

The Effect of Trails and Group Size on the Performance and Behaviour of the Forest Tent
Caterpillar (*Malacosoma disstria* Hübner)

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Abstract

The Effect of Trails and Group Size on the Performance and Behaviour of the Forest Tent Caterpillar (*Malacosoma disstria* Hübner)

Alice Le Huu

The presence of trails and group size play a role in the biology of forest tent caterpillars (*Malacosoma disstria*). Therefore, the effect of the presence of trails and group size on caterpillar performance and behaviour were studied in a laboratory setting. Since forest tent caterpillars may undergo an ontogenetic shift from gregarious to solitary individuals as they mature, these effects were tested on second and fourth instar larvae.

For this study, caterpillars were placed in environments with pre-laid trails and of different group sizes. Caterpillars were reared through a larval instar and their performance was measured at the end of the larval stage. Second instar larvae developed faster, and were smaller, when reared in the presence of trails and at larger group sizes. In contrast, fourth instar larvae did not show any differences in development rate.

For behavioural observations, larvae were placed in similar environments to those in the performance experiments. The behaviour and foraging efficiency of caterpillars was monitored at regular intervals for four hours. For second instars, results suggest that differences in growth and development time in the presence of trails may be linked to foraging success. Young caterpillars are highly dependent on trails to locate their food while older ones are not. Behavioural observations show differences in frequency and duration of certain behaviours between second and fourth instar larvae. Changes in the performance and behaviour between larval stages suggest an ontogenetic shift may occur between second and fourth instars.

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Chapter 1

General Introduction

Tent caterpillars are well renowned for their elaborate silk tents; they live in large colonies and build highly visible shelters. Tent caterpillars are often referred to as a colonial species because they are highly gregarious insects that have sophisticated social behaviours including shelter building, thermoregulation, cooperative foraging and antipredator defense (Fitzgerald 1995). However, they are not considered to be a communal species because they lack cooperative brood care, reproductive division of labor, and parent offspring aggregation (Costa and Fitzgerald 1996).

Due to its tremendous economic impact on forests, the forest tent caterpillar (FTC), *Malacosoma disstria*, is the subject of intense study. Most of the damage to forests occurs because they are early spring feeders. Forest tent caterpillars emerge from their egg masses just as the first leaves of the season are appearing (Fitzgerald 1995). As a result, most of their larval stage is spent feeding on highly nutritious spring leaves. In mid summer, the forest tent caterpillars pupate and moths emerge from their cocoon in late spring or early summer (Hodson 1939). These adult moths do not feed: mating takes place within a few hours of eclosion and females lay a single, large cluster of eggs before dying (Snodgrass 1961). The eggs remain in diapause over winter until the following spring when the larvae will emerge from their eggs (Hodson 1939). The egg hatching is timed to coincide with the budburst of host trees (Fashingbauer et al. as cited in Fitzgerald 1995).

The FTC is an outbreaking forest pest that is responsible for severe deforestation in the United States and in southern Canada (Rothman and Roland 1998, Fitzgerald

1995). In Canada, population outbreaks occur intermittently, and last about 6.5 years (Hodson 1941). FTC primarily feed on trembling aspen (*Populus tremuloides*) and sugar maple (*Acer saccharum*) (Goyer et al. 1987). Gregory and Wargo (1986) found that the initial defoliation of young spring leaves is harmful to trees; some species such as trembling aspen and sugar maple can produce new leaves, while others trees cannot. Newly refoliated aspen trees will usually have smaller and fewer leaves. In addition, these trees will produce less wood and store less energy reserves (Gregory and Wargo 1986). Churchill et al. (1964) suggests that refoliated trees also suffer from an increase in mortality due to increased susceptibility to pathogens. The effect of defoliation may not be immediately apparent, but studies suggest that mortality of defoliated trees may increase in successive years (Churchill et al. 1964). It is thus evident that FTC population outbreaks may have a severe effect on Canada's maple and forestry industry therefore, from an economic standpoint, it is important to determine the causes or factors that influence FTC outbreaks. Research has focused on numerous aspects of FTC biology with the hopes of containing, or controlling these outbreaks. Viral pathogens, weather, and induced plant chemical defenses have all been implicated in FTC population outbreaks, but the causes of these outbreaks remain unclear (Beisner and Myers 2000). It has been suggested that in tent caterpillars, the benefits of group living, such as increased foraging success and protection from predators may play a role in increasing FTC populations (Fitzgerald 1995). Since the benefits of group living appear to play a role in population dynamics, we will be studying this aspect of FTC biology. Group living is a common characteristic among outbreaking forest pests and may thus be integral to the understanding of population fluctuations (Smits 2002).

