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Title: Seasonal trends in honeydew-foraging strategies in the red wood ant, *Formica yessensis* (Hymenoptera: Formicidae)

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Abstract The red wood ants *Formica yessensis* are known to support super colonies comprising thousands of nests, contain approximately 360 million workers, and over one million queens along the Ishikari coast, Hokkaido, northern Japan. Previous studies revealed the abundance of prey insects in Ishikari is very limited; suggesting that honeydew collected from aphids is a critical resource for *F. yessensis*. Furthermore, several reports suggested *F. yessensis* performs a generation change between late July and early August at the study site. The present study examined seasonal changes in *F. yessensis* honeydew-foraging workers and specifically addressed the following: information transfer to aphid trees; fidelity to aphid trees; and changes in *F. yessensis* body size. Observation of marked ants revealed that information transfer to aphid trees occurred by direct guidance from older to younger foragers. Seasonal sampling indicated that honeydew-foraging ant body size decreased with progressive seasons. Large gaster coefficient of variation (CV) values showed two honeydew-foraging ant worker types were present in the super colony. The results revealed older foragers exhibited a large body size, which decreased in number towards autumn. Younger workers exhibited a smaller body size, and initiated honeydew foraging after emergence. Honeydew is a critical resource, therefore information transfer to aphid tree location, and honeydew-foraging were the first priority tasks observed in *F. yessensis* at the study site.

Keywords Honeydew-foraging, *Formica yessensis*, generation change, fidelity, body size, gaster

Introduction

Consistent with many large colonial organisms specialised life histories and social system, *Formica yessensis* worker ants are sterile. Consequently workers do not engage in mating practices, but maximize efforts on identifying and collecting food sources (Traniello 1989). Compared to prey insects, honeydew is a suitable and effective feeding resource; and foraging locations are stable. Therefore, an optimal strategy in *F. yessensis* is the transfer of geographic information regarding aphid tree locations from older to younger foraging generations. However, little is known about how information transfer occurs in ants. *F. yessensis* support super colonies comprising thousands of nests, approximately 360 million workers, and over a million queens distributed along the Ishikari coast, Hokkaido, northern Japan (43°N, 141°E). Cherix (1987) compared the diets of *F. yessensis* super colonies distributed at Ishikari (the study site) and Hakkenzan (adjacent forest sites). The number of prey insects in Ishikari was approximately 1/28 that of Hakkenzan, suggesting honeydew is a critical resource for *F. yessensis* in Ishikari.

The annual activity cycle, and development of nest structure for *F. yessensis* in Ishikari, including some environmental phenology was characterised by Ito (1973). Extra-nest activities by post-hibernating workers initiate in mid-April, following thaw. Full-scale activity starts in late May, when *Tuberculatus quercicola* aphid honeydew becomes available on *Quercus dentata*. Budding is generally practiced generally from May through July, and new workers emerge in late July to mid-September (Fig. 1). Corresponding to Ito's (1973) description, the worker number in attending aphid colonies increases until late June, followed by a decline that continues until late July. In late July,

early August, worker number sharply increases again. This period of emergence of new ant nest workers coincides with an increase in aphid colony workers in late July. Therefore, it is reasonable to hypothesize that new workers correspond to a younger generation of ant workers. Genotypic analyses using microsatellite markers revealed distinct genetic differentiation among samples of *F. yessensis* individuals representing seasonal populations collected in spring and summer (Yao and Akimoto 2009). These results suggested summer honeydew foragers were not members of the spring population. However, principle coordinate analysis for spring and summer population samples indicated a genetic relationship among populations. In *F. yessensis*, the daughter queen has been observed returning to natal nests (Higashi 1983). Consequently, information transfer regarding aphid tree locations is likely inherited, and occurs between late July and early August. This raises the question of whether or not morphological differences exist between older and younger generations of workers. If morphological differences are favoured in younger generations, body size variation in ants attending aphid colonies is expected to change with the progression of seasons.

