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# Summer and fall ants have different physiological responses to food macronutrient content

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ABSTRACT

Seasonally, long-lived animals exhibit changes in behavior and physiology in response to shifts in environmental conditions, including food abundance and nutritional quality. Ants are long-lived arthropods that, at the colony level, experience such seasonal shifts in their food resources. Previously we reported summer- and fall-collected ants practiced distinct food collection behavior and nutrient intake regulation strategies in response to variable food protein and carbohydrate content, despite being reared in the lab under identical environmental conditions and dietary regimes. Seasonally distinct responses were observed for both no-choice and choice dietary experiments. Using data from these same experiments, our objective here is to examine colony and individual-level physiological traits, colony mortality and growth, food processing, and worker lipid mass, and how these traits change in response to variable food protein–carbohydrate content. For both experiments we found that seasonality *per se* exerted strong effects on colony and individual level traits. Colonies collected in the summer maintained total worker mass despite high mortality. In contrast, colonies collected in the fall lived longer, and accumulated lipids, including when reared on protein-biased diets. Food macronutrient content had mainly transient effects on physiological responses. Extremes in food carbohydrate content however, elicited a compensatory response in summer worker ants, which processed more protein-biased foods and contained elevated lipid levels. Our study, combined with our previously published work, strongly suggests that underlying physiological phenotypes driving behaviors of summer and fall ants are likely fixed seasonally, and change circannually.

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## 1. Introduction

Macronutrients (protein, carbohydrates, and lipids) are the key drivers of growth and reproduction for most animals (Simpson and Raubenheimer, 2012), but often the individual foods available to foraging animals contain macronutrients in suboptimal amounts and/or ratios (Behmer and Joern, 2012). More studies are showing that many animal species regulate their macronutrient intake (Behmer, 2009; Simpson and Raubenheimer, 2012), and that a given species regulates its macronutrient intake depending on its immediate physiological demands (Clark et al., 2013; Maklakov et al., 2008). Shifts in seasonality (e.g., photoperiod, temperature, water availability) are accompanied by some degree of change in the quality and abundance of foods available to animals. For example, in temperate grasslands, in the spring and summer, herbivores encounter actively growing plants with high protein (p) and carbohydrate (c) content (Behmer and Joern, 2012; Lenhart et al., 2015). In contrast, as winter approaches, plants stop growing, and protein and carbohydrate content drops dramatically.

In response, animals are observed to respond, both behaviorally and physiologically, to these changes (Basson and Clusella-Trullas, 2015; Cook et al., 2011), which are often entrained to seasonal shifts in photoperiod, temperature, and associated environmental conditions (DeCoursey, 2004; Helm et al., 2013; Kumar et al., 2010), and that interact with circadian processes that operate on a broader timescale (i.e., circannually) (Danks, 2005; Saunders, 1977). For example, pregnancy or lactation in vertebrates is timed to periods when protein-rich foods are abundant (Bell et al., 2000; Dewey, 1997), while animals preparing to migrate or hibernate often encounter an abundance of high-energy food prior to periods of food dearth (Hernandez, 2009). However, prolonging nutrient availability past timing of these periods can delay expected behavioral and physiological responses (Humphries et al., 2003; Vuarin et al., 2015).

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For most animals the causal relationship between seasonal environmental cues, food abundance/quality, and changes in animal behavior and physiology remain unclear, in part because experiments that disentangle their individual effects are often difficult to conduct. Social insect colonies offer a number of characteristics that make them a tractable model for addressing this issue. First, a social insect colony has traits that can be considered analogous to a solitary animal. For instance, sterile workers and fertile queens are often compared to animal somatic and reproductive tissues, respectively (Seeley, 1995). Individual workers can be analogous to nutrient storage cells, holding lipid reserves or liquid carbohydrates (Hölldobler and Willson, 1990). These, and many other examples (Gillooly et al., 2010; Hölldobler and Wilson, 2009; Seeley, 1995; Shik et al., 2012) form the basis for many referring to a social insect colony as a 'superorganism' (Wheeler, 1928). Second, because an individual colony is often long-lived (Jemielity et al., 2005; Keller and Genoud, 1997), it will experience seasonal changes in photoperiod, temperature, and associated environmental conditions (e.g., changes in rainfall patterns), including associated shifts in the nutritional quality/abundance of food. Third, like solitary animals, social insect colonies practice selective feeding (Kay, 2004; Roces, 1990; Wilder and Eubanks, 2010), and tightly regulate their protein–carbohydrate intake (Cook and Behmer, 2010; Cook et al., 2010, 2012; Dussutour and Simpson, 2008, 2009). Ants utilize a broad range of different food types (Hölldobler and Willson, 1990), and fire ants exhibit seasonal changes in their behavior and physiology (Tschinkel, 1993) that are likely accompanied by shifts in food selection tied to regulating colony nutrient intake (Cook et al., 2011).

Previously we demonstrated fire ants collected in the summer and fall had distinct behavioral responses to food macronutrient variability that were independent of environmental cues (Cook et al., 2011). In that study polygynous field colonies were collected during summer and fall and brought to the lab, where they were then split into replicate experimental queen-right lab colonies standardized for size and demography. These experimental colonies were maintained under similar temperature, humidity, and light regimes, and both subjected to the same cafeteria-type experiments. The first experiment was a no-choice experiment, with colonies constrained to a single food with a fixed p:c ratio to observe how workers respond behaviorally to regulating colony nutrient intake when feeding on foods having protein–carbohydrate ratios that differ relative to self-selected p:c ratios. When the food p:c ratio was balanced (1:1), summer and fall colonies collected equal amounts of food. However, as the p:c ratio of the experimental foods became increasingly imbalanced summer colonies collected significantly more food. In contrast, fall colonies always collected the same amount of food, regardless of its p:c ratio. The second experiment was a choice experiment, which gauged self-selected p:c intake by providing colonies with nutritionally sub-optimal, but complimentary food-pairings. Both summer and fall colonies actively regulated their protein–carbohydrate intake and both selected carbohydrate-biased intake targets (~1:1.5). A significant observation made for both experiments was that summer ants consumed more carbohydrate when it was abundant relative to the protein need of the colony (Cook et al., 2011).

Following these findings, we predict summer and fall fire ants have distinct physiologies independent of environmental cues, underling their contrasting behavioral responses to variable food macronutrient content. Contrasting seasonal physiologies, either apart from or combined with nutrient deficits or excesses experienced by summer or fall colonies in our previous experiments (above), are predicted to have consequences for colony performance. The directionality of consequences are predicted to align based on the nutritional needs of summer and fall fire ant colonies outlined previously (Cook et al., 2011). Here, using data from those same experiments, we test these predictions by comparing changes in workforce and brood biomasses, worker mortality and lipid content, and how summer and fall colonies manipulated unconsumed foods having variable macronutrient content.

## 2. Materials and methods

### 2.1. Experimental fire ant colonies

For a more thorough description of methods used to establish lab fire ant colonies for the experiments detailed below, see Cook et al. (2011). Briefly, six polygynous summer and fall colonies were collected between June 5 and June 15, 2009, and between October 20 and October 31, 2009, respectively. Colonies were returned to the lab, and a drip-floatation method (Banks et al., 1981) was employed to remove workers, brood, and queens from nest soil. Experimental colonies consisted of a single wingless queen, 1000 mg (~500) workers (haphazardly chosen), 200 mg larvae, and 100 mg pupae. Larvae and pupae included in experimental colonies ranged in size and developmental stage, but larvae and pupae of reproductives were purposely avoided. To control for field colony effects, for each of the six colonies collected, one experimental colony (above) was randomly allocated to each of the eight dietary treatments (see below). Simultaneously, a subset of workers was haphazardly collected from each source colony for later lipid extraction (see below).

Individual experimental colonies were housed in a 24.6 cm × 19.2 cm × 9.5 cm plastic box, provided with a 15 cm diameter lidded and covered Petri dish filled approximately half-full with hardened Castone® dental stone as an artificial nest; the Castone® substrate was moistened regularly to maintain high humidity inside nest chambers. Water was provided *ad libitum*. Colonies were housed in an insectary under a 12 h:12 h L:D diel cycle (using fluorescent lighting), and kept at 26 °C and with ambient humidity (45–60%).