All tent caterpillars emerge from the egg mass and form large colonies. They rest together and move together between feeding sites. The FTC differs from other tent caterpillars because it does not construct a silk tent for shelter. Instead, the FTC constructs silk mats a short distance from its feeding site where the colony rests when it is not foraging. Fitzgerald (1993) classifies FTC as unique because they are the only species of *Malacosoma* that are nomadic foragers. They travel between food sources, and will often abandon a source before it is completely depleted. After feeding, a colony will construct a temporary silk mat (Fitzgerald 1993). The silk mat acts as a resting place where FTC can huddle together for thermoregulation; however, FTC will abandon their silk mat while searching for new food sources (Fitzgerald 1995). They will then construct a new silk mat a short distance from their new food source (Fitzgerald and Costa 1986). In contrast, most other *Malacosoma* species are central place foragers (Fitzgerald 1995). This means that they use a tent as a fixed base for foraging bouts (Fitzgerald and Edgally 1979a). Caterpillars will make repeated foraging bouts, but individuals will return to their shelter once they have fed (Fitzgerald and Peterson 1983).

Larval FTC emerge from the egg and aggregate in colonies throughout their first three larval instars. A study performed by Fitzgerald and Costa (1986) demonstrated that lab reared FTC colonies are highly cohesive: out of 256 colony days, first and second instars will spend 86% of the time in a single cohesive unit, 12% of the time split into two groups, and 3% in groups of 3. However, as the larvae mature past their third instar, the colony fragments. Fourth instars are much more independent and are often observed foraging alone (Fitzgerald and Costa 2000).

The shift from group to solitary living may be the result of a delicate interplay between the costs and benefits of group living. Living in colonies may provide advantages in the field by increasing thermoregulation, for antipredator behaviour, and improved foraging and mobility (Robison 1993). Ecological theory predicts that there are also individual fitness costs associated with an increase in group size. For example, there will be increased competition for resources which may lead to aggression among group members, as well as increased disease transmission (Giraldeau and Caraco 1999). Increased disease transmission may be an important cost of group living for forest tent caterpillars. FTC are susceptible to infection by nuclear polyhedrosis viruses, and it has been suggested that a diseased first instar larva may contain sufficient virus to kill one million other first instars (Stairs 1972).

Thermoregulation may be an important advantage of sociality in young FTC. It has been shown that in eastern tent caterpillars, *Malacosoma americanum*, at the center of an aggregation are protected from wind by the bodies of their colony mates (Joos et al. 1988). Since FTC are active in early spring, they encounter low temperatures. They may aggregate in order to decrease their body heat loss. Costa (1997) suggests that group living can also improve antipredator defense: tent caterpillars flick the anterior portion of their body from side to side when attacked by a predator. This flicking behaviour is believed to ward off predators or to discourage parasitoids from approaching (Costa 1997). This type of behaviour is frequently observed in aggregated tent caterpillars; and is less common in solitary caterpillars (Fitzgerald 1993). Large colonies which engage in this flicking display are more likely to scare off predators than a single individual engaging in this type of behaviour (Fitzgerald 1995).

Robison (1993) found that lab reared FTC grow more slowly and have lower survival rates when raised in groups below five individuals. This effect was particularly strong in first instars. Survival for first instar FTC reared in isolation was very low (Robison 1993). Fitzgerald (1993) personal observation of isolated FTC is that individuals tend to wander extensively. This suggests that the caterpillar may be searching for its colony mates (Fitzgerald 1993). Therefore, it appears that colony aggregation is an integral aspect of FTC biology and may be important even under optimal laboratory conditions.