In addition to information transfer, ant-aphid tree fidelity is a critical strategy to maintain stable resources. Fidelity to honeydew has been examined in *Camponotus pennsylvanicus* (Fowler & Roberts 1980), *Ectatomma ruidum* (Schatz et al. 1995), *Lasius fuliginosus* (Quinet & Pasteels 1996), and *Formica obscuripes* (McIver & Yandell 1998). Wilson (1971) demonstrated in highly polygynous populations, colony boundaries frequently appear in individuals that move freely among nests. Therefore, in mutualisms between *F. yessensis* and *T. quercicola*, an aphid tree can have foragers composed of

workers that belong to different nests. Moreover, as insects and their larvae are low in abundance in spring but high in summer, aphid tree fidelity may diminish based on insect prey availability.

The aims of the present study is to examine the mutualistic relationships between *F. yessensis* ants and *T. quercicola* aphids using the following approaches: (1) fidelity to honeydew by means of a marking methodology; and (2) examine the morphological differences between older and younger foragers by comparing workers collected in June, August, and September. Fidelity to honeydew, and seasonal body size variation were discussed in terms of resource availability, and environmental factors.

Materials and methods

Marking

Ten *Q. dentata* trees were chosen in the field, and used to monitor marked workers (Fig. 2). Workers foraging honeydew, or moving on *Q. dentata* were collected with tweezers from each tree, and marked by a paint marker (Uni Mitsubishi Pencil). Workers were marked on the dorsal surface of the gaster, and released to the trunk of the tree from which the worker was collected. From each tree, 1000 workers were marked. Different colours were applied on each of ten trees. Marking was performed twice on each tree from 6-12 June and 29-31 July 2011.

Weekly observations of marked individuals were made from 9:00 to 14:00. For

each tree, the ant number on aphid colonies per *Q. dentata* shoot, where seven-eight leaves gathered, was counted. The counting did not exceed 20 shoots supporting aphid colonies. If the number of aphid colonies was fewer than 20, the number of ants climbing up the trunk was counted for 20min. Observations were conducted from 25 June to 4 October 2011. The fidelity coefficient defined by Quinet & Pasteels (1996) was employed: $100 \times (Co/Co+De)$, where Co (conservants) refers to the number of marked ants observed on the site where they were marked; and De (deviants), the number of marked ants observed at other sites.

Ant collection and measurement

Morphological differences between older and younger foragers were investigated by collecting a total of 50 *F. yessensis* workers per tree from *T. quercicola* aphid colonies. If the number of *T. quercicola* aphid colonies did not exceed 20, *F. yessensis* ants observed on the tree trunk were collected. Ant collection was performed three times in 2011; 18-25 June, 3 August, and 23-26 September. Ant samples were preserved in a collection tube with 100% ethanol and stored in -20°C freezer.

Worker ant head width was measured as a body size index. In addition to measuring of head width, three body parts (head, thorax, and gaster) were weighed to evaluate each average weight and the coefficient of variation (CV) between seasons. The head was removed from each worker to measure head width. All legs were removed from each worker to extract genomic DNA for another experiment. The remaining body i.e.,

the thorax and gaster, was divided into two parts. Subsequently the head, thorax, and gaster were dried at 56°C for a minimum 3 hr. Each body part was weighed with an Ultramicro Balance SE-2 (Sartorius). The head, thorax, and gaster weights were summed as body weight. The head width length measurement, and body parts weight were ordered based on each *Q. dentata* tree (trees 1-10), and the medians were used for statistical analyses. Each CV value was converted to an arcsine-square root. Because few ants were found on trees 6 and 9 in autumn, the total number of scores used in statistical tests resulted in 28.

Statistical analyses

The effects of season and tree on body size were examined applying a two-way ANOVA. The ANOVA model analysed the effects of the independent variables ‘tree’ and ‘month’ (factors), on the dependent variable ‘body size’. A three-way ANOVA was applied to investigate body parts with large CV values. The ANOVA model contained three factors, ‘body parts’, ‘tree’, and ‘month’, and the interaction term ‘body parts×month’.