### 2.2. Experimental foods

Experimental foods consisted of five agar-based synthetic foods, which contained the same quantities of micronutrients, salts, vitamins, and lipids from whole egg powder, but varied in protein and carbohydrate content (Cook et al., 2011). The foods' protein component consisted mainly of a 1:1 mixture of whey protein and casein, and the carbohydrate component was sucrose. The following combinations of protein and carbohydrate, expressed as percentage of total food dry weight, represent the p:c ratios of the five experimental foods: (1) 54% protein and 18% digestible carbohydrate (p54:c18), (2) p42:c32, (3) p37:c37, (4) p33:c43, and (5) p19:c57 (see Table S1).

### 2.3. Experimental protocol

#### 2.3.1. Food collection and consumption

No-choice and choice experiments were performed simultaneously over a five-week period. In the no-choice experiment, experimental ant colonies were restricted to one of the five diets. In the choice experiment, ant colonies were presented with one of three food pairings: (1) p42:c32 with p19:c57, (2) p19:c57 with p54:c18, and (3) p33:c43 with p54:c18. Colonies were provided with fresh food (1 $cm^3$ cubes, centered in a small plastic weighing dish), every day, for five weeks. Any food remaining after 24 h was removed, dried for 48 h at 40 °C, and weighed. The daily amount of food collected was determined by subtracting the weight of remaining dried food from the food's initial dry weight. Food's initial dry

weight was calculated using the equation from a linear regression of the wet and dry weights of 20 replicate 1 $cm^3$ cubes of experimental foods.

*2.3.2. Colony performance*

In both experiments, dead workers were collected weekly from each colony by carefully aspirating corpses; worker heads were counted under a stereoscope to obtain total number of dead workers. Statistical analysis of worker mortality was conducted using data from weeks one, three, and five. At the end of each experiment, workers and total brood (separated into larvae and pupae under a stereoscope) were collected and weighed. In analyses, these values were compared against the weights of workers, larvae, and pupae of each colony at the start of experiments.

*2.3.3. Worker lipid content*

At the end of each experiment (5 weeks), approximately 50–100 workers were haphazardly collected from total amassed workers into vials containing silica gel desiccant, and dried to constant weight. Lipids were extracted from these samples, and also from samples of source colony workers (above). Whole-body lipids were extracted by soaking each sample three times for 24 h in chloroform. After the third soak, workers were re-dried and weighed. Worker lipid content was expressed as mg lipid/mg dry body mass, by subtracting the lipid free dry weight from the dry weight prior to lipid extraction. As a measure for the change in worker lipid content, the lipid content of workers taken from experimental colonies after five weeks was compared to the lipid content of source colony workers.

*2.3.4. Manipulation of food macronutrient content*

For many of the experimental colonies we observed that not all foods collected were consumed. Furthermore, these uneaten foods appeared as two distinct types: (1) hoarded and (2) discarded. Hoarded food was usually found inside the nest chamber, was similar in color to unmanipulated foods, and was course in texture. In contrast, discarded food was invariably found outside the nest chamber. Additionally, discarded food was typically lighter in color and had a finer texture. At the end of the experiments, both hoarded and discarded foods were collected from colonies, dried thoroughly, and weighed to the nearest 0.01 mg. They, as well as dried samples of experimental foods, were ground to a fine powder in liquid nitrogen, and then re-dried. Protein and carbohydrate were extracted and assayed using spectrophotometry as reported previously (Cook et al., 2011). In analyses, we compared the p:c ratios of hoarded and discarded foods to the p:c ratio of single foods (no-choice), and that of food pairings (choice).

*2.4. Statistical analyses*

Prior to statistical analyses, we determined whether data were normally distributed and had equal variances across groups using the Shapiro–Wilks test and O'Brien test, respectively. If data did not meet these criteria, they were transformed (signified in text). If common data transformations did not meet these criteria, data were analyses using non-parametric tests. For measures of colony performance, we used two-factor repeated measures ANOVA to test for significant effects of season, and diet treatment on worker mortality. We used two-factor ANOVA to test for significant effects of these same factors on post-experiment worker lipid content, the change in worker lipid content, and also for measures of total worker and brood mass. We used separate *t*-tests to determine between-group differences in the change in worker lipid content. For the no-choice experiments, we used nominal logistic regression of proportional data to examine the tendency for summer and fall colonies to either hoard or discard foods based on food p:c ratio. To determine the extent to which feeding ants manipulated the p:c ratio of collected no-choice and choice foods we used separate non-parametric Wilcoxon tests that compared the mean p:c ratio of hoarded and discarded food to the known p:c ratio of unmanipulated experimental foods. Where applicable, appropriate post hoc tests were conducted to determine within-group differences. All analyses were conducted using the software package Jump 7.02 (SAS Institute, Inc.).

## 3. Results

*3.1. No-choice experiment*

*3.1.1. Colony Performance*

Season significantly affected most measures of colony performance, but not food macronutrient content, or an interaction between the two factors (Table 1). Total worker mortality was significantly higher in summer colonies (Fig. 1A), but the mean total worker mass remaining after five weeks was similar for summer and fall colonies (Fig. 1B). For colonies having brood (larvae and/or pupae) at the end of the experiments (80% and 100% in summer and fall colonies, respectively), total brood mass was significantly greater in summer colonies (Fig. 1C).

*3.1.2. Worker lipid content*

Workers from summer source colonies contained more lipids than workers from fall source colonies at the start of the experiment ($F_{1,34} = 41.56$, $P < 0.001$; Table S2). At the end of the experiment lipid content was similar for summer and fall workers (Table 2), although workers from summer colonies feeding on relatively carbohydrate-rich foods tended to have higher lipid content (Fig. 2A; Table S2). The change in worker lipid content was significantly affected by season only (Table 2); fall workers increased their lipid levels after five weeks, and workers of summer colonies tended to maintain body lipids at source worker levels (Fig. 2B; Table S2).

*3.1.3. Manipulation of food macronutrient content*

Eighty and sixty percent of summer and fall colonies, respectively contained hoarded foods, and 57% and 66% contained discarded foods; sets of percentages were statistically similar ($X^2 = 0.89$, df = 1, $P = 0.345$, and $X^2 = 1.76$, df = 1, $P = 0.185$, respectively). The mean (± s.e.m) quantities of both hoarded and discarded foods were significantly greater in summer colonies

**Table 1**
Performance of colonies from no-choice experiment. Results from two-way ANOVA testing the effect of season and food p:c ratio on total mortality, total worker mass, and brood mass (larvae and pupae) after five weeks.

| Variable | Source | df | *F*-ratio | *P*-value |
|---|---|---|---|---|
| | *Between-factors* | | | |
| Worker mortality | Season | 1 | 40.37 | <0.001 |
| | Food p:c ratio | 1 | 1.16 | 0.342 |
| | Season-by-Food p:c ratio | 1 | 0.84 | 0.506 |
| | *Within-factors* | | | |
| Worker mortality | Time | 2 | 10.28 | <0.001 |
| | Time-by-Season | 2 | 3.67 | 0.033 |
| | Time-by-Food p:c ratio | 8 | 0.56 | 0.807 |
| | Time-by-Season-by-Food p:c ratio | 8 | 1.28 | 0.261 |
| Worker mass (mg) | Season | 1 | 0.55 | 0.460 |
| | Food p:c ratio | 4 | 1.21 | 0.317 |
| | Season-by-Food p:c ratio | 4 | 0.62 | 0.651 |
| Brood mass (mg) | Season | 1 | 4.55 | 0.038 |
| | Food p:c ratio | 4 | 0.72 | 0.582 |
| | Season-by-Food p:c ratio | 4 | 1.54 | 0.207 |

