Citation for the published paper:
Fedderwitz, Frauke; Björklund, Niklas; Ninkovic, Velemir; Nordlander; Göran. (2015) The structure of feeding behavior in a phytophagous insect (Hylobius abietis). Entomologia experimentalis et applicata. Volume: 155, pp 229-239.
http://dx.doi.org/10.1111/eea.12302.

Fedderwitz F, Björklund N, Ninkovic V & Nordlander G (2015) The structure of feeding behavior in a phytophagous insect (*Hylobius abietis*). Entomologia Experimentalis et Applicata 155: 229-239.,

The structure of feeding behavior in a phytophagous insect (*Hylobius abietis*)

Frauke Fedderwitz[1]*, Niklas Björklund[1], Velemir Ninkovic[2] & Göran Nordlander[1]

[1]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden, and [2]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

***Correspondence:** Frauke Fedderwitz, Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, SE-750 07 Uppsala, Sweden. E-mail: frauke.fedderwitz@slu.se

Running head: *Structure of insect feeding behavior*

Key words: feeding intervals, food intake, herbivore, prandial correlation, short-term feeding, Coleoptera, Curculionidae, *Picea abies*, Pinaceae

Accepted: 2 March 2015

Abstract

Analysis of the feeding behavior of animals using such a high temporal resolution that meals can be defined may improve our understanding of the mechanisms regulating feeding. Meals can be distinguished in an ethologically meaningful manner by using the 'meal criterion', the shortest non-feeding interval between feeding bouts recognized as meals. However, such a criterion has only been determined for a few insect species. Applying a recent method developed for assessing meal criteria for vertebrates, we determined the meal criterion for *Hylobius abietis* (L.) (Coleoptera: Curculionidae) based on data from video recordings of single individuals feeding on seedlings of Norway spruce, *Picea abies* (L.) Karst. (Pinaceae). The pine weevil is an economically important pest insect because it feeds on the stem bark of planted conifer seedlings. Weevils had 4-5 meals per day. Each meal lasted about 24 min during which about 13 mm^2 of bark per meal were removed. Females had longer total meal durations and longer non-feeding intervals within meals than males. Girdling seedlings did not affect the weevils' feeding properties. The size of meals was significantly correlated to the duration of non-feeding intervals before and after them. This study is one of few describing the feeding behavior of an insect at a temporal resolution that allows individual meals to be distinguished. With more meal-related data from insects available, differences in meal properties may be interpreted based on phylogeny, ecology, and physiology. Our results may also assist in the setup and interpretation of studies of plant-insect interactions, and facilitate the evaluation and development of methods to protect plants against herbivores.

Introduction

The food intake of herbivores is affected not only by their access to food, but also by the handling time and the host plants' reactions to their feeding (e.g., Nuorteva, 1972; Clauss et al., 2007; Zas et al., 2011). Thus knowledge about the feeding patterns of herbivores may help to understand their interaction with plants, including the role of host plant resistance (Mithöfer et al., 2005).

Studies on short-term feeding behavior, i.e., feeding patterns at the level of feeding events and meals, are useful for investigating the mechanisms controlling limitations in food acquisition and meal properties (Tolkamp et al., 2000; Howie et al., 2009). Feeding events, such as bites into food, can be structured into discrete bouts of ingestion, so called meals, which can be used as units in studies on feeding behavior (e.g., Simpson, 1981; Raubenheimer & Bernays, 1993; Mitchell & Low, 1994; Bernays & Singer, 1998; Tomlin et al., 1998). This facilitates comparisons of results obtained from multiple studies, as meals are independent of the original unit in which they were measured (Howie et al., 2009). Generally, there are numerous short non-feeding intervals during meals (intrameal intervals) and fewer longer non-feeding intervals between meals (intermeal intervals) (e.g., Simpson, 1990). Thus, meals can be distinguished in an ethologically meaningful manner by using the 'meal criterion', the shortest non-feeding interval between feeding bouts recognized as meals (Tolkamp et al., 2011), determined using the distribution of durations of non-feeding intervals. This can also provide important information on pre- and post-prandial correlations, i.e., the relationships between meal sizes and intermeal intervals before and after meals, which are influenced by satiety and hunger, respectively (Le Magnen & Tallon, 1966; Savory, 1981).

The short-term feeding behavior of about 10 insect species has been studied using meal criteria (Bowdan, 1988a; Simpson et al., 1988, 1989; Raubenheimer & Bernays, 1993; Bright et al., 1994; Mitchell & Low, 1994; Wright et al., 1999; Raubenheimer & Browne, 2000; Jones & Raubenheimer, 2002; Nagata & Nagasawa, 2006; Colasurdo et al., 2007). These meal criteria were determined using log-survivorship and log-frequency distributions of feeding events (e.g., Sibly et al., 1990; Mitchell & Low, 1994), underpinned by the assumption that the events start at random times. However, it is not likely that feeding events start at random times since the feeding motivation will be low directly after a meal, i.e. due to satiety. Recently, a method based on normal or Weibull distributions has been developed for vertebrates, which agrees better with the concept of satiety (Tolkamp et al., 1998, 2011). This method has so far not been used with insects.

In this study, we investigated the short-term feeding behavior of the pine weevil, *Hylobius abietis* (L.) (Coleoptera: Curculionidae), one of the most economically important forest pests in Europe (Långström & Day, 2004). Adults feed on the tender bark of several conifer species and may kill up to 90% of seedlings during the first 3 years after plantation (Petersson & Örlander, 2003). Several aspects of its feeding behavior have been examined, including the effects of tree species (Manlove et al., 1997; Månsson & Schlyter, 2004; Zas et al., 2011, 2012), chemical composition of the bark and seedling fertilization (Wainhouse et al., 2005; Zas et al., 2008; Sampedro et al., 2009), and various methods designed to reduce the damage the weevil causes (Nordlander et al., 2009; Zas et al., 2014). However, the meal criterion of this species has not been determined, and thus the opportunities to investigate the mechanisms controlling the feeding pattern through studies of its short-term feeding behavior has not been exploited. Therefore, we examined the short-term feeding behavior of the pine weevil on seedlings in a controlled environment using video recordings. The aims of this study were to (1) determine the meal criterion for the pine weevil using the new methodology described above which takes the non-random temporal distribution of feeding initiation into consideration, (2) determine fundamental parameters of feeding structure including how often and how long they eat, and the amounts they eat per meal, (3) test if the sizes of pine weevil meals are related (pre- and/or post-prandially) to intermeal intervals, (4) test if pine weevil feeding patterns are influenced by previous injury to seedlings and/or their sex.