Results

Fidelity to aphid trees was revealed in younger and older generations. A high fidelity to aphid trees was detected in marked workers during early June and late August; 96-100% in the younger generation, and 95-100% in the older generation (Table 1). Mixed older and younger foragers in aphid trees were observed between 4 and 31 August (Fig. 3).

A significant seasonal effect was found in head width ($F_{2,16} = 17.92$, $P < 0.0001$), and body weight ($F_{2,16} = 21.84$, $P < 0.0001$). Head width length and body weight were high in June, and low in August and September (head width, 1.31 ± 0.1 mm for June, 1.19 ± 0.06 mm for August, 1.12 ± 0.04 mm for September; body weight, 1.14 ± 0.22 mg for June, 0.89 ± 0.12 mg for August, 0.7 ± 0.08 mg for September) (Fig. 4).

The gaster exhibited the largest weight among body parts ($F_{2,66} = 33.07$, $P < 0.0001$; 0.35 ± 0.06 mg for head, 0.35 ± 0.06 mg for thorax, 0.46 ± 0.08 mg for gaster). CV for all body parts decreased seasonally, with the exception of the gaster. The collective CV was high in June and August and low in September ($F_{2,66} = 14.57$, $P < 0.0001$; 0.4 ± 0.08 for June, 0.42 ± 0.08 for August, 0.33 ± 0.07 for September) (Fig. 5).

Discussion

The present study showed that high fidelity to aphid trees (*Q. dentata*) was transferred from older to younger foragers by means of older foragers guidance. I observed many workers emerging from nests during the summer, however fidelity to aphid trees was maintained. In *Formica* species, it has been reported that daughter queens return to natal nests after mating. Therefore, geographical information may be transferred from generation to generation. More importantly, direct guidance from older to younger workers assures the information of aphid tree location. Honeydew is a more stable food resource relative to insect prey in terms of search cost. However, not all *Q. dentata* trees maintain aphid colonies until autumn. Indeed, no aphid colony was found on two of the ten trees in late September, and attending ants were not observed on those trees. In ant nests experiencing difficulty foraging new protein resources, the capacity to pass on knowledge of honeydew resource locations (*i.e.*, nest sites) would be advantageous, and

favoured by natural selection. *F. yessensis* workers actively entering and exiting the nest sites have been observed at the mounds near the study trees for 11 consecutive years; and new nests appear to be established close to natal nests adjacent to trees where honeydew might be available throughout the seasons. Experimental studies have shown that in *F. paralugubris* B, new queens can mate and remain in their natal nest or seek adoption in a foreign nest following a mating flight (Cherix et al. 1991). If *F. yessensis* queens possess similar philopatric behavior, genetic similarities may be maintained in a nest that exploits an aphid population on a host tree as a honeydew resource. It is suggested that the susceptible and narrowly distribution *Q. dentata* has enabled aphid colonies to persist on a limited number of trees for a long period of time, resulting in a long-term mutualistic interaction between *T. quercicola* aphids, and attending *F. yessensis* ants.

The present study showed that the body size of honeydew-foraging ant workers was not uniform throughout the summer through fall seasons, but ranged from 1.31-1.12 mm. It has been reported in some ant species that division of labour is related to body size (Herbers 1979; McIver & Loomis 1993), and age (Seid & Traniello 2006; Vieira et al. 2010; Amador-Vargas 2012). However, honeydew-foraging tasks in *F. yessensis* workers showed no relationship to body size or age. This may be responsible for differences in energy requirements between collecting honeydew, and hunting and carrying prey. When ants demand honeydew by tapping their antennae, aphids quickly respond to the requirement. Therefore, compared to hunting and carrying insects, collecting honeydew likely requires less energy. Consequently, large body size may not be required for honeydew collection.

Larger body size was observed in workers emerging in spring. This is due to selection for larger body size in ants to over-winter; during the long hibernation

(approximately six months from November to late April) larger workers have lipid stores to survive the winter months (Blanchard et al. 2000). In the period before new workers emerged, larger workers were observed undergoing multiple tasks, including hunting, foraging honeydew, and carrying insect prey. Spring workers were observed on oak trees seeking prey in mid-May, even when aphids were absent on trees. Furthermore, marked workers, which had collected honeydew in June, were recorded carrying pupae to new nests for nest budding in mid-July.