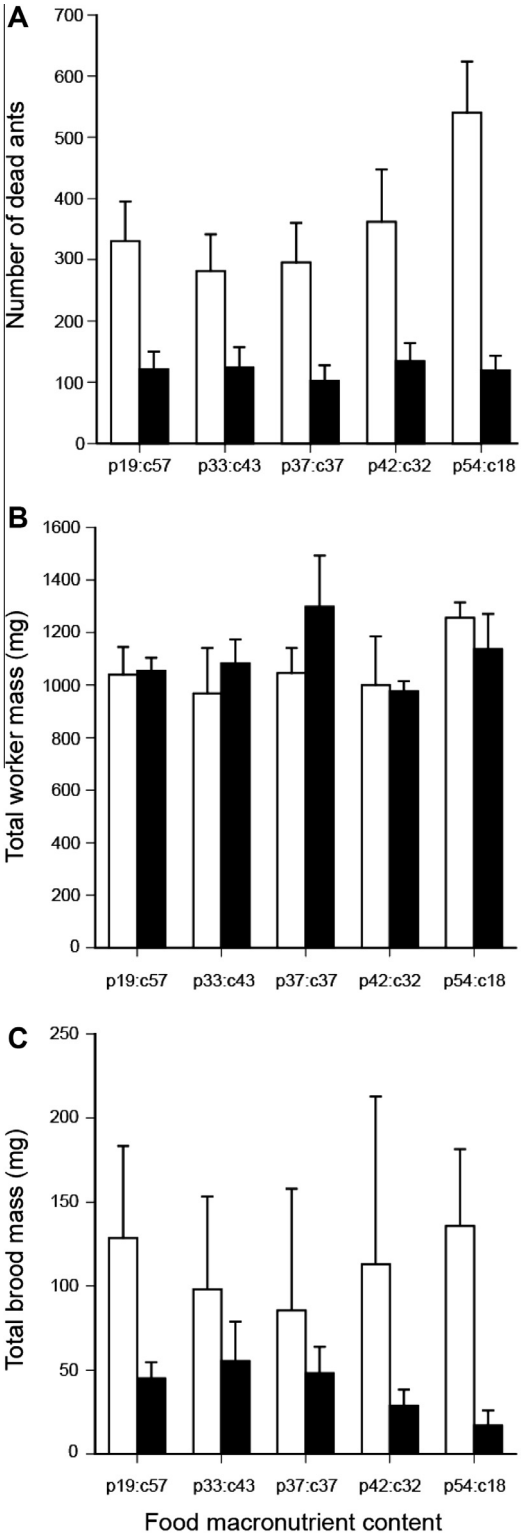


**Fig. 1.** Performance of colonies from no-choice experiment. (A) Mean (± s.e.m.) weekly number of dead ants, (B) mean (± s.e.m.) mass of total workers, and (C) mean (± s.e.m.) mass of total brood (larvae + pupae) at the end of the experiment (5 weeks). Summer collected (open columns) and fall collected (filled columns) ants were kept for 5 weeks on diets with different protein–carbohydrate (p:c) ratios (e.g., p19:c57 = 19% protein and 57% carbohydrate); total macronutrient content was similar across the diets (72–76%).

**Table 2**
Lipid content of workers from no-choice experiment. Results from two-way ANOVA testing the effects of season and food p:c ratio on worker lipid content, and change in lipid content after five weeks.

| Variable | Source | df | *F*-ratio | *P*-value |
|---|---|---|---|---|
| Worker lipid levels | Season | 1 | 2.98 | 0.090 |
| | Food p:c ratio | 4 | 2.13 | 0.091 |
| | Season-by-Food p:c ratio | 4 | 2.60 | 0.047 |
| Change in worker lipids | Season | 1 | 5.41 | 0.024 |
| | Food p:c ratio | 4 | 0.87 | 0.486 |
| | Season-by-Food p:c ratio | 4 | 1.22 | 0.312 |

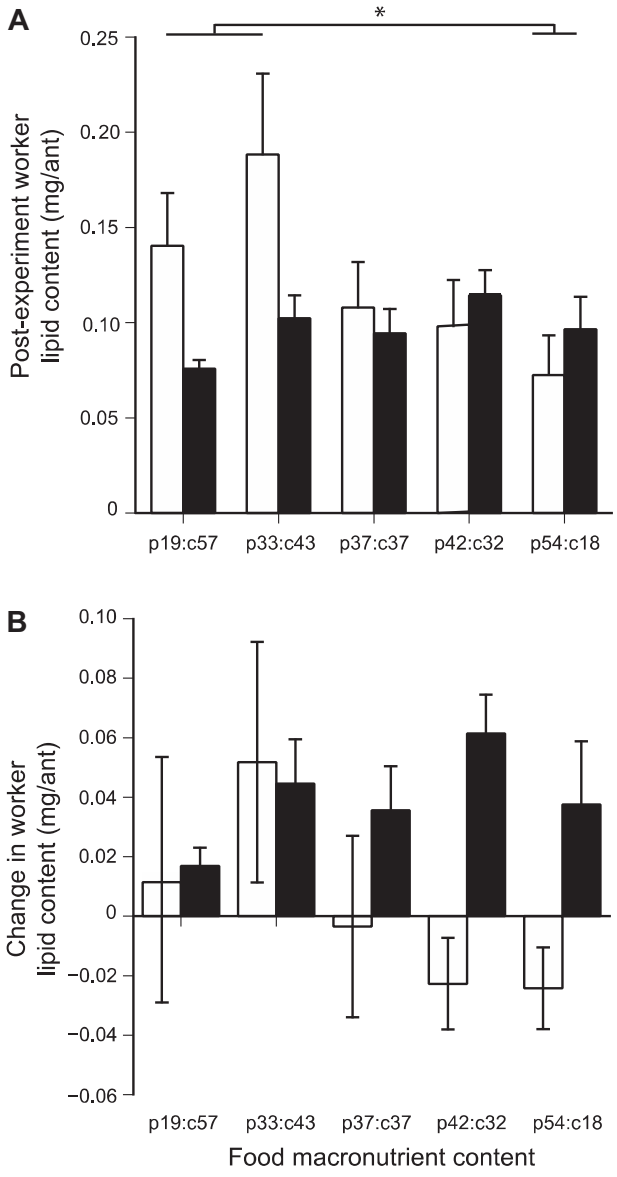


**Fig. 2.** Lipid content of workers from choice experiment. (A) Mean (± s.e.m.) lipid content, and (B) mean (± s.e.m.) change in lipid, at the end of the experiment. Summer collected (open columns) and fall collected (filled columns) ants were kept for 5 weeks on diets with different protein–carbohydrate (p:c) ratios (e.g., p19:c57 = 19% protein and 57% carbohydrate); total macronutrient content was similar across the diets (72–76%). Horizontal line connects treatment groups differing significantly in Student's post hoc tests.

(Fig. 3A and B), but other factors did not significantly affect these quantities (Table 3). The p:c ratios of hoarded foods from summer and fall colonies were similar (Table 3), and both closely matched the p:c ratio of experimental foods (Fig. 3C; Table S3). The p:c ratio of discarded foods from summer or fall colonies were similar (Table 3), but increased significantly as food p:c ratio increased (Fig. 3D; Table S3).