Materials and methods

Insects and plant material

Pine weevils were collected in central Sweden (Boda såg, Svärdsjö, 60°43’ N, 015°52’ E) during spring migration a few weeks before the start of the experiment, then stored in darkness at 10 °C and fed with branches of Scots pine [*Pinus sylvestris* L. (Pinaceae)]. Such storage conditions interrupt the weevils’ reproductive development and synchronize oviposition to the following period, which is useful for experimental purposes because their feeding increases during the oviposition period (Bylund et al., 2004). About a week before their use in experiments, they were placed individually in Petri dishes (9 cm diameter) together with small Scots pine twigs and a tube with water and transferred to a chamber maintained at room temperature (22 °C) with an artificial light-dark cycle (L18:D6, not in phase with the natural light cycle). Weevils that had eaten during this week were used in the following experiments.

The plants used in the experiments were 1.5-year-old Norway spruce, *Picea abies* (L.) Karst. (Pinaceae) seedlings obtained from a commercial nursery (Sjögränd, Bergvik Skog, Falun, Sweden). They originated from seeds collected in a seed orchard and were intended for planting in central Sweden. They were kept outdoors and transferred to the chamber providing experimental conditions 1 day before use. They had a mean height of ca. 30 cm and a lower stem diameter of ca. 0.4 cm.

Set-up and experimental procedure

The experiment was conducted at room temperature (22 °C) from June to September 2011. Weevil behavior was observed in transparent plastic cylinders (8 cm high, 11 cm diameter) surrounding the middle part of a seedlings' stem (Figure 1). This prevented the weevils from accessing the stem bases and top shoots and thus offered a more homogeneous feeding source (Kaakinen et al., 2004). To ensure high visibility of weevil activity, two video cameras (Handycam HDR-XR550VE; Sony, Tokyo, Japan) were focused on the seedling with a 45° angle between them. Light intensity of 59 μmol m^{-2} s^{-1} was used for light phase conditions and 0.1 μmol m^{-2} s^{-1} for dark phase conditions (measured by Sky 200 SKP; Skye Instruments, Llandrindod Wells, UK). In addition, a red light was used during the dark phase to increase recording visibility. The observation sessions were chosen to start 11 h after the start of the light phase to temporally separate potential effects of disturbance when an observation session was initiated from those of a change in light intensity. Thus, each observation session started with 7 h of light phase, followed by a 6-h dark phase, and then another 11 h light phase (i.e., a light-dark cycle of L18:D6).

To habituate the weevils to conditions in the cylinder, they were placed singly in it, together with a seedling, 24 h before the start of each observation session. A tube with water and a refuge made of a dark V-shaped metal plate (2.5 cm wide, 3.5 cm long, maximum height 2.5 cm) were placed at the bottom of each cylinder. To avoid the effects of any feeding scars, seedlings were exchanged with new ones after the habituation period.

Each weevil was observed in two sessions: once with a non-girdled seedling and once with a seedling girdled by manually removing a 5-mm ring of outer and inner bark under the first node 24 h prior to video recording. This kind of girdling mimics a type of pine weevil damage that is often observed on planted seedlings (Eidmann, 1974). Half of the weevils of each sex were placed in cylinders with girdled seedlings and the other half with non-girdled seedlings during their first observation session. Weevil behavior was video-recorded for 24-h sessions, after which the area of each final feeding scar, which could have resulted from several meals, was measured. Between observation sessions, the weevils were placed for 48 h in Petri dishes with pieces of Scots pine and a tube with water. In total 13 male and 12 female weevils were video recorded. However, the short-term feeding behavior was only determined for those six males and six females that fed from both types of seedlings and thereby a balanced dataset was obtained.

Analysis

The start and end of every feeding event during each 24-h session of continuous observation were noted by visual examination of the video-recordings (The Observer XT 10; Noldus Information Technology, Wageningen, The Netherlands). Feeding was defined as manipulation of bark or needles with the weevil's mouth parts. Video files were watched at up to 16× normal speed, but during periods when the weevils were feeding the playback speed was slowed, allowing recordings with a precision of 0.04 s. If an observation session ended with a non-feeding period it was merged with the first non-feeding period of the same session in the analysis to minimize underestimation of the length of non-feeding intervals and represent the continuity between days. This minimizes a potential source of bias, as non-feeding intervals are more likely to be cut off by the experimental design than feeding intervals, due to their greater length and diel distribution (Fedderwitz et al., 2014). Non-feeding intervals that were shorter than 1 s were excluded from all further analysis, as they probably represented chewing or swallowing events that could not be detected on the video.

Meal criterion

The meal criterion was determined using the approach described in Tolkamp et al. (2011). The durations of all non-feeding intervals were ln-transformed. Two population models with combinations of Gaussian and 3-parameter Weibull distributions were used to find the best-fit likelihood estimates for the model parameters (R version 2.12.1; The R Foundation for Statistical Computing, Vienna, Austria). The model based on two Weibull distributions provided the best fit to the data. In addition, meal criteria were determined separately for girdled and non-girdled seedlings and for male and female weevils. We also performed a bootstrap analysis (1 000 permutations) to obtain a 95% confidence interval for the meal criterion based on the entire dataset.

Meal properties

Based on the calculated meal criterion for the entire dataset, meals were identified in the observation sessions, thereby allowing the calculation of meal properties listed in Table 1. Other feeding properties were also measured (listed in Table 1). For each property, linear mixed-effects models were fitted using R software. Explanatory variables were weevil sex and girdled/non-girdled seedlings. Weevil identity was used as a random factor to account for between-individual differences. Models were reduced to only include weevil sex as a fixed factor for calculating transformed mean values and standard errors.

Prandial correlations

Pre- and post-prandial Spearman's correlation coefficients were calculated using the entire dataset. Then, to determine the effects of prandial correlations on the structure of feeding events, the meal size and pre-prandial intervals were divided into three equally sized classes, i.e., large, medium, and small meals and long, medium, and short pre-prandial intervals.

Total feeding amount

The differences between the amount of bark removed per observation session by pine weevils on girdled and non-girdled seedlings were tested with a paired t-test (Minitab version 16.1.0; Minitab, State College, PA, USA), as each individual fed on both a girdled and a non-girdled seedling. To compare amounts removed by males and females, a two-sample t-test of averages per individual was used.

Feeding scar area

It was not possible to measure the area of individual feeding scars made during most individual meals because the scars were frequently connected to each other at the end of an observation session. Therefore, in the following analyses, the sizes of individual meals were estimated from the duration of the meals and the average feeding rate during an observation session. The reliability of these estimations was supported by a non-significant Spearman's correlation between feeding rate and the size of 17 individual meals for which it was possible to measure the feeding scar area (median size = 15 mm^2, median duration = 25 min 38 s; $r_s = -0.03$, $P = 0.90$; $n = 17$; Minitab). The estimates were further supported by the non-significant difference between the measured size of these 17 meals and their estimated sizes, based on the duration of the meals and the average feeding rate per observation session ($t = 1.40$, d.f. = 16, $P = 0.18$).