Forest tent caterpillars spin silk from their labial glands, and the pheromone 5β -cholestane-3-one, is secreted from a sternal secretory site located between their prolegs (Fitzgerald 1995; Fitzgerald and Webster 1993). This steroid is relatively non-volatile and is detected through contact chemoreception (Fitzgerald and Peterson 1988). Forest tent caterpillars have been shown to use a complex system of silk and pheromone trails for communication (Fitzgerald and Webster 1993). A pheromone is a chemical released by an organism into its environment enabling it to communicate with other members of its own species (Wyatt 2003). The use of pheromones in communication suggests that the signal is beneficial to the sender and the receiver (Dusenbery as cited in Wyatt 2003). Forest tent caterpillar larvae continuously deposit silk and pheromone trails during locomotion. Caterpillar may benefit from laying down pheromone trails from their food to their silk mats (Fitzgerald 1995). If a caterpillar were to return to its food source, it would waste less time locating its food. Colony mates may also benefit from contact with the pheromone trails. The chemical trails are believed to be used in communication with colony mates and to help maintain colony cohesiveness during synchronous

foraging bouts (Fitzgerald and Costa 1986). Fitzgerald (1993) suggests that group foraging may increase foraging efficiency in the field. The silk trails may increase FTC purchase over physical obstacles that prevent them from reaching food sources (1993).

Although trail following behaviour has been demonstrated in many social caterpillars, it has been extensively studied in the Eastern tent caterpillar. This species deposits silk and pheromone while foraging in a manner similar to the FTC. If foraging is successful, upon its return to the tent, the eastern tent caterpillar will reinforce the trail by repeatedly rubbing its sternum against the substrate (Fitzgerald and Edgerly 1979). As a result, the caterpillar will leave a stronger pheromone trail for its colony mates to follow (Fitzgerald 1995). Consequently, eastern tent caterpillars can distinguish between trails which lead to profitable and to non-profitable food sources (Fitzgerald and Webster 1993). A higher number of colony members are recruited to the most profitable resources with low competition and high nutritive value (Fitzgerald and Peterson 1983).

Although the role of trail following in FTC has not been directly investigated, it is possible that FTC use silk trails in order to increase their foraging efficiency in similar manner to the eastern tent caterpillar. Although comparisons may be drawn between the FTC and the eastern tent caterpillar, it is important to note that there are some fundamental differences between the two species. As mentioned previously, the eastern tent caterpillar is a central place forager which returns to its shelter after each foraging bout. In contrast, the FTC is not constrained by a shelter and can move from food source to food source. Fitzgerald (1995) suggests that trails are more important to the eastern tent caterpillar because they return to their tent several times, whereas FTC are nomadic. It will thus be useful for the eastern tent caterpillar to reinforce successful trails so that it

does not waste energy searching for another food source (Fitzgerald 1995). However, trails may have a use beyond elective recruitment in the FTC. Trails are believed to be important in maintaining colony cohesion when the FTC travel from one feeding site to another. Since the FTC do not maintain physical contact while they travel, the trails may enable them to follow their colony mates (Fitzgerald and Costa 1986). In the field, silk may also be important in providing a stable substrate for FTC to grip as individuals move across obstacles on their way to new feeding sites (Fitzgerald and Webster 1993). Groups of FTC may benefit from trails by maintaining colony cohesion during foraging bouts (Robison 1993). By maintaining contact during foraging bouts, groups FTC can gather on a silk mat and engage in behaviour that promotes thermoregulation for the whole colony.

Despite the advantages of group living, there are also many disadvantages. Group living may also facilitate the transmission of viral and bacterial infections (Beisner and Myers 2000). Solitary caterpillars are less likely to come into contact with diseased individuals and contaminated feces than caterpillars living in a large group (Costa 1997). In the field, an increase in group size may result in competition for limited amounts of food resources. In the first instar, an entire FTC colony can survive on a single leaf; however, as caterpillars grow and consume more food, competition may become increasingly intense (Fitzgerald 1995, Robison 1993). This may lead to food shortages or an increased amount of energy spent foraging for food, and less energy incorporated into growth (Rothman 1997). The response of second and fourth instars to selection pressures may change. As caterpillars grow, fourth instars may experience more intraspecific competition because they are much larger and need to consume more food. This may cause the caterpillar to abandon its colony mates and engage in solitary foraging. It is

thus evident that living in colonies has some disadvantages. A shift in the relative costs and benefits of group-living, might explain the decrease in social behaviour as caterpillars grow (Despland and Hamzeh 2004).