It has been reported that new emergence workers care for eggs and pupae, in *F. yessensis* younger workers emerged from nests and foraged for honeydew. This pattern does not follow normal polyethism patterns in ants, where new emergence workers begin tasks inside the nests. However, *F. yessensis* exhibits highly aggressive behaviour, and its mortality risks caused by predators is negligible even considering its small body size. In addition, assigning many small workers to aphids is reasonable in terms of worker production costs.

Gasters exhibited the highest CV in weight, indicating that gasters are the most flexible body part. This large CV may be partly explained by the existence of several honeydew collecting worker types. McIver & Yandell (1998) reported that honeydew was often transferred mouth to mouth to other ants from honeydew-foraging workers called transporters. The two types of workers may differ in physical carrying capacity for honeydew, resulting in large variation in the extent of the distended gaster. CV for all body parts but the gaster decreased with progressive seasons, indicating that honeydew-foraging workers gradually became uniform in body size. These results indicated that in autumn, honeydew foragers were replaced with new emergence workers, and older foraging ants no longer took part in honeydew foraging.

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Figure captions

Fig. 1 Annual cycle of colony activities and nest development of nest in *F. yessensis*.

Figure 1 in Ito (1973) was rearranged. Arrows indicate ant collection (C1-3) and marking (M1-2 periods. (Adobe Illustrator CS5.1 was used to create this figure)

Fig. 2 Tree distribution sampled in the present study. Distances among trees (m) are given as solid line. The number in each circle is an assigned sample number. Broken and solid curved lines indicate ant movement from the original ant marking tree to other trees in spring and summer. (Adobe Illustrator CS5.1 was used to create this figure)

Fig. 3 Seasonal changes in (a) the number of non-marked, spring-marked, and summer-marked ants observed on 10 sampled trees. The number of spring- and summer-marked ants was extracted from (a) and given again in (b). (Kaleida Graph 4.1.3 was used to produce tif fail and Adobe Illustrator CS5.1 was used to convert the tif file to eps file.)

Fig. 4 Seasonal changes in (a) head width (mm), and (b) body weight (mg) of *F. yessensis*.

Means $\pm$ SD. Data points with different letters indicate a significant difference between seasons was detected using a multiple comparisons test (Tukey's HSD test). (Kaleida Graph 4.1.3 was used to produce tif fail and Adobe Illustrator CS5.1 was

used to convert the tif file to eps file.)

Fig. 5 (a) Body part comparisons (head, thorax, and gaster). (b) Seasonal changes in the CV in each body part. Data points with different letters indicate a significant difference between seasons was detected using a multiple comparisons test (Tukey's HSD test). (Kaleida Graph 4.1.3 was used to produce tif fail and Adobe Illustrator CS5.1 was used to convert the tif file to eps file.)

Table 1. Fidelity to aphid trees in June and August.

	June					August				
	ants observ	ants mark ed	colour	ants marked in other tree	fidelity	ants observ ed	ants mark ed	colour	ants marked in other tree	fidelity
Free										
1	553	66	white	1 (yellow)	100	1180	125	light blue	1 (red)	100
2	335	26	light blue	0	96	1837	75	orange	0	99
3	400	50	yellow	0	96	1236	38	red	0	97
4	1115	28	pink	0	100	2622	142	yellow	0	100
5	590	20	green	0	100	1935	26	white	0	100
6	904	72	silver	1 (light blue)	100	655	41	blue	1 (orange)	100
7	418	28	red	0	100	2028	39	silver	0	100
8	238	44	blue	0	98	1151	75	green	1 (pink)	100
9	300	48	orange	1 (blue)	100	344	21	pink	0	95
10	2055	23	gold	1 (yellow)	100	4521	151	greenish- yellow	0	100