A Spearman's correlation analysis was used to determine the correlation between feeding scar area and meal duration based on the 17 meals for which it was possible to measure the feeding scar area of individual meals.

Movement after meals

To evaluate the frequency and duration of time periods that weevils spent on the seedling in relation to feeding we also recorded the duration of each interval between a pine weevil climbing on and off the seedling without feeding, the time it spent on the seedling before each meal, and the time it spent on the seedling after each meal until it either left the seedling or started a new meal. The intervals between a meal and the weevil leaving the seedling or starting to feed again, as well as the intervals between consecutive meals when weevils did and did not leave the seedling, were compared using Mann-Whitney tests (Minitab).

Results

This study focused on the analysis of feeding patterns at such a high temporal resolution that individual meals can be distinguished. However, results of feeding properties per 24-h observation session are also presented but due to the low sample size the power to detect differences is limited (Table 2).

Meal criterion

The meal criterion calculated using the entire dataset was 4 min and 8 s (95% CI: 2 min 8 s - 6 min 33 s; n = 1 205; Figure 2). The meal criteria for weevils on girdled seedlings (5 min 31 s; n = 621) and non-girdled seedlings (3 min 13 s; n = 584), as well as for males (3 min 54 s; n = 362) and females (4 min 20 s; n = 843) all have overlapping 95% confidence intervals. It was therefore concluded that the meal criterion based on the entire dataset was representative for all subsets.

Meal properties

Girdling of the seedlings did not affect any of the measured meal or other feeding properties and neither did the interaction of girdled/non-girdled seedlings and weevil sex ($P>0.05$ for all properties listed in Table 1). However, feeding patterns differed between males and females (Figure 3). The total meal duration during an observation session was significantly shorter for males than for females, and accordingly the total intermeal duration was longer (Table 2). Furthermore, fewer feeding occurrences within meals were observed for males, and both the average and total durations of their intrameal intervals were significantly shorter (Table 3). No other meal or feeding properties significantly differed between the sexes (Tables 2 and 3).

Male weevils had about four meals per day, each lasting about 25 min (median values; Tables 2 and 3). The intermeal intervals were about 6.5 h. During a meal by males, seven intrameal intervals with a duration of 9 s each were observed and feeding occurrences lasted slightly more than 2 min (all medians). Female weevils had about five meals per day, each lasting about 22 min, with an intermeal interval of approximately 4 h (all medians). There were 11 intrameal intervals, each lasting 13 s and the duration of their feeding occurrence was slightly less than 2 min (all medians).

Prandial correlations

The pre- and post-prandial correlations were both significant ($r_s = 0.33$, $P<0.001$ and $r_s = 0.23$, $P = 0.01$, respectively; both $n = 117$). When the meals were grouped into three equally sized classes according to the durations of the pre-prandial interval, the meals following medium pre-prandial intervals were more similar in size to those following long pre-prandial intervals than those following short pre-prandial intervals (16, 18, and 9 mm^2, respectively; medians) (Figure 4A). When the post-prandial intervals were grouped into three equally sized classes according to meal size, the post-prandial intervals following medium-sized meals were more similar in duration to those following small meals than to those following large meals (87, 64, and 160 min, respectively; medians) (Figure 4B).

Total feeding amount

Weevils removed 61 mm^2 of bark per observation session (median values based on means for individuals; $n = 12$), corresponding to about 6% of the bark available in the cylinders. The minimum amount of bark removed was 6 mm^2 and the maximum 114 mm^2. There was no significant difference in the total area of feeding scars between girdled and non-girdled seedlings (55.9 ± 8.6 and 59.5 ± 7.4 mm^2, respectively; $t = -0.32$, d.f. = 11, $P = 0.76$) nor between male and female weevils (49.2 ± 5.8 vs. 66.3 ± 8.7 mm^2, respectively; $t = 1.64$, d.f. = 8, $P = 0.14$).

Feeding scar area

The feeding of a pine weevil typically resulted in separate feeding scars that were produced during one or several meals. By the end of the observation sessions the median area of feeding scars made by males was 23 mm^2, very similar to the median area of scars known to be the result of a single male meal (21 mm^2) and the calculated median male meal size (median meal duration × median feeding rate = 22 mm^2). In contrast, the median feeding scar made by females by the end of an observation session (31 mm^2) was substantially larger than the median area of scars known to be the result of a single female meal (14 mm^2) and the calculated median female meal size (median meal duration × median feeding rate = 14 mm^2). There was a strong positive correlation between feeding scar area and meal duration (based on data from scars known to be the result of a single meal by the end of an observation period: $r_s = 0.63$, $P = 0.005$; $n = 17$).

Movement after meals

After 17% of the meals, the weevils did not leave the seedling before starting another meal. The time between meals was shorter when weevils did not leave the seedling before eating another meal than when they did [median: 32 ($n = 19$) vs. 119 min ($n = 74$); W = 3 818, $P<0.01$]. Weevils spent 32 min on seedlings after a meal when they did not leave the seedling before taking another meal and only 49 s when they left the seedling before the next meal (W = 4 553, $P<0.01$; medians). Four meals were excluded from this analysis, because the weevils stayed on the seedling after these meals until the observation session ended (median: 32 min 29 s).

Discussion

In most studies of short-term feeding behavior, log-survivorship or log-frequency analysis has been used to determine meal criteria (e.g., Sibly et al., 1990; Mitchell & Low, 1994; Tolkamp et al., 1998; Tolkamp et al., 2011). However, in recent years an approach taking the non-random initiation of feeding times into consideration has been developed, thereby providing a more biologically meaningful definition of a meal (Tolkamp et al., 2011). This may facilitate comparisons between distantly related chewing herbivores, as the underlying triggers of hunger and satiety are the same when food access is unlimited. Previously this method has only been used to analyze the feeding behavior of vertebrates (Tolkamp et al., 2011). Here we extend the methodology by applying it to calculate the meal criterion for a phytophagous chewing insect, the pine weevil.