The purpose of this research is to study possible costs and benefits associated with group living in forest tent caterpillars. During population outbreaks, FTC are more likely to experience crowded conditions and increased interactions with other caterpillars. An investigation of the effects of group living on forest tent caterpillars may contribute to an understanding of the population dynamics. Fitzgerald (1995) has suggested that rapid population growth may occur under favorable weather conditions and may be facilitated by larval gregariousness which reduces predation and increases speed of development (Fitzgerald 1995). This research project will be focus on the performance and behaviour of the forest tent caterpillar under different group sizes in order to assess the possible costs and benefits associated with group living. Benefits found to be associated with group living may be investigated further to determine if they are implicated in population surges. In addition, since the number of trails in the caterpillar's environment is linked to its population size, trails may have an indirect impact on forest tent population dynamics.

Consequently, the following laboratory experiments focus on the effects of trails and group living on FTC performance and behaviour. For these experiments, the performance of the forest tent caterpillar will refer to its rate of development, growth over the duration of the instar stage, and the caterpillar's efficiency of conversion of digested material. These effects were studied for early and late instars to assess if an ontogenetic change occurs between these two life stages. Experiments were designed to separate the effects of group size from those of silk and pheromone trails. The first experiment

examines the effect of trails and group living on growth and development of second and fourth instar larvae. The second experiment will examine the mechanisms involved by studying the time budgets of second and fourth instars.

Chapter 2

The Effect of Trails and Group Size on the Performance of the Forest Tent Caterpillar

2.1 Abstract

Forest tent caterpillars (*Malacosoma disstria*) demonstrate highly gregarious behaviour in their early instars; however, grouping decreases at their fourth instar. Caterpillars appear to rely on a complex system of pheromone trails for communication. We tested the effect of trails and group size on the performance of the caterpillar for second and fourth instar larvae. Insects were reared in environments with pre-laid trails or in control treatments. Each treatment had three different group sizes.

Trails increased growth in second and fourth instar larvae. Trails also accelerated development for second instars. In large groups, second instar larvae had an increased rate of development. However, their weight gain over the second stadium was decreased. Fourth instar caterpillars did not show any significant difference in their rate of development due to group size. However, in large groups, their weight gain over the fourth stadium was decreased.

Second instars may demonstrate accelerated development in order to avoid possible food shortages due to crowding. In contrast, fourth instars had reduced growth over their stadium, so this implies that there is a cost to crowding. In the forest tent caterpillar, differences in the performance of second and fourth instars may indicate an ontogenetic shift. Results from this study suggest that the ontogenetic shift in FTC may be plastic due to the cost and benefit tradeoffs seen between second and fourth instar.

2.2 Introduction

Although a theoretical framework for the costs and benefits of group living exists, it is often difficult to demonstrate these effects empirically. In an attempt to assess the costs and benefits of group living, two factors that are integral to FTC biology, group size and trails, were tested to see if they improve FTC performance. Previously, grouping has been shown to be important in FTC survival and development, especially in young larvae. Under laboratory conditions, Robison (1993) noted that first instars grow more slowly and have lower survival rates when they are raised in groups of less than five individuals. The mechanism behind this effect remains unclear, although it has been suggested that foraging efficiency may be involved (Robison 1993). The present experiment will further investigate the effects of grouping and trails by examining the variables of growth, rate of development, and gravimetric analysis, as a measure of FTC performance. The growth of the individual is the weight gained over the duration of the stadium, while the rate of development is the duration of the larval stadium. Gravimetric analysis is the quantitative analysis of food use by weight (Fitzgerald 1995). Waldbauer (1968) measured food use with the approximate digestibility of the food; the efficiency of conversion of digested material into body mass; and the efficiency of conversion of ingested material into body mass (Waldbauer 1968). These variables may then be used to assess net costs and benefits of group living.

This experiment was designed to separate the effect of trails and group size. These two variables cannot be completely separated because over time, the presence of group members results in the formation of trails. Therefore, this experiment attempted to

determine if the presence of trails at the onset of the experiment had an effect on the FTC performance.