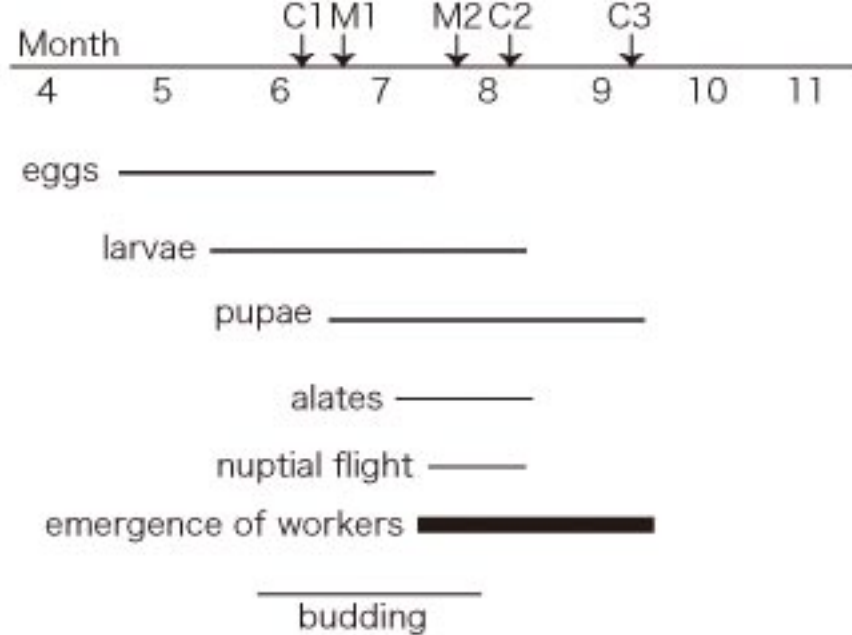


Fig. 1