The estimated meal criterion for the pine weevil (4 min 8 s) is in the lower range of meal criteria reported for other phytophagous insects, which include approximately 2 min for the fifth instars of the tobacco hornworm caterpillar [*Manduca sexta* (L.)] and the larvae of the silkworm (*Bombyx mori* L.), 3 min for the German cockroach [*Blattella germanica* (L.)], 4 min for the caterpillar of the cotton bollworm [*Helicoverpa armigera* (Hübner)], 5 min for the Colorado potato beetle [*Leptinotarsa decemlineata* (Say)], over 6 min for the migratory locust [*Locusta migratoria* (L.)], 8 min for the western horse lubber grasshopper [*Taeniopoda eques* (Burmeister)], 2-9 min for the first instar of the American bird grasshopper [*Schistocerca americana* (Drury)], about 12 min for the plains lubber grasshopper [*Brachystola magna* (Girard)], and up to about 16 min for the forest tent caterpillar (*Malacosoma disstria* Hübner) (Simpson, 1982; Reynolds et al., 1986; Chapman & Beerling, 1990; Raubenheimer & Bernays, 1993; Bright et al., 1994; Mitchell & Low, 1994; Raubenheimer & Browne, 2000; Jones & Raubenheimer, 2002; Nagata & Nagasawa, 2006; Colasurdo et al., 2007). However, all these meal criteria were estimated from log-survivorship analyses, which generally result in shorter estimated meal criteria, e.g., the meal criterion for cows increases from about 8 min to 35 min when estimated with a log-normal distribution instead of a log-survivorship analysis (Tolkamp & Kyriazakis, 1999).

We observed that some of the pine weevil meals did not have any intrameal intervals (16 out of 117 meals). Wright et al. (1999) observed no intrameal intervals in meals of the Costa Rican weevil [*Exopthalmus jekelianus* (White)]. The Colorado potato beetle did not have any intrameal intervals in approximately half of its meals (Mitchell & Low, 1994), whereas Chapman & Beerling (1990) observed intrameal intervals in all recorded meals of the first instar of the American bird grasshopper. These differences in intrameal structures are interesting, but more research is needed before this variation can be understood.

The pine weevil had about four meals per day, relatively few compared to other frequencies recorded for insect species: about 1.4 meals in 5 h for the German cockroach (Jones & Raubenheimer, 2002), five in 10 h for the Costa Rican weevil (Wright et al., 1999), 10 per day for the adult migratory locust (Simpson, 1982), 7-9 per day for advanced first instars of the American bird grasshopper (Chapman & Beerling, 1990), about six in 7 h for cotton bollworm caterpillars based on meal and intermeal durations (Raubenheimer & Browne, 2000), nine in 7 h for the Colorado potato beetle (Mitchell & Low, 1994), two per hour for tobacco hornworm caterpillars based on meal and intermeal durations (Reynolds et al., 1986), and up to 163 meals in 48 h for the forest tent caterpillar (Colasurdo et al., 2007).

The meals of the pine weevil lasted about 24 min, on average, close to the 20 min recorded for the Colorado potato beetle (Mitchell & Low, 1994) and the 33 min for the forest tent caterpillar on a balanced artificial diet (Colasurdo et al., 2007), but substantially longer than the recorded durations (2-18 min) for meals of Costa Rican weevil, the first instar of

American bird grasshopper, tobacco hornworm caterpillar, the caterpillar of the cotton bollworm, the larvae of the silkworm, German cockroach, and fifth instar migratory locusts (Simpson, 1982; Reynolds et al., 1986; Chapman & Beerling, 1990; Wright et al., 1999; Raubenheimer & Browne, 2000; Jones & Raubenheimer, 2002; Nagata & Nagasawa, 2006). In total, female pine weevils spent more time in meals than male pine weevils, which is in accordance with previous studies (Merivee et al., 1998; Bylund et al., 2004; Toivonen & Viiri, 2006). No such differences between the sexes were observed in the Colorado potato beetle (Szentesi & Jermy, 1993).

Both feeding rate and meal duration can influence meal size. Whether the main factors governing the termination of meals are the amount ingested or the time spent feeding (in the absence of interruptions by external agents, e.g., predators) can be determined from analyses of variations in feeding rates and meal sizes. For example, meals of the migratory locust are terminated after a certain time rather than after a certain amount has been ingested (Simpson, 1982). We calculated the meal sizes of pine weevils from their durations, which makes it more difficult to draw any such conclusions, but it was possible to determine the feeding rate directly for 17 out of 117 meals (both groups with similar median duration). Data for these 17 meals indicate that meal size is correlated linearly to feeding duration, but meal size is not correlated to feeding rate. Previous studies have found that female weevils have a higher feeding rate (Wainhouse et al., 2004) and make smaller feeding scars than males (Bylund et al., 2004; Toivonen & Viiri, 2006). However, neither was observed in the present study, indeed there were indications of the opposite. Like the pine weevil, feeding rate of the Colorado potato beetle appears to be roughly constant for small meals, but for larger meals it is substantially more variable (Mitchell & Low, 1994). The migratory locust, first instars of the American bird grasshopper, and the Australian sheep blowfly [*Lucilia cuprina* (Wiedemann)] also have variable feeding rates and quite constant meal durations (Simpson et al., 1988; Simpson et al., 1989; Chapman & Beerling, 1990). The feeding rates of these three insects per feeding occurrence do not increase with meal size – except when the migratory locust feeds very large meals –, but the overall feeding rate per meal increases, due to increases in the proportion of time spent feeding per meal and therewith a decrease of the time spent in intrameals (Simpson et al., 1988; Chapman & Beerling, 1990). In the Australian sheep blowfly, the feeding rate on food with a better quality is increased due to more time spent feeding within a meal (Simpson et al., 1989). Similarly, forest tent caterpillars increase the time spent feeding within a meal on protein-rich food (Colasurdo et al., 2007). Thus, several factors can influence interactions between feeding rate, meal duration, and the resulting meal size. For example, the insect's physiology influences how fast it can obtain and process food, and the composition of available food, such as deterrent or toxin contents, can profoundly affect meal durations or feeding rate (Szentesi & Bernays, 1984; Usher et al., 1988; Glendinning & Slansky, 1995; Jones & Raubenheimer, 2002).

Prandial correlations provide indications of the mechanisms underlying the initiation and termination of meals and predictions of both meal sizes (from durations of preceding non-feeding intervals) and durations of following non-feeding intervals (from meal sizes). Both pre-prandial and post-prandial correlations were significant for the pine weevil. Meals were much smaller after short pre-prandial intervals than after medium or long pre-prandial intervals. Similarly, post-prandial intervals were much shorter after small and medium-sized meals than after large meals. Both types of correlation are also significant for Colorado potato beetle, migratory locust, and cotton bollworm (Simpson, 1982; Simpson & Ludlow, 1986; Mitchell & Low, 1994; Raubenheimer & Browne, 2000). In contrast, Costa Rican weevils have a significant post-prandial correlation, but no pre-prandial correlation (Wright et al., 1999), whereas the opposite pattern has been observed for Australian sheep blowfly, tobacco hornworm, and American bird grasshopper (Reynolds et al., 1986; Bowdan, 1988a,b Simpson et al., 1989; Chapman & Beerling, 1990).

