccal pellets were irregularly fluorescent and more difficult to extract. Thus, the infrabuccal pocket appears to be most effective at sequestering particles 3 µm diameter or greater.

When fed microspheres 0.05 - 10 µm diameter under both social and solitary conditions, the ants' crops remained replete with fluorescent particles even 20 hours post-feeding. As trophallaxis is thought to enhance filtration of the ingluvium (Eisner and Happ 1962), the prolonged retention of particles in the crops of isolated workers was anticipated. The high concentration of particles in the crops of ants in social groups was more surprising, but can be attributed to the conditions of the test. Since all (or nearly all) experimental animals fed at the test solutions, hungry nestmates with which a replete forager could engage in trophallaxis were lacking, thereby limiting opportunities for food exchange during the 20-hour post-feeding interval.

Despite their long retention in the crop, particles 3 - 10 µm diameter were never found in the mid- and hindgut, demonstrating the ability of the proventriculus to exclude particles of this size class. Particles 0.05 to 1 µm diameter, in contrast, were readily detected in the mid- and hindguts of both major and minor workers. These results strongly suggest that the efficiency of the proventriculus as a filtering mechanism breaks down between 1 and 3 µm. This threshold is undoubtedly correlated with the size of the sepal canals, which act as viaducts to the midgut. Diagrams prepared from morphological examinations of the proventriculus in *C. americanus*, a carpenter ant comparable in size to *C. pennsylvanicus*, show canals ranging from about 5 - 10 µm diameter (Eisner and Wilson 1952). The anatomy of the proventriculus in *C. pennsylvanicus* must be investigated to confirm this hypothesis.

Caste was shown to exert an effect only when 1 µm particles were consumed in small social groups. Under those conditions, fluorescence was detected in the mid- and hindguts of minor workers but not in major workers. One must be cautious in interpreting the results. It is most tempting to conclude that the filtering ability of the proventriculus is affected by caste. A relationship between caste and filtering ability has been demonstrated only once previously in ants: filtration of particles from the ingluvium is positively correlated with body size in the leaf-cutting ant, *A. octospinosus* (Quinlan

and Cherrett 1978). The present results suggest an inverse correlation between body size and filtering ability, however, the utility of which is not immediately apparent. An alternative explanation is that majors, which are less active than minors, can meet their energetic requirements with quantities of food so small that they cannot be detected over the natural fluorescence of the gut tissue.

These results demonstrate the complexity and efficiency with which *C. pennsylvanicus* filters particulate matter from the communal food supply. In general, particles 45 µm or greater are not ingested, particles 10 µm or greater are sequestered in the infrabuccal pocket, and particles 1 µm or greater are excluded from the midgut. The relevance is clear for innovative control strategies dependent on consumption, such as bait and microencapsulation technologies or some microbial control agents. The relevance for colony nutrition and the possible adaptive significance of the serial filtration system are less clear, but therefore more interesting. It seems possible that the colony is using its filtration system to achieve two ends: 1) to exclude abrasive matter from the midgut, which in this group is unprotected by a peritrophic membrane, and 2) to exploit the nutritional component of the material thus collected. Many potentially nutritious food sources, like pollen grains, fungal spores, and arthropod hemocytes, protein granules, and tissue fragments are at least 1 µm in size (Weber and Hess 1976)(Stanley and Linskens 1974)(Woodring 1985). The serial filtration mechanism of the colony can easily capture and concentrate particles of that dimension. To a colony having larvae able to digest solid food, such as *S. invicta* (Petralia and Vinson 1978, Glancey et al. 1981), and struggling with a chronic shortage of nitrogenous food, such particulate matter may represent a valuable food reservoir.

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