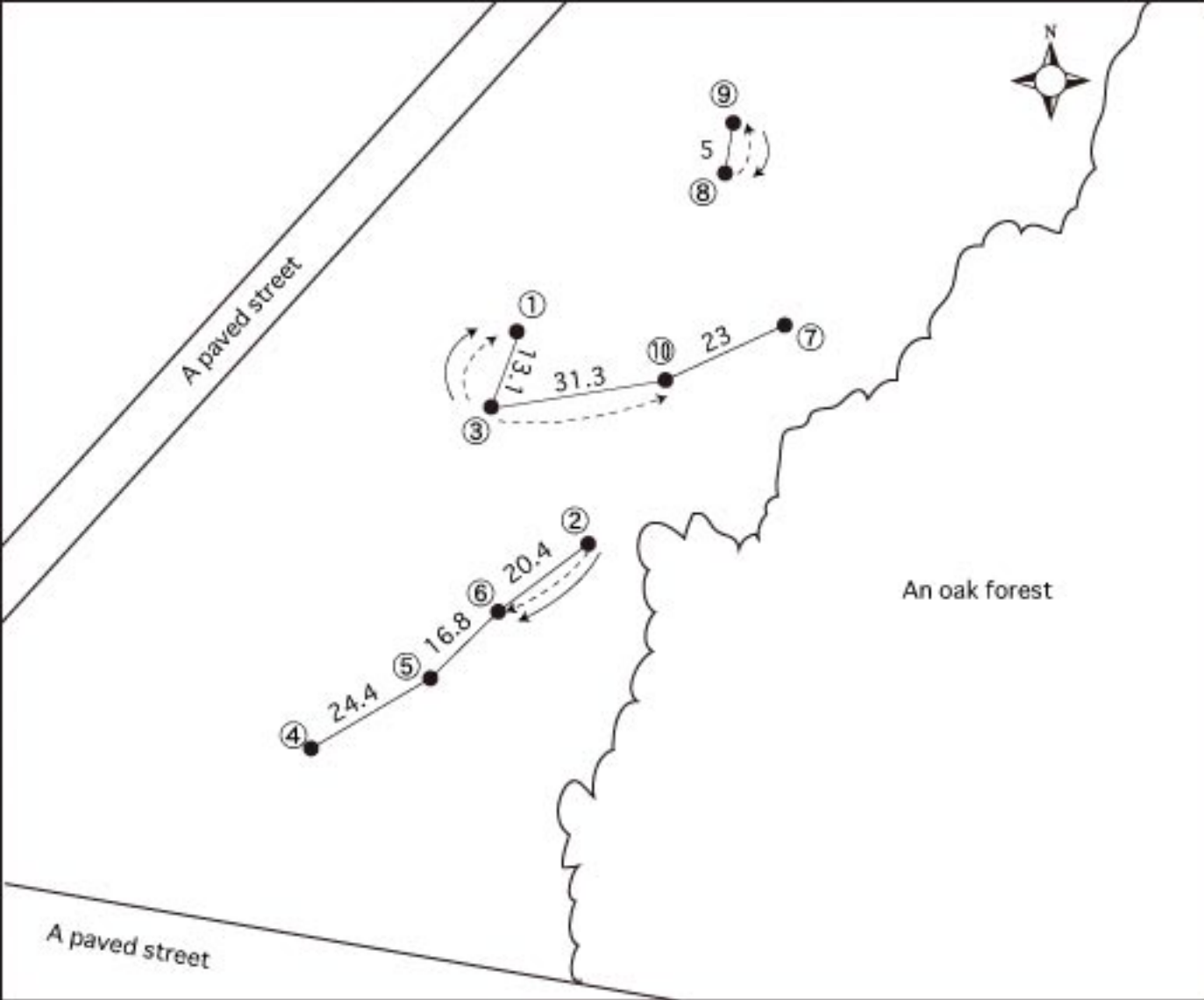


Fig. 2

Fig. 3

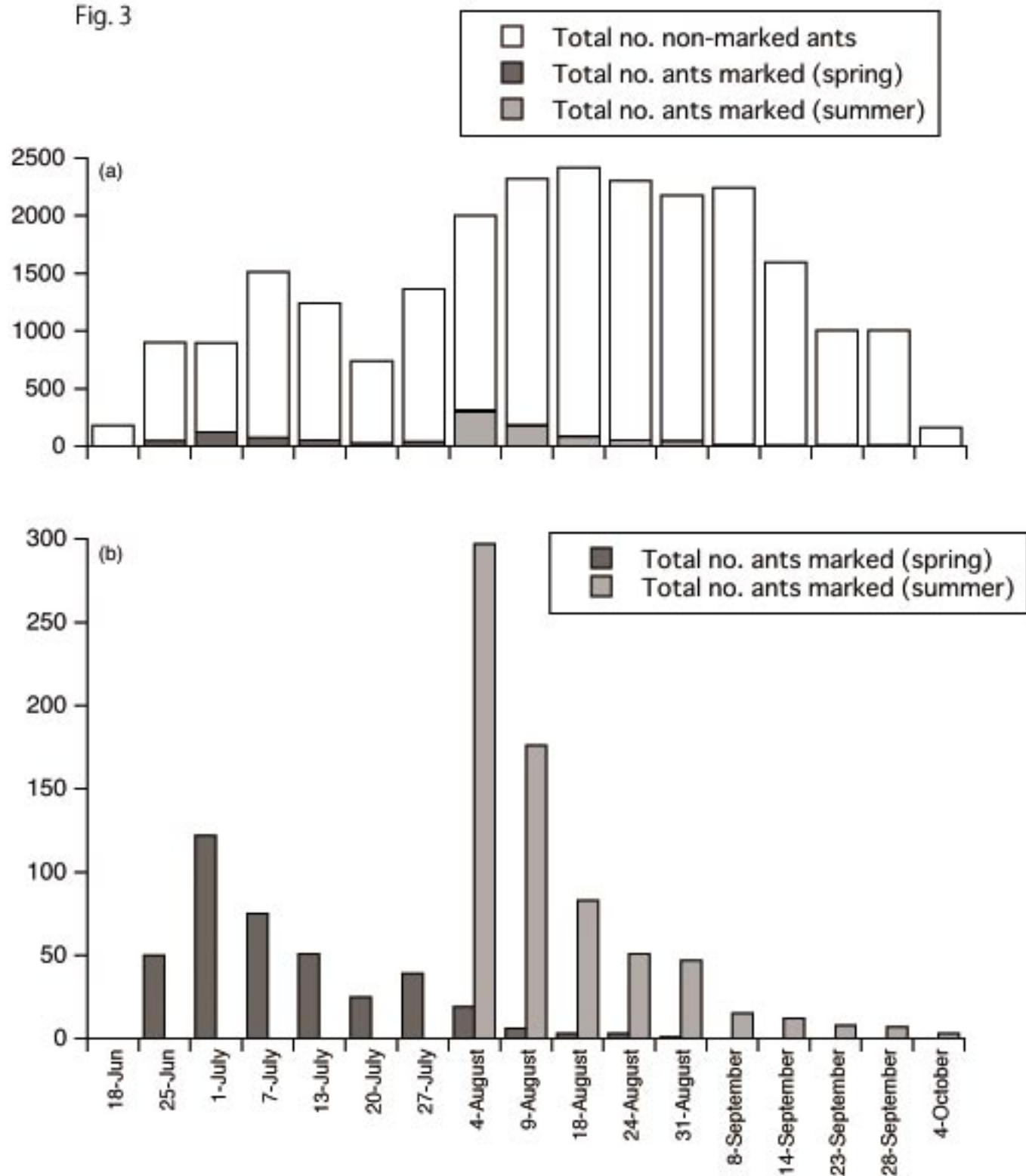