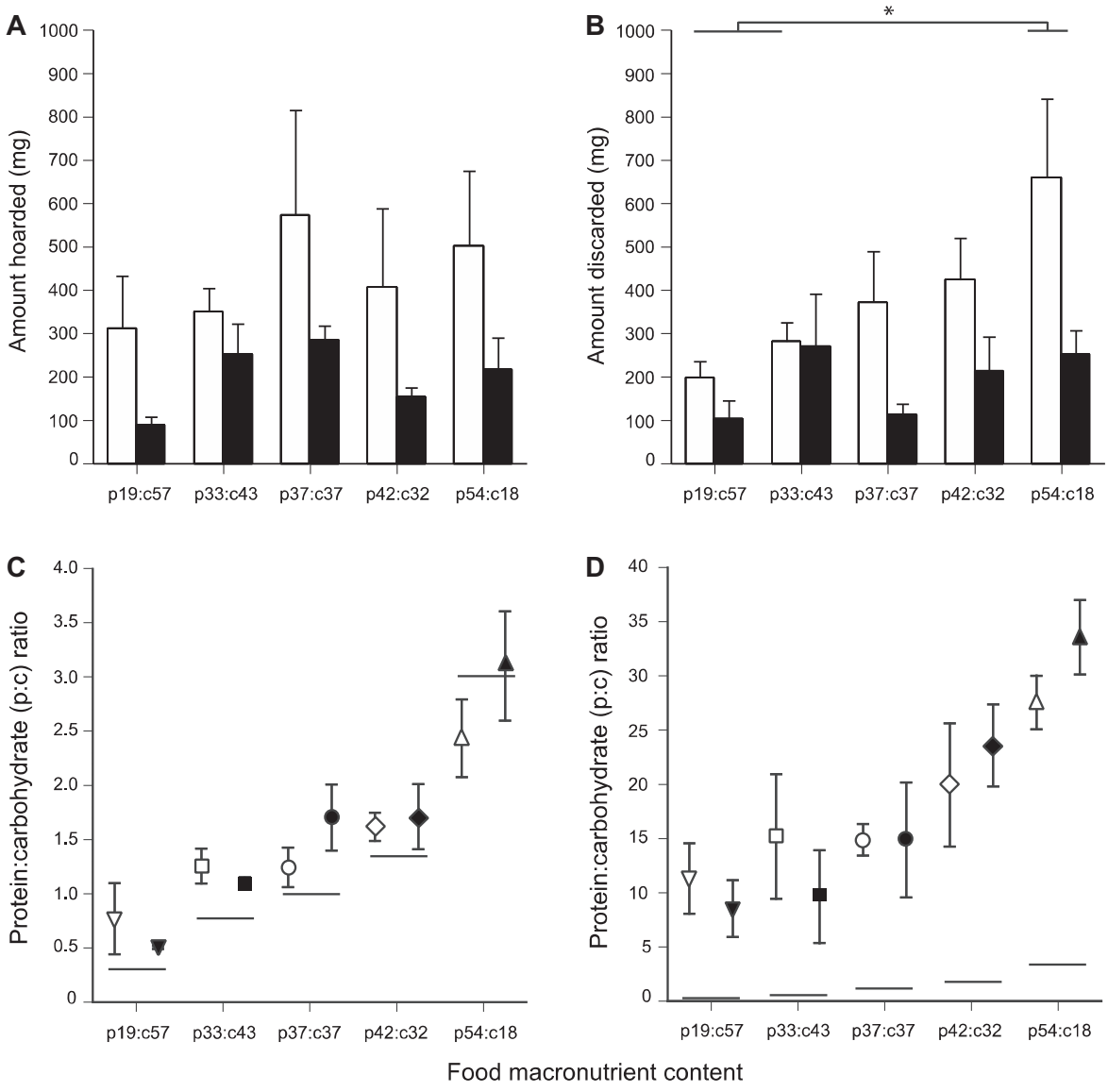


**Fig. 3.** Manipulation of macronutrient content of no-choice foods. (A) Mean (± s.e.m.) amounts of hoarded food (unconsumed food piled inside of nests), (B) mean (± s.e.m.) amounts of discarded foods (unconsumed food kept piled outside the nest), (C) mean (± s.e.m.) protein–carbohydrate (p:c) ratio of hoarded foods, and (D) mean (± s.e.m.) p:c ratio of discarded food. Summer collected (open columns) and fall collected (filled columns) ants were kept for 5 weeks on diets with different protein–carbohydrate (p:c) ratios (e.g., p19:c57 = 19% protein and 57% carbohydrate); total macronutrient content was similar across the diets (72–76%). For reference, the p:c ratio for each experimental food is shown as a horizontal line. Horizontal line connecting treatment groups indicates significant differences in Student's post hoc tests.

### 3.2. Choice experiment

#### 3.2.1. Colony performance

Only season significantly affected measures of colony performance (Table 4). Worker mortality was almost three times greater in summer colonies (Fig. 4A), and after five weeks mean total worker mass was significantly less in summer colonies (Fig. 4B). For colonies having brood (larvae and/or pupae) at the end of the experiments (72% and 100% in summer and fall colonies, respectively), total brood mass was similar for summer and fall colonies (Fig. 4C).

#### 3.2.2. Worker lipid content

Workers from summer source colonies contained more lipids than workers from fall source colonies at the start of the experiment ($F_{1,34} = 41.56$, $P < 0.001$; Table S2). Post-experiment worker lipid content was similar between summer and fall workers (Fig. 5A), but was significantly lower in workers from colonies feeding on the protein-biased food pairing (Table 5). The change in lipid content was similar for summer and fall workers (Fig. 5B), and nutrient content of food pairings did not significantly affect the change in worker lipids in either summer or fall colonies (Table 5). Summer workers maintained lipid content at source colony levels, but fall worker significantly increased lipid content relative to source colony levels on all except the protein-biased food pairing (Table S4).

#### 3.2.3. Manipulation of food-pairing macronutrient content

Forty-four and seventy-two percent of summer and fall colonies, respectively contained hoarded foods, and 94% and 62% contained discarded foods; sets of percentages were statistically similar ($X^2 = 2.90$, df = 1, $P = 0.089$, and $X^2 = 0.01$, df = 1, $P = 0.994$, respectively). The mean total quantity of hoarded food was similar in summer and fall colonies (Fig. 6A), and other factors did not affect the quantity of foods hoarded (Table 6). In contrast, the mean total quantity of discarded food was significantly greater in

**Table 3**
Manipulation of macronutrient content of no-choice foods. Results from two-way ANOVA testing the effects of season and food p:c ratio on quantities of hoarded and discarded foods, and P:C ratio of hoarded and discarded food, after five weeks.

| Variable | Source | df | *F*-ratio | *P*-value |
|---|---|---|---|---|
| Amount of hoarded food | Season | 1 | 6.30 | 0.017 |
| | Food p:c ratio | 4 | 1.28 | 0.299 |
| | Season-by-Food p:c ratio | 4 | 0.37 | 0.826 |
| Amount of discarded food | Season | 1 | 14.10 | <0.001 |
| | Food p:c ratio | 4 | 2.02 | 0.096 |
| | Season-by-Food p:c ratio | 4 | 0.09 | 0.883 |
| P:C ratio of hoarded food | Season | 1 | 0.04 | 0.837 |
| | Food p:c ratio | 4 | 19.71 | <0.001 |
| | Season-by-Food p:c ratio | 4 | 1.41 | 0.251 |
| P:C ratio of discarded food | Season | 1 | 0.22 | 0.645 |
| | Food p:c ratio | 4 | 10.57 | <0.001 |
| | Season-by-Food p:c ratio | 4 | 3.27 | 0.024 |

**Table 4**
Performance of colonies from choice experiment. Results from two-way ANOVA testing the effect of season and food p:c ratio on total mortality, total worker mass, and brood mass (larvae and pupae) after five weeks.

| Variable | Source | df | *F*-ratio | *P*-value |
|---|---|---|---|---|
| Worker mortality | Season | 1 | 4.05 | <0.001 |
| | Food pairing | 2 | 0.03 | 0.898 |
| | Season-by-Food p:c ratio | 2 | 0.03 | 0.896 |
| Worker mass | Season | 1 | 6.21 | 0.018 |
| | Food pairing | 2 | 0.38 | 0.684 |
| | Season-by-Food p:c ratio | 2 | 1.11 | 0.343 |
| Brood mass | Season | 1 | 1.07 | 0.310 |
| | Food pairing | 2 | 1.26 | 0.299 |
| | Season-by-Food p:c ratio | 2 | 0.48 | 0.620 |

**Table 5**
Lipid content of workers from choice experiment. Results from two-way ANOVA testing the effects of season and food p:c ratio on worker lipid content, and change in lipid content after five weeks.

| Variable | Source | df | *F*-ratio | *P*-value |
|---|---|---|---|---|
| Worker lipid levels | Season | 1 | 0.86 | 0.360 |
| | Food pairing | 2 | 4.24 | 0.024 |
| | Season-by-Food p:c ratio | 2 | 0.11 | 0.898 |
| Change in worker lipids | Season | 1 | 1.85 | 0.184 |
| | Food pairing | 2 | 2.43 | 0.105 |
| | Season-by-Food p:c ratio | 2 | 0.08 | 0.925 |

summer colonies (Fig. 6B), but other factors did not significantly affect the quantity of discarded foods (Table 6). The p:c ratio of hoarded foods in summer and fall colonies did not differ, and were similar to those of experimental foods (Fig. 6C; Table S5). The p:c ratio of discarded foods from summer and fall colonies was also similar (Fig. 6D), but the p:c ratio of discarded foods from summer colonies fed the carbohydrate-biased food pairing was significantly higher than that of the experimental food pairing (Table S5).