The pine weevils created one or several new feeding scars and sometimes returned to old scars during a meal. To estimate how many meals were needed to make a median-sized feeding scar, we compared the areas of feeding scars known to be the result of a single meal, the areas of feeding scars measured at the end of an observation session, and the areas of feeding scars calculated from the median meal duration and median feeding rate. There was good agreement between these estimates for scars made by males, thus most feeding scars made by males had a similar area as those known to be the result of a single meal. Female weevils, however, required 2.2 meals to create a median sized feeding scar. Consequently, the number of feeding scars made by a male weevil after 24 h could be used as a proxy for the number of meals, whereas it is more complicated for female weevils.

Most pine weevils that did not stay on the seedling to eat another meal left the seedling shortly after each meal was finished. Additionally, weevils that stayed on the seedling fed again sooner than those that left. These differences in behaviors may reflect a conflict between increased opportunities to meet a mate when staying on the seedling and the higher energy use when leaving the seedling.

None of the examined meal or other feeding properties significantly differed between weevils on girdled vs. non-girdled seedlings. Girdling of a seedling may affect food quality and therewith feeding patterns. Accordingly, the food quality (glucose concentration) negatively affects the size and frequency of meals, and intrameal durations in the Australian sheep blowfly (Simpson et al., 1989). The time interval between the first and second wounding chosen in the present study might not have been long enough to reach peak levels of induced defenses (Lewinsohn et al., 1991), but represents the naturally occurring situation for seedlings planted on clear cuts (Nordlander, 1991; Björklund et al., 2005), which might explain why no differences in feeding patterns were found.

Establishing meal criteria provides opportunities to explore short-term feeding patterns at high resolution, rather than merely examining the end results of feeding. Meal criteria have only been determined for a few insect species as yet, but the accumulation of data for more species should facilitate conclusions about differences in meal properties based on phylogeny, ecology, and physiology. In this study, we determined the meal criterion for the pine weevil with a method originally developed for vertebrates, thereby validating its use for invertebrates. Basic knowledge about feeding patterns could also assist in the setup and interpretation of studies of herbivore-plant interactions, and it may facilitate the evaluation and development of methods to protect plants against herbivores.

Acknowledgements

We thank Henrik Nordenhem and Anders Eriksson for assistance with the practical work, Mikael Andersson Franko for statistical support, and Allan Nordlund for measuring the light conditions. We appreciate the comments of Diem Nguyen, Johan Stenberg, and Marie Winsa on the manuscript. This study was financed by the Swedish Foundation for Strategic Research (Parasite Resistant Tree project) and the Swedish Forestry Sector (The Swedish *Hylobius* Research Program).

References

Bernays EA & Singer MS (1998) A rhythm underlying feeding behaviour in a highly polyphagous caterpillar. Physiological Entomology 23: 295-302.

Björklund N, Nordlander G & Bylund H (2005) Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil, *Hylobius abietis*. Physiological Entomology 30: 225-231.

Bowdan E (1988a) The effect of deprivation on the microstructure of feeding by the Tobacco hornworm caterpillar. Journal of Insect Behavior 1: 31-50.

Bowdan E (1988b) Microstructure of feeding by tobacco hornworm caterpillars, *Manduca sexta*. Entomologia Experimentalis et Applicata 47: 127-136.

Bright KL, Bernays EA & Moran VC (1994) Foraging patterns and dietary mixing in the field by the generalist grasshopper *Brachystola magna* (Orthoptera: Acrididae). Journal of Insect Behavior 7: 779-793.

Bylund H, Nordlander G & Nordenhem H (2004) Feeding and oviposition rates in the pine weevil *Hylobius abietis* (Coleoptera: Curculionidae). Bulletin of Entomological Research 94: 307-317.

Chapman RF & Beerling EAM (1990) The pattern of feeding of first instar nymphs of *Schistocerca americana*. Physiological Entomology 15: 1-12.

Clauss M, Schwarm A, Ortmann S, Streich WJ & Hummel J (2007) A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 148: 249-265.

Colasurdo N, Dussutour A & Despland E (2007) Do food protein and carbohydrate content influence the pattern of feeding and the tendency to explore of forest tent caterpillars? Journal of Insect Physiology 53: 1160-1168.

Eidmann HH (1974) *Hylobius abietis* L., Großer Brauner Rüsselkäfer, Vol. 2: Die Forstschädlinge Europas (ed. by W Schwenke), pp. 277-293. Paul Parey, Hamburg, Germany.

Fedderwitz F, Björklund N, Ninkovic V & Nordlander G (2014) Diel behaviour and time budget of the adult pine weevil, *Hylobius abietis*. Physiological Entomology 39: 103-110.

Glendinning JI & Slansky F (1995) Consumption of a toxic food by caterpillars increases with dietary exposure: support for a role of induced detoxification enzymes. Journal of Comparative Physiology A 176: 337-345.

Howie JA, Tolkamp BJ, Avendaño S & Kyriazakis I (2009) A novel flexible method to split feeding behaviour into bouts. Applied Animal Behaviour Science 116: 101-109.

Jones SA & Raubenheimer D (2002) Short-term responses by the German cockroach, Blattella germanica, to insecticidal baits: behavioural observations. Entomologia Experimentalis et Applicata 102: 1-11.

Kaakinen S, Jolkkonen A, Iivonen S & Vapaavuori E (2004) Growth, allocation and tissue chemistry of *Picea abies* seedlings affected by nutrient supply during the second growing season. Tree Physiology 24: 707-719.

Långström B & Day KR (2004) Damage, control and management of weevil pests, especially *Hylobius abietis*: Bark and Wood Boring Insects in Living Trees in Europe, A Synthesis (ed. by F Lieutier, KR Day, A Battisti, J-C Grégoire & HF Evans) Springer, Dordrecht, The Netherlands, pp. 415-444.

Le Magnen J & Tallon S (1966) La périodicité spontanée de la prise d'aliments ad libitum du rat blanc. Journal of Physiology 58: 323-349.

Lewinsohn E, Gijzen M & Croteau R (1991) Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosynthesis among species. Plant Physiology 96: 44-49.

Manlove JD, Styles J & Leather SR (1997) Feeding of the adults of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). European Journal of Entomology 94: 153-156.

Månsson PE & Schlyter F (2004) Hylobius pine weevils adult host selection and antifeedants: feeding behaviour on host and non-host woody Scandinavian plants. Agricultural and Forest Entomology 6: 165-171.

Merivee E, Sibul I & Luik A (1998) Diel dynamics of feeding and locomotor activities in the large pine weevil, *Hylobius abietis*. Baltic Forestry 4: 59-61.