Fig. 4

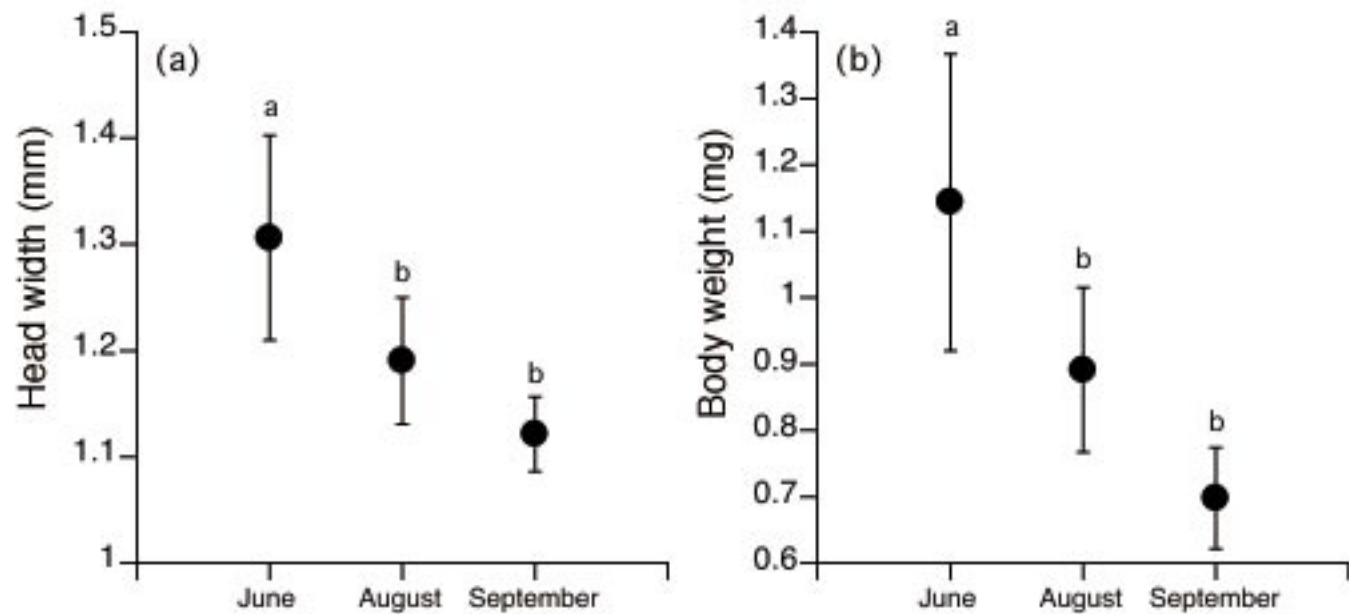


Fig. 5