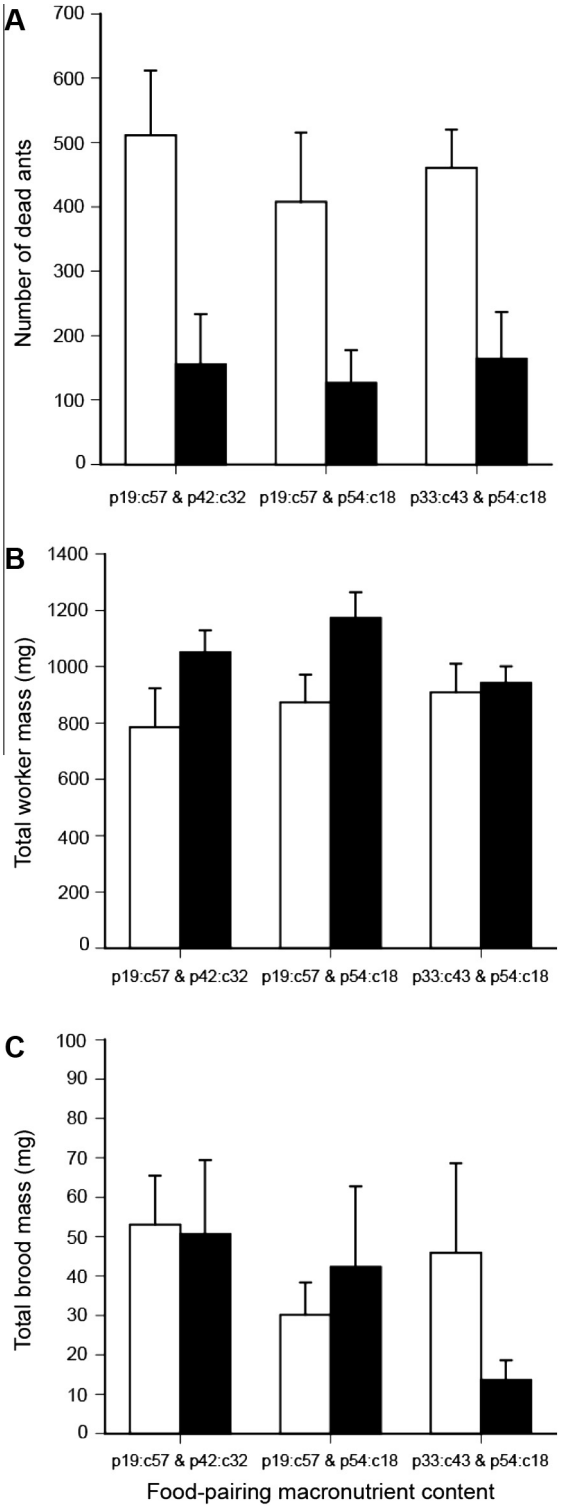


**Fig. 4.** Performance of colonies from choice experiment. (A) Mean (± s.e.m.) total number of dead ants, (B) mean (± s.e.m.) mass of total workers, and (C) mean (± s.e.m.) mass of total brood (larvae + pupae) at the end of the experiment (5 weeks). Summer collected (open columns) and fall collected (filled columns) ants were kept for 5 weeks on diets with different protein–carbohydrate (p:c) ratios (e.g., p19:c57 = 19% protein and 57% carbohydrate); total macronutrient content was similar across the diets (72–76%).

## 4. Discussion

The objective of our study was to test if the underlying physiologies of summer and fall fire ants differ, and whether this distinction, either apart or together with variable food protein–carbohydrate content, has consequences for colony performance. Results for most measured variables show highly significant seasonal effects and only marginal dietary effects. This suggests that behavioral and physiological characteristics of ants may be seasonally fixed. In some instances a dietary effect was observed. In such

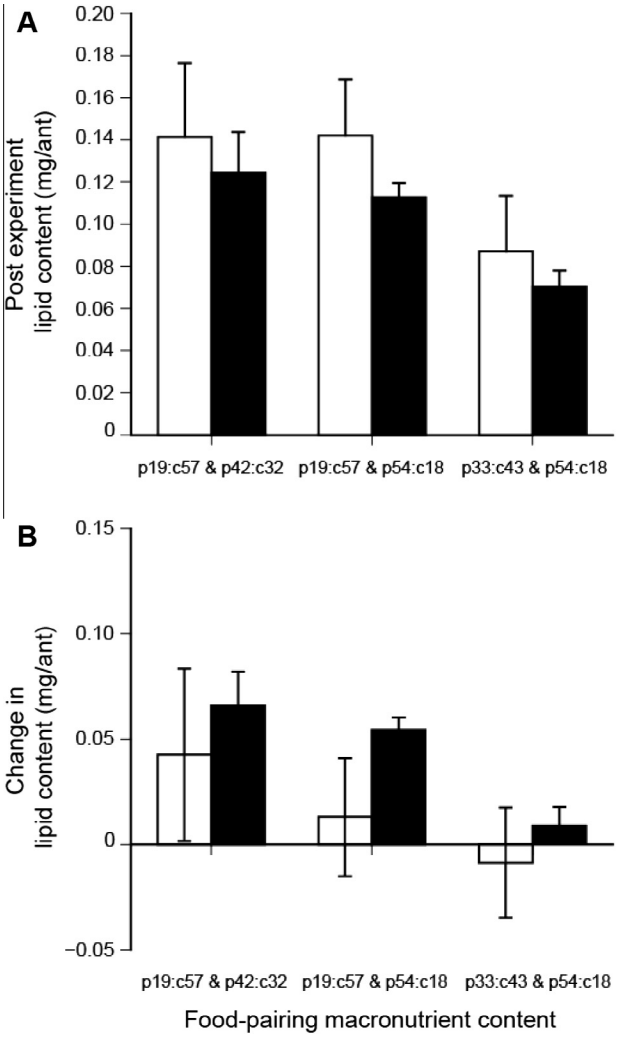


**Fig. 5.** Lipid content of workers from choice experiment. (A) Mean (± s.e.m.) lipid content, and (B) mean (± s.e.m.) change in lipid, at the end of the experiment. Summer collected (open columns) and fall collected (filled columns) ants were kept for 5 weeks on diets with different protein–carbohydrate (p:c) ratios (e.g., p19:c57 = 19% protein and 57% carbohydrate); total macronutrient content was similar across the diets (72–76%).

cases we refer to results reported here for food manipulation, and those from our previous study (Cook et al., 2011).

One of our most notable findings was the much higher mortality of summer ants, dying approximately three times faster than fall ants, despite both experiencing equivalent environmental conditions and diets. Additionally, food collection and consumption likely did not affect mortality rates; in the no-choice experiment summer and fall colonies collected and consumed very similar amounts of the p37:c37 food, yet worker mortality remained significantly higher in summer colonies. This suggests the metabolic activity is greater in summer ants than fall ants, independent of temperature and nutritional effects. It is not likely that summer and fall ant metabolic rates converged to correlate with the standard experimental temperature of 26 °C, since fire ant respiration temperature coefficient ($Q_{10}$) is stable across a range of temperatures (Vogt and Appel, 1999). Also, several other ant species' metabolic rates were unchanged by shifts in temperature (Mackay and Sassaman, 1984). In our lab studies higher metabolism of summer ants may be inferred through increased food collection (Cook et al., 2011), and consumption of large quantities of carbohydrate when abundant. A colony's workforce creates its engine, which for summer colonies appears at 'high rev' primed to take advantage of increased foraging opportunities and resource availability during those months. For insects, high mass-specific metabolic rate can lead to elevated oxidative stress (De Block and Stoks, 2008), which could shorten longevity (Hulbert et al., 2007; Shik et al., 2012), which for ant colonies, could result in reduced total colony size (Bockoven et al., 2015; Gillooly et al., 2010; Shik et al., 2012). In ants however, consumption of excess carbohydrate confers positive effects on worker longevity (Cook et al., 2010; Dussutour and Simpson, 2009; Konrad et al., 2009), perhaps conferred indirectly *via* other physiological pathways affected by high carbohydrate consumption (Corona et al., 2007; Vafopoulou, 2014). Our results show this for the first week, but over time mortality rose sharply. As for summer ants feeding on the p57:c19 food, their mortality was trending higher than others, but perhaps if our experiment continued for an additional two weeks we might have seen significantly higher mortality in colonies feeding on this food (Cook et al., 2010; Dussutour and Simpson, 2009, 2012). The finding that all ants that have so far been studied with respect to protein–carbohydrate regulation demonstrate carbohydrate-biased intakes (Cook and Behmer, 2010; Cook et al., 2010, 2011; Dussutour and Simpson, 2009, 2012) suggests there is a strong functional significance to individual ants limiting their protein intake.