Mitchell BK & Low R (1994) The structure of feeding behavior in the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Journal of Insect Behavior 7: 707-729.

Mithöfer A, Wanner G & Boland W (2005) Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. Plant Physiology 137: 1160-1168.

Nagata S & Nagasawa H (2006) Effects of diet-deprivation and physical stimulation on the feeding behaviour of the larvae of the silkworm, Bombyx mori. Journal of Insect Physiology 52: 807-815.

Nordlander G (1991) Host finding in the pine weevil *Hylobius abietis*: effects of conifer volatiles and added limonene. Entomologia Experimentalis et Applicata 59: 229-237.

Nordlander G, Nordenhem H & Hellqvist C (2009) A flexible sand coating (Conniflex) for the protection of conifer seedlings against damage by the pine weevil *Hylobius abietis*. Agricultural and Forest Entomology 11: 91-100.

Nuorteva M (1972) Die Geschwindigkeit der Nahrungspassage durch den Darmkanal bei *Hylobius abietis* L. Imagines (Col., Curculionidae). Annales Entomologici Fennici 38: 113-118.

Petersson M & Örlander G (2003) Effectiveness of combinations of shelterwood, scarification, and feeding barriers to reduce pine weevil damage. Canadian Journal of Forest Research 33: 64-73.

Raubenheimer D & Bernays EA (1993) Patterns of feeding in the polyphagous grasshopper *Taeniopoda eques*: a field study. Animal Behaviour 45: 153-167.

Raubenheimer D & Browne LB (2000) Developmental changes in the patterns of feeding in fourth- and fifth-instar *Helicoverpa armigera* caterpillars. Physiological Entomology 25: 390-399.

Reynolds SE, Yeomans MR & Timmins WA (1986) The feeding behaviour of caterpillars (*Manduca sexta*) on tobacco and on artificial diet. Physiological Entomology 11: 39-51.

Sampedro L, Moreira X, Martins P & Zas R (2009) Growth and nutritional response of *Pinus pinaster* after a large pine weevil (*Hylobius abietis*) attack. Trees - Structure and Function 23: 1189-1197.

Savory CJ (1981) Correlations between meals and inter-meal intervals in Japanese quail and their significance in the control of feeding. Behavioural Processes 6: 23-36.

Sibly RM, Nott HMR & Fletcher DJ (1990) Splitting behavior into bouts. Animal Behaviour 39: 63-69.

Simpson SJ (1981) An oscillation underlying feeding and a number of other behaviors in fifth-instar *Locusta migratoria* nymphs. Physiological Entomology 6: 315-324.

Simpson SJ (1982) Patterns in feeding: a behavioral analysis using *Locusta migratoria* nymphs. Physiological Entomology 7: 325-336.

Simpson SJ (1990) The pattern of feeding. Biology of grasshoppers (ed. by RF Chapman & A Joern) , pp. 73-104. John Wiley and Sons, New York, NY, USA.

Simpson SJ, Browne LB & van Gerwen ACM (1989) The patterning of compensatory sugar feeding in the Australian sheep blowfly. Physiological Entomology 14: 91-105.

Simpson SJ & Ludlow AR (1986) Why locusts start to feed: a comparison of causal factors. Animal Behaviour 34: 480-496.

Simpson SJ, Simmonds MSJ, Wheatley AR & Bernays EA (1988) The control of meal termination in the locust. Animal Behaviour 36: 1216-1227.

Szentesi A & Bernays EA (1984) A study of behavioural habituation to a feeding deterrent in nymphs of *Schistocerca gregaria*. Physiological Entomology 9: 329-340.

Szentesi A & Jermy T (1993) A comparison of food-related behaviour between geographic populations of the Colorado potato beetle (Coleoptera, Chrysomelidae), on six solanaceous plant species. Entomologia Experimentalis et Applicata 66: 283-293.

Toivonen R & Viiri H (2006) Adult large pine weevils *Hylobius abietis* feed on silver birch *Betula pendula* even in the presence of conifer seedlings. Agricultural and Forest Entomology 8: 121-128.

Tolkamp BJ, Allcroft DJ, Austin EJ, Nielsen BL & Kyriazakis I (1998) Satiety splits feeding behaviour into bouts. Journal of Theoretical Biology 194: 235-250.

Tolkamp BJ, Allcroft DJ, Barrio JP, Bley TAG, Howie JA et al. (2011) The temporal structure of feeding behavior. American Journal of Physiology 301: R378-R393.

Tolkamp BJ & Kyriazakis I (1999) To split behaviour into bouts, log-transform the intervals. Animal Behaviour 57: 807-817.

Tolkamp BJ, Schweitzer DPN & Kyriazakis I (2000) The biologically relevant unit for the analysis of short-term feeding behavior of dairy cows. Journal of Dairy Science 83: 2057-2068.

Tomlin ES, Alfaro RI, Borden JH & He F (1998) Histological response of resistant and susceptible white spruce to simulated white pine weevil damage. Tree Physiology 18: 21-28.

Usher BF, Bernays EA & Barbehenn RV (1988) Antifeedant tests with larvae of *Pseudaletia unipuncta*: variability of behavioral response. Entomologia Experimentalis et Applicata 48: 203-212.

Wainhouse D, Boswell R & Ashburner R (2004) Maturation feeding and reproductive development in adult pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). Bulletin of Entomological Research 94: 81-87.

Wainhouse D, Staley J, Johnston J & Boswell R (2005) The effect of environmentally induced changes in the bark of young conifers on feeding behaviour and reproductive development of adult *Hylobius abietis* (Coleoptera: Curculionidae). Bulletin of Entomological Research 95: 151-159.

Wright GA, Raubenheimer D & Simpson SJ (1999) An analysis of the pattern of feeding in the Costa Rican weevil, *Exopthalmus jekelianus*, on the tropical tree, *Cedrela odorata*. Physiological Entomology 24: 391-397.

Zas R, Björklund N, Nordlander G, Cendan C, Hellqvist C & Sampedro L (2014) Exploiting jasmonate-induced responses for field protection of conifer seedlings against a major forest pest, *Hylobius abietis*. Forest Ecology and Management 313: 212-223.

Zas R, Moreira X & Sampedro L (2011) Tolerance and induced resistance in a native and an exotic pine species: relevant traits for invasion ecology. Journal of Ecology 99: 1316-1326.

Zas R, Sampedro L & Moreira X (2012) The role of induced defences in the success of an exotic pine: the importance of recognizing your enemies. IOBC/WPRS Bulletin 83: 37-41.

Zas R, Sampedro L, Moreira X & Martíns P (2008) Effect of fertilization and genetic variation on susceptibility of *Pinus radiata* seedlings to *Hylobius abietis* damage. Canadian Journal of Forest Research 38: 63-72.

Figure captions

Figure 1 Experimental setup. A small tube with water and a dark-colored refuge for the pine weevils inside the cylinder are not shown.

Figure 2 Distribution of non-feeding intervals (n = 1 205) in all pine weevil observation sessions and fitted model for two Weibull distributions. The arrow indicates the intersection and thus the location of the meal criterion.

Figure 3 Schematic feeding pattern of male and female pine weevils during 24 h; medians based on mean values for individuals (*$P<0.05$ between sexes; linear mixed-effects models) (see also Tables 2 and 3).

Figure 4 (A) Pre-prandial and (B) post-prandial correlations. Pre-prandial correlation based on the distribution of pine weevils' meal size (bin width 5 mm^2, n = 117) and post-prandial correlation based on the distribution of intermeal intervals after a meal (bin width 30 min, n = 117). Data were split into groups of equal sizes based on intermeal intervals before the meal for pre-prandial correlations and based on meal size for post-prandial correlations. Dashed lines represent median values.

Table 1 Definition of meal and other feeding properties

Feeding/meal property	Unit	Definition
No. meals		No. all meals during one observation session
Total meal duration	h	Summed durations of all meals during one observation session
No. intermeal intervals		No. all intermeal intervals (i.e., non-feeding intervals between meals) during one observation session
Total duration of intermeal intervals	h	Summed durations of all intermeal intervals during one observation session
Total area eaten	mm^2	Area of bark removed during one observation session
Feeding scar area	mm^2	Area of individual feeding scars after an observation session
Feeding rate	$mm^2\ s^{-1}$	Amount of bark removed per unit time (see text for details)
Meal duration	s	Duration of a single meal including feeding and non-feeding intrameal intervals
Intermeal interval duration	min	Duration of a single intermeal interval
Feeding amount	mm^2	Area of bark removed during one meal (meal duration × feeding rate)
Feeding percentage	%	Percentage of time spent feeding within a meal
No. feeding occurrences		No. single feeding occurrences per meal separated by intrameals
Total feeding within a meal	min	Duration of all feeding within one meal
Duration of feeding occurrence	min	Duration of a single feeding occurrence
No. intrameals		No. intrameal intervals during one meal
Total duration of intrameals	min	Duration of all intrameal intervals during one meal
Duration of intrameal	min	Duration of one intrameal interval

Table 2 Meal and other feeding properties for male and female pine weevils (per 24-h observation session). Medians are based on untransformed means per individual. Grand means are based on transformed data (Mean^T)

Feeding/meal property	Median		Mean^T ± SE			
	Male	Female	Male	Female	t (d.f. = 10)[1]	P
No. meals	3.50	5.25	1.32 ± 0.20[2]	1.78 ± 0.17[2]	1.75	0.11
Total meal duration (min)	64.59	102.78	7.73 ± 0.84[3]	10.84 ± 0.84[3]	2.62	0.03
No. intermeal intervals[4]	3.50	5.25	1.56 ± 0.16[2]	1.93 ± 0.14[2]	1.73	0.12
Total duration of intermeal intervals (h)	22.70	21.93	22.66 ± 0.26	21.64 ± 0.26	2.74	0.02
Total area eaten (mm^2)	41.75	66.25	49.17 ± 7.61	66.25 ± 7.61	1.59	0.14
Feeding scar area (mm^2)	23.18	31.33	2.96 ± 0.19[2]	3.27 ± 0.19[2]	1.16	0.27
Feeding rate[5] (mm^2/min)	0.90	0.62	1.15 ± 0.19[3]	0.75 ± 0.19[3]	1.46	0.18

[1] Linear mixed-effects models.

[2] ln-transformation was used to meet normality and homoscedasticity requirements.

[3] Square-root transformation was used to meet normality and homoscedasticity requirements.

[4] The first and last intermeal per observation were combined to minimize underestimation of the length of non-feeding intervals and represent continuity between days.

[5] One observation session was excluded because the calculated feeding rate (16.33 mm^2/min) was considered to be an outlier.

Table 3 Meal properties for male and female pine weevils (per meal). Medians are based on untransformed means per individual. Grand means are based on transformed data data (Mean^{T})

Meal property	Median		Mean^{T} ± SE			
	Male	Female	Male	Female	t (d.f. = 10)[1]	P
Meal duration (min)	24.58	22.42	3.97 ± 0.43[3]	4.21 ± 0.39[3]	0.42	0.69
Intermeal interval duration[4] (min)	393.51	258.49	5.23 ± 0.31[2]	4.75 ± 0.28[2]	1.15	0.28
Feeding amount (mm^2)	13.81	11.75	3.44 ± 0.38[3]	3.15 ± 0.36[3]	0.55	0.60
Feeding percentage (%)	94.51	89.01	7.54 ± 0.28[3]	7.01 ± 0.23[3]	1.44	0.18
No. feeding occurrences	8.04	12.33	2.00 ± 0.11[2]	2.33 ± 0.09[2]	2.39	0.04
Total feeding within a meal (min)	23.17	19.48	3.81 ± 0.44[3]	3.96 ± 0.41[3]	0.24	0.82
Duration of feeding occurrence (min)	2.21	1.71	0.09 ± 0.17[2]	-0.22 ± 0.16[2]	1.34	0.21
No. intrameals	7.04	11.35	1.80 ± 0.13[2]	2.17 ± 0.11[2]	2.14	0.06
Total duration of intrameals (min)	1.27	2.75	0.92 ± 0.11[3]	1.33 ± 0.09[3]	2.80	0.02
Duration of intrameal (s)	9.23	13.31	1.67 ± 0.08[b]	1.93 ± 0.08[b]	2.56	0.03

[1] Linear mixed-effects models.

[2] ln-transformation was used to meet normality and homoscedasticity requirements.

[3] Square-root transformation was used to meet normality and homoscedasticity requirements.

[4] The first and last intermeal per observation were combined to minimize underestimation of the length of non-feeding intervals and represent continuity between days.