Interestingly, despite having much higher worker mortality, summer lab colonies on average maintained their initial biomass, which after five weeks was not significantly different than that of fall colonies. In other words, summer colonies had a high rate of worker turnover, with the queen's reproductive output matching losses to mortality in many cases. Brood biomass was significantly greater in summer colonies, corresponding with bursts in colony somatic growth and production of sexual alates in many natural colonies of resident ant species (Børgesen, 2000; Ricks and Vinson, 1972; Tschinkel, 1993, 1999), suggesting a high metabolic output of colonies as a whole during summer. In our study, high variability in brood mass of summer colonies from no-choice experiment precluded insights to how fire ant queens might respond physiologically to variable food macronutrient content. For all no-choice foods, queens of some replicate lab summer colonies were highly fecund, but others were not. This high variability may be reflective of the wide range of persistent growth potentials observed in field-collected fire ant source colonies (Bockoven et al., 2015). Through a more active mechanism, some data suggest that an influx of weakly or non-inseminated fire ant queens can occur during spring and early summer seasons, and by fall and into winter seasons, the number of these queens dwindles (Glancey and Lofgren, 1988; Goodisman and Ross, 1999). This may explain the much higher and lower variability in brood masses of summer and fall lab colony replicates, respectively Moreover, a higher reproductive output of summer over fall fire ant queens may be explained by an endogenous, seasonal cycle of egg production (Ricklefs and Wikelski, 2002), similar to that observed for queens of several other temperate ant species (Kipyatkov and Lopatina, 2009).

As fall approaches it makes evolutionary sense that long-lived social insect colonies, similar to solitary animals, halt reproduction and slow growth in favor of sequestration of resources, making them available when the environment is lacking. In our study, hoarding by lab colonies likely represents foods in different stages of processing rather than true food hoarding/stashing. However, aside from external food storage, several ant species can sequester excess ingested carbohydrates as lipid reserves (Cook et al., 2010; Hahn, 2006), and for social insects generally, seasonal nutrient storage is a central characteristic of colony life history (Børgesen, 2000; Hunt and Nalepa, 1994; Tschinkel, 1993, 1999). In contrast to storage, during period of carbohydrate dearth stored lipids may be mobilized and used as an energy source *via* lipolysis

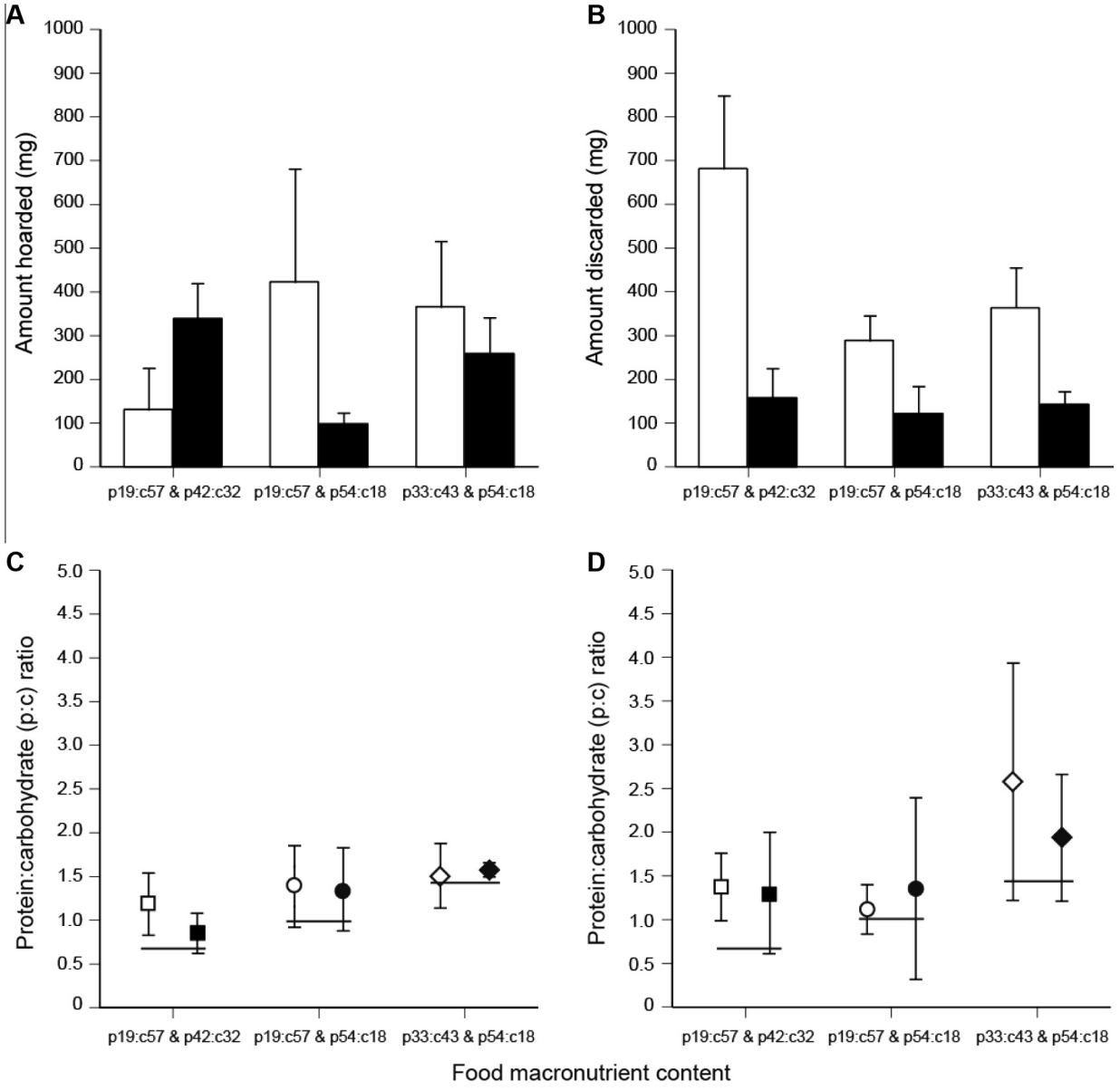


**Fig. 6.** Manipulation of macronutrient content of choice food-pairings. (A) Mean (± s.e.m.) amounts of hoarded food (unconsumed food piled inside of nests), (B) mean (± s.e.m.) amounts of discarded foods (unconsumed food kept piled outside the nest), (C) mean (± s.e.m.) protein–carbohydrate (p:c) ratio of hoarded foods, and (D) mean (± s.e.m.) p:c ratio of discarded food. Summer collected (open columns) and fall collected (filled columns) ants were kept for 5 weeks on diets with different protein–carbohydrate (p:c) ratios (e.g., p19:c57 = 19% protein and 57% carbohydrate); total macronutrient content was similar across the diets (72–76%). For reference, the p:c ratio for each experimental food is shown as a horizontal line.

(Arrese and Soulages, 2010). Summer ants feeding on more protein-biased no-choice foods appeared to use large amounts of lipids stores for this purpose, while some summer ants feeding on carbohydrate-rich no-choice foods gained lipid mass. In contrast fall ants feeding even on protein-rich no-choice foods gained lipid mass, suggesting that lipogenic processes are favored in fall fire ant colonies, or that lower metabolic rate of these ants favored lipid gain, even on low carbohydrate foods. High variability in the change of summer ant lipid content precluded a significantly different physiological response across no-choice treatments. What underlying mechanisms could help explain this observation? Similar to honeybees (Toth et al., 2005; Toth and Robinson, 2005) but not some bumblebees (Couvillon et al., 2011), ant species show a decrease in worker lipid content during behavioral ontogeny in the social context; younger, more corpulent 'nurse' ants are located near brood underground (Blanchard et al., 2000; Tschinkel, 1987), and likely have different physiologies (Hölldobler and Willson, 1990). Mixed-aged members of summer lab colonies may have responded differently to nutrient excesses/deficits. Fall ants did not show this trend; perhaps fall colonies during sequestration, are comprised of a similarly aged cohort of long-lived workers, similar to what is observed for 'winter' honeybees (Amdam and Omholt, 2002; Mattila et al., 2001), or all workers reduce foraging activities, compelled by underlying physiological mechanisms (Ricklefs and Wikelski, 2002).

A striking observation for fall lab colonies reported in our previous study (Cook et al., 2011) was that they collected very similar amounts of no-choice foods, not compensating behaviorally like summer lab colonies to food variable macronutrient content. All foods had the same amount of lipids; perhaps fall colonies were regulating intake of lipids rather than protein and carbohydrates. A mechanism used by diapausing insects to overwinter involves

**Table 6**
Manipulation of macronutrient content of choice food-pairings. Results from two-way ANOVA testing the effects of season and food p:c ratio on quantities of hoarded and discarded foods, and P:C ratio of hoarded and discarded food, after five weeks.

| Variable | Source | df | *F*-ratio | *P*-value |
|---|---|---|---|---|
| Amount of hoarded food | Season | 1 | 0.06 | 0.807 |
| | Food pairing | 2 | 0.72 | 0.493 |
| | Season-by-Food p:c ratio | 2 | 1.43 | 0.255 |
| Amount of discarded food | Season | 1 | 16.10 | <0.001 |
| | Food pairing | 2 | 2.23 | 0.125 |
| | Season-by-Food p:c ratio | 2 | 0.54 | 0589 |
| P:C ratio of hoarded food | Season | 1 | 1.85 | 0.194 |
| | Food pairing | 2 | 4.44 | 0.032 |
| | Season-by-Food p:c ratio | 2 | 0.30 | 0.404 |
| P:C ratio of discarded food | Season | 1 | 0.30 | 0.587 |
| | Food pairing | 2 | 3.90 | 0.036 |
| | Season-by-Food p:c ratio | 2 | 0.19 | 0.829 |

active substitution of saturated lipid components into cellular membranes (Kostal and Simek, 1998). This inherently lowers metabolic rate and oxidative tissue damage, thereby aiding increased longevity (Hulbert et al., 2007; Jemielity et al., 2005; Keller and Genoud, 1997). Lipids in experimental diets were constituents of whole egg powder, which contains both abundant saturated and unsaturated fatty acids.

Finally, protocols having standardized environmental conditions and foods allowed our experiments to disentangle the separate effects of environmental cues and macronutrient availability on fire ant seasonal behavior and physiology. It is clear through this and our previous study (Cook et al., 2010) that the behavioral responses of summer and fall fire ants are driven by underlying physiologies running at different times on an endogenous, circannual biological clock. Macronutrient availability had only transient effects on measured responses, except for elevated worker lipids and amounts of discarded foods, both of which were related to increased carbohydrate consumption (Vuarin et al., 2015). Interestingly, *Solenopsis invicta* is native to South America, as are other highly motile ant species (e.g., *Linepithema humile*, the Argentine ant) (McGlynn, 1999), and has become ecologically dominant in invasive habitats and regions of the Northern Hemisphere (Holway, 1999; Porter and Savignano, 1990). A feature of fire ant colonies in their invasive range is the production of an early batch of winter brood, which may aid their ecological dominance (Tschinkel, 2006). Interestingly, summer and winter collected fire ant queens were similar in some aspects of their physiology, both contrasting with those of spring and fall collected queens (authors' unpubl. data). Conducting studies similar to those described herein with fire ants in their native range would be of importance for understanding the relative strengths of proximate and ultimate forces shaping circannual behavior and physiology.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2016.02.001.

## References


Amdam, G.V., Omholt, S.W., 2002. The regulatory anatomy of honeybee lifespan. J. Theor. Biol. 216, 209–228.

Arrese, E.L., Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55, 207.

Banks, W.A., Lofgren, C.S., Jouvenaz, D.P., Stringer, C.E., Bishop, P.M., Williams, D.F., Wojcik, P.D., Glancey, B.M., 1981. Techniques for collecting, rearing, and handling imported red fire ants. U.S. Dept. Agric. Tech. AAT-S-21, 1–9.

Basson, C.H., Clusella-Trullas, S., 2015. The behavior-physiology nexus: behavioral and physiological compensation are relied on to different extents between seasons. Physiol. Biochem. Zool. 88, 384–394.

Behmer, S.T., 2009. Animal behaviour: feeding the superorganism. Curr. Biol. 19, R366–R368.

Behmer, S.T., Joern, A., 2012. Insect herbivore outbreaks viewed through a physiological framework: insights from orthoptera. Insect Outbreaks Revisited, 1–29.

Bell, A.W., Burhans, W.S., Overton, T.R., 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proc. Nutr. Soc. 59, 119–126.

Blanchard, G.B., Orledge, G.M.F.A.U.R., Reynolds, S.E.F.A.U.F., Franks, N.R., 2000. Division of labour and seasonality in the ant Leptothorax albipennis: worker corpulence and its influence on behaviour. Anim. Behav. 59, 723–738.

Bockoven, A.A., Wilder, S.M., Eubanks, M.D., 2015. Intraspecific variation among social insect colonies: persistent regional and colony-level differences in fire ant foraging behavior. PLoS ONE 10 e0133868.

Børgesen, L.W., 2000. Nutritional function of replete workers in the pharaoh's ant, Monomorium pharaonis (L.). Insect Soc. 47, 141–146.

Clark, R.M., McConnell, A., Zera, A.J., Behmer, S.T., 2013. Nutrient regulation strategies differ between cricket morphs that trade-off dispersal and reproduction. Funct. Ecol. 27, 1126–1133.

Cook, S.C., Behmer, S.T., 2010. Macronutrient regulation in the tropical terrestrial ant Ectatomma ruidum (Formicidae): a field study in Costa Rica. Biotropica 42, 135–139.

Cook, S.C., Eubanks, M.D., Gold, R.E., Behmer, S.T., 2010. Colony-level macronutrient regulation in ants: mechanisms, hoarding and associated costs. Anim. Behav. 79, 429–437.

Cook, S.C., Eubanks, M.D., Gold, R.E., Behmer, S.T., 2011. Seasonality directs contrasting food collection behavior and nutrient regulation strategies in ants. PLoS ONE 6, e25407.

Cook, S.C., Wynalda, R.A., Gold, R.E., Behmer, S.T., 2012. Macronutrient regulation in the Rasberry crazy ant (Nylanderia sp nr. pubens). Insect Soc. 59, 93–100.

Corona, M., Velarde, R.A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K.A., Robinson, G.E., 2007. Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. Proc. Natl. Acad. Sci. USA 104, 7128–7133.

Couvillon, M.J., Jandt, J.M., Bonds, J., Helm, B.R., Dornhaus, A., 2011. Percent lipid is associated with body size but not task in the bumble bee Bombus impatiens. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 197, 1097–1104.

Danks, H.V., 2005. How similar are daily and seasonal biological clocks? J. Insect. Physiol. 51, 609–619.

De Block, M., Stoks, R., 2008. Compensatory growth and oxidative stress in a damselfly. Proc. R. Soc. London B: Biol. Sci. 275, 781–785.