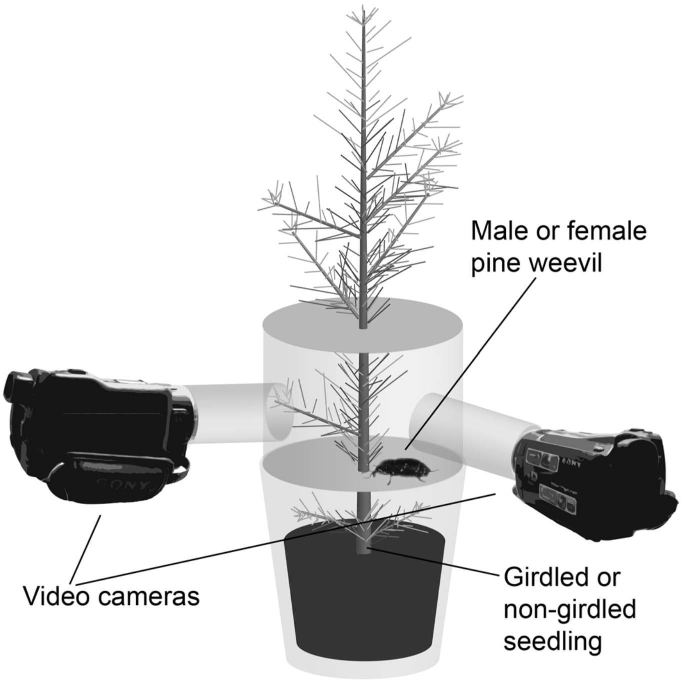

Figure 1

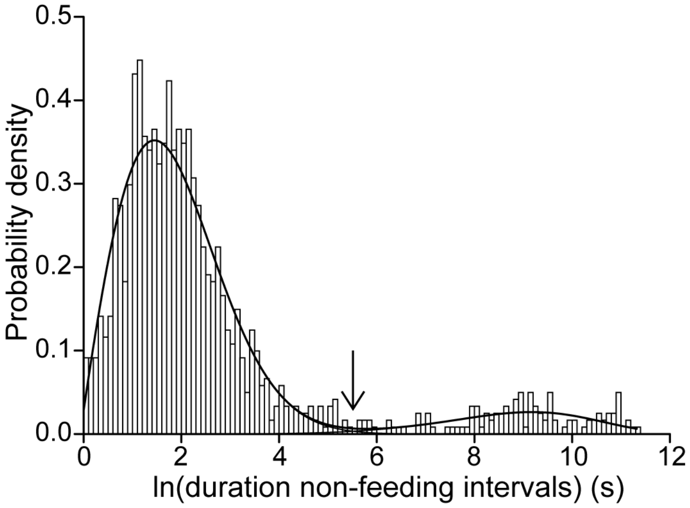

Figure 2

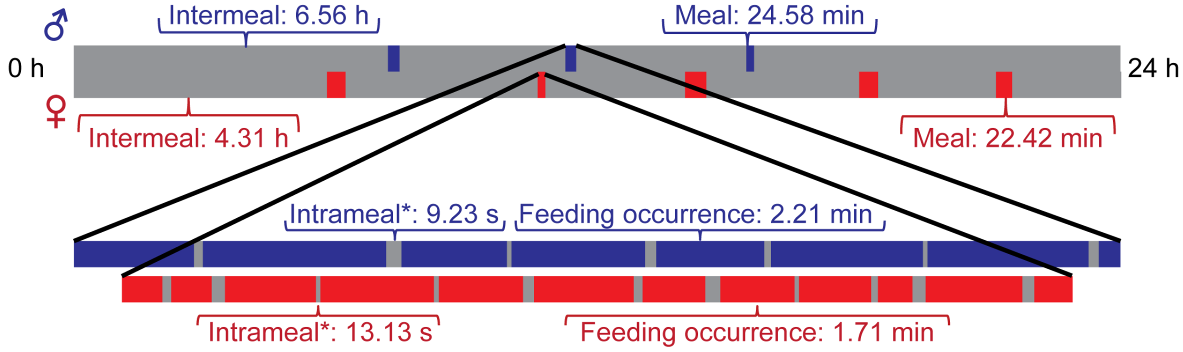

Figure 3

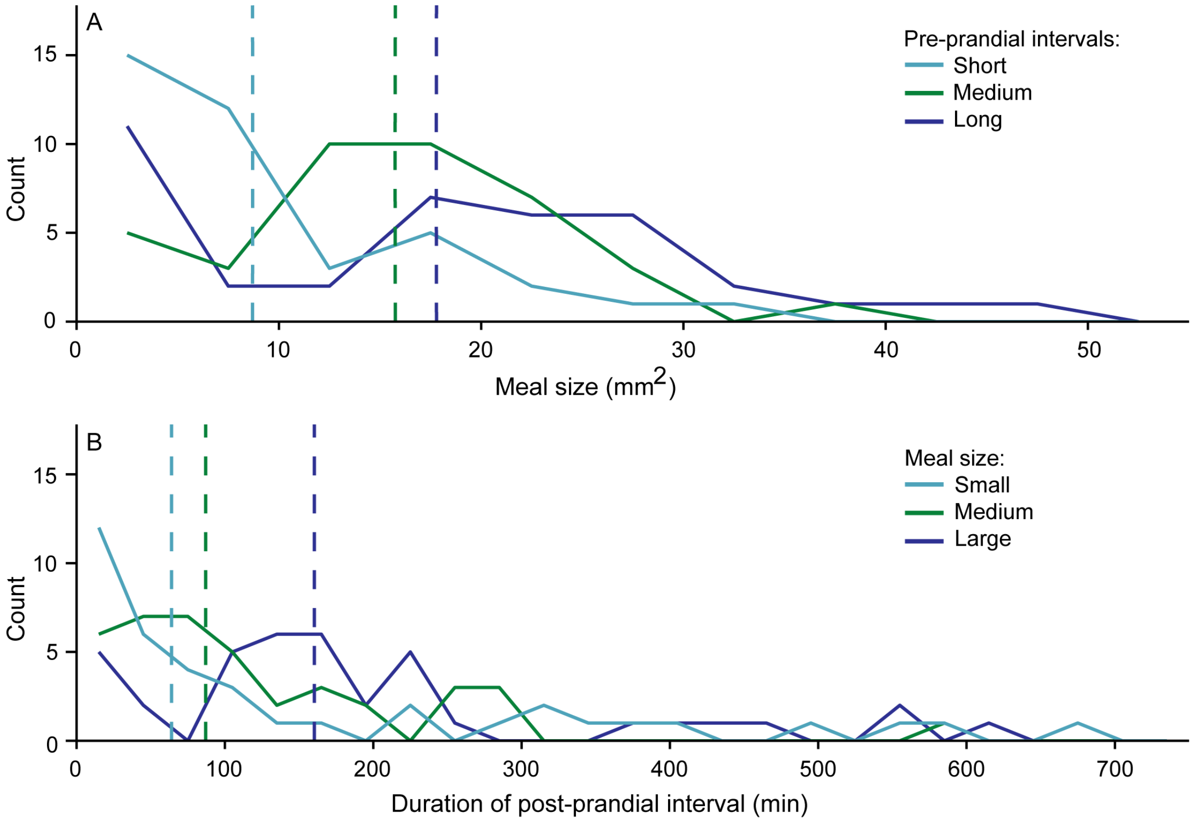

Figure 4