DeCoursey, P.J., 2004. The behavioral ecology and evolution of biological timing systems. In: DeCoursey, P.J., Dunlap, J.C., Loros, J.J. (Eds.), Chronobiology: Biological Timekeeping. Sinauer Associates, Sunderland, MA, p. 382.

Dewey, K.G., 1997. Energy and protein requirements during lactation. Annu. Rev. Nutr. 17, 19–36.

Dussutour, A., Simpson, S.J., 2008. Carbohydrate regulation in relation to colony growth in ants. J. Exp. Biol. 211, 2224–2232.

Dussutour, A., Simpson, S.J., 2009. Communal nutrition in ants. Curr. Biol. 19, 740–744.

Dussutour, A., Simpson, S.J., 2012. Ant workers die young and colonies collapse when fed a high-protein diet. Proc. R. Soc. London B: Biol. Sci. 279, 2402–2408.

Gillooly, J.F., Hou, C., Kaspari, M., 2010. Eusocial insects as superorganisms: insights from metabolic theory. Commun. Integr. Biol. 3, 360–362.

Glancey, B.M., Lofgren, C.S., 1988. Adoption of newly-mated queens: a mechanism for proliferation and perpetuation of polygynous red imported fire ants. Fla. Entomol. 71, 581–587.

Goodisman, M.A.D., Ross, K.G., 1999. Queen recruitment in a multiple-queen population of the fire ant Solenopsis invicta. Behav. Ecol. 10, 428–435.

Hahn, D.A., 2006. Two closely related species of desert carpenter ant differ in individual-level allocation to fat storage. Physiol. Biochem. Zool. 79, 847–856.

Helm, B., Ben-Shlomo, R., Sheriff, M.J., Hut, R.A., Foster, R., Barnes, B.M., Dominoni, D., 2013. Annual rhythms that underlie phenology: biological time-keeping meets environmental change. Proc. R. Soc. London B: Biol. Sci. 280, 20130016.

Hernandez, A., 2009. Summer-autumn feeding ecology of pied flycatchers Ficedula hypolueca and spotted flycatchers Muscicapa striata: the importance of frugivory in a stopover area in north-west Iberia. Bird Conserv. Int. 19, 224–238.

Hölldobler, B., Willson, E.O., 1990. The Ants. Harvard University Press, Massachussets, USA.

Hölldobler, B., Wilson, E.O., 2009. The Superorganism. WW Norton & Company Limited, New York.

Holway, D.A., 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. Ecology 80, 238–251.

Hulbert, A., Pamplona, R., Buffenstein, R., Buttemer, W., 2007. Life and death: metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87, 1175–1213.

Humphries, M.M., Kramer, D.L., Thomas, D.W., 2003. The role of energy availability in mammalian hibernation: an experimental test in free-ranging eastern chipmunks. Physiol. Biochem. Zool. 76, 180–186.

Hunt, J.H., Nalepa, C.A., 1994. Nourishment and evolution in insect societies. Westview [etc.], Boulder [etc.].

Jemielity, S., Chapuisat, M., Parker, J.D., Keller, L., 2005. Long live the queen: studying aging in social insects. Age 27, 241–248.
Kay, A., 2004. The relative availabilities of complementary resources affect the feeding preferences of ant colonies. Behav. Ecol. 15, 63–70.
Keller, L., Genoud, M., 1997. Extraordinary lifespans in ants: a test of evolutionary theories of ageing. Nature 389, 958–960.
Kipyatkov, V.E., Lopatina, E.B., 2009. Temperature and photoperiodic control of diapase induction in the ant Lepisiota semenovi (Hymenoptera, formicidae) from Turkmenistan. J. Evol. Biochem. Phys. 45, 238–245.
Konrad, R., Wackers, F.L., Romeis, J., Babendreier, D., 2009. Honeydew feeding in the solitary bee Osmia bicornis as affected by aphid species and nectar availability. J. Insect Physiol. 55, 1158–1166.
Kostal, V., Simek, P., 1998. Changes in fatty acid composition of phospholipids and triacylglycerols after cold-acclimation of an aestivating insect prepupa. J. Comp. Physiol. B. 168, 453–460.
Kumar, V., Wingfield, J.C., Dawson, A., Ramenofsky, M., Rani, S., Bartell, P., 2010. Biological clocks and regulation of seasonal reproduction and migration in birds. Physiol. Biochem. Zool. 83, 827–835.
Lenhart, P.A., Eubanks, M.D., Behmer, S.T., 2015. Water stress in grasslands: dynamic responses of plants and insect herbivores. Oikos 124, 381–390.
Mackay, W.P., Sassaman, C.A., 1984. Paradoxical acclimation and seasonal comparisons of oxygen-consumption rates in 3 species of pogonomyrmex harvester ants (Hymenoptera, Formicidae). J. Comp. Physiol. 154, 399–407.
Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessmann, J., Clissold, F., Raubenheimer, D., Bonduriansky, R., Brooks, R.C., 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. Curr. Biol. 18, 1062–1066.
Mattila, H., Harris, J., Otis, G., 2001. Timing of production of winter bees in honey bee (Apis mellifera) colonies. Insect Soc. 48, 88–93.
McGlynn, T.P., 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. J. Biogeogr., 535–548
Porter, S.D., Savignano, D.A., 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology 71, 2095–2106.
Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. Trends Ecol. Evol. 17, 462–468.
Ricks, B.L., Vinson, S.B., 1972. Digestive enzymes of imported fire ant, Solenopsis-Richteri (Hymenoptera – Formicidae). Entomol. Exp. Appl. 15, 329–334.
Roces, F., 1990. Leaf-cutting ants cut fragment sizes in relation to the distance from the nest. Anim. Behav. 40, 1181–1183.
Saunders, D.S., 1977. Insect clocks. Comp. Biochem. Phys. A 56, 1–5.
Seeley, T.D., 1995. The wisdom of the hive: the social physiology of honey bee colonies. Harvard University Press.
Shik, J.Z., Hou, C., Kay, A., Kaspari, M., Gillooly, J.F., 2012. Towards a general life-history model of the superorganism: predicting the survival, growth and reproduction of ant societies. Biol. Lett. 8, 1059–1062.
Simpson, S.J., Raubenheimer, D., 2012. The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity. Princeton University Press.
Toth, A.L., Robinson, G.E., 2005. Worker nutrition and division of labour in honeybees. Anim. Behav. 69, 427–435.
Toth, A.L., Kantarovich, S., Meisel, A.F., Robinson, G.E., 2005. Nutritional status influences socially regulated foraging ontogeny in honey bees. J. Exp. Biol. 208, 4641–4649.
Tschinkel, W., 1987. Seasonal life history and nest architecture of a winter-active ant, Prenolepis imparis. Insect Soc. 34, 143–164.
Tschinkel, W.R., 1993. Sociometry and sociogenesis of colonies of the fire ant Solenopsis invicta during one annual cycle. Ecol. Monogr. 63, 425–457.
Tschinkel, W.R., 1999. Sociometry and sociogenesis of colonies of the harvest ant, Pogonomyrmex badius: distribution of workers, brook and seeds within the nest in relation to colony size and season. Ecol. Entomol. 24, 222–237.
Tschinkel, W.R., 2006. The Fire Ants. Belknap Press of Harvard University Press.
Vafopoulou, X., 2014. The coming of age of insulin-signaling in insects. Front. Physiol. 5, 216.
Vogt, J.T., Appel, A.G., 1999. Standard metabolic rate of the fire ant, Solenopsis invicta Buren: effects of temperature, mass, and caste. J. Insect Physiol. 45, 655–666.
Vuarin, P., Dammhahn, M., Kappeler, P.M., Henry, P.Y., 2015. When to initiate torpor use? Food availability times the transition to winter phenotype in a tropical heterotherm. Oecologia 179, 43–53.
Wheeler, W.M., 1928. The social insects, their origin and evolution. In: Paul, K. (Ed.), Trench, Trubner & Co., Ltd.; Harcourt, Brace and Company, New York, London.
Wilder, S.M., Eubanks, M.D., 2010. Might nitrogen limitation promote omnivory among carnivorous arthropods? Comment. Ecology 91, 3114–3117.