Observations of FTC colonies have shown that second instars are highly gregarious while fourth instars are more solitary (Fitzgerald 1993). This may be due to different responses to selective pressures between the two stages of development (Despland and Hamzeh 2004). Differences in responses should not be surprising since fourth instar caterpillars are subject to different environmental conditions. For example, fourth instars occur in the summer; consequently, they are subject to higher temperatures than second instar larvae. In addition, fourth instar larvae must consume an entire leaf on their own, while a colony of second instar larvae can subsist on a single leaf (Fitzgerald 1993). Fourth instar caterpillars are more likely to experience competition for resources, since they require larger amounts of food. Even if they are solitary in nature, fourth instars are likely to encounter other FTC during their foraging bouts and experience intraspecific competition. Since fourth instars consume large quantities of food, the costs of group living may exceed the benefits in fourth instar larvae, explaining the observed shift from gregarious to solitary behaviour.

In this experiment, fourth instars are artificially subjected to crowded conditions in the hopes of measuring the cost and benefits of group living at each instar stage. To assess if the costs of group living differed between developmental stages, the effects of group size and trails on FTC performance was determined for second and fourth instar caterpillars.

2.3 Methods and Materials

2.3.1 Experimental Insects

FTC egg masses which had completed diapause were purchased from the Great Lakes Forestry Center operated by the Canadian Forest Service. In an effort to kill viral pathogens that remain on the exterior of the egg masses, eggs were placed in Javex for 90 seconds, then rinsed with water for 3-5 minutes. Finally, egg batches were washed with 1% Javex and placed in plastic containers with cardboard lids. The bottom of the container was filled with Addy's diet, and caterpillars hatched directly on their food (Addy 1969). Each morning, containers were checked for newly hatched larvae. Larvae remained in 23 ml cups until they molted into their second instar larvae, then were transferred into 250 ml cups with cardboard lids. Every second week, culture insects were transferred into new plastic cups, and the artificial diet was replaced. Containers were monitored daily, and each successive molt to a larval stage was recorded. Newly molted second and fourth instar caterpillars were removed from the culture and used for the experiments.

2.3.2 Experimental Design

The following experimental protocol was performed for newly molted second and fourth instar caterpillars. For the first experiment, a two-way factorial design was used to determine the effect of two variables; presence of trails and different group sizes, on the performance of a focal individual. The performance variables measured were the

development time and the wet weight gain of a focal individual caterpillar. For each trail and group size treatment there were ten replicates.

During the experiment, second instar larvae were housed in petri dishes with a diameter of 9 cm, while fourth instar caterpillars were placed in petri dishes with a diameter of 15 cm. Fourth instars were placed in larger petri dishes because they are much larger and more mobile than second instars.

Moist paper towel lined the bottom of the petri dishes, and a piece of wax paper was placed on this paper towel. Nylon netting was then suspended over the wax paper. The food and experimental insects were placed onto the nylon netting to prevent them from coming into contact with the wax paper and wet paper towel that lined the bottom of the petri dish.

2.3.3 Trail Treatments and Experimental Diet

To create the trail treatments, ten caterpillars from a variety of egg batches and of the same larval stadium as the experimental insects, were placed on the nylon netting for two hours. Once two hours had elapsed, these caterpillars were removed from the petri dish and the experimental insects were placed in the petri dish. In each of the replicates, a focal individual was identified and some poster paint was used to mark its abdominal setae. Care was taken to ensure that the mobility of second and fourth instar larvae was unaffected by the paint on its setae. The weight of the focal individual was recorded, as was the total mass of the group.

Small cubes of food were left out at room temperature for two hours before the experiment, prior to weighing. This allowed for excess moisture on the surface of the

food to evaporate and the weight of the food to stabilize. Food was placed onto the nylon netting on the opposite side of the petri dish from where the focal individual was located.

2.3.4 Experimental Protocol

Petri dishes were housed in growth chambers at 22⁰C with 8:16 night to day photoperiod. A container of water was placed at the bottom of the growth chamber to ensure that the environment remained humid. Each morning, the dishes were monitored for newly molted caterpillars, and the paper towel lining the bottom of the petri dish was moistened with water to prevent the diet from drying out. Focal individuals that had molted were removed from the dish, weighed and the date of the molt was recorded. They were then placed in microcentrifuge tubes and frozen. As individual group members molted, they were removed from the dish and frozen separately. Once all of the caterpillars had molted, the food and feces were removed, and dried for gravimetric analysis.

2.3.5 Gravimetric Analysis

Gravimetric analysis was performed to assess assimilation and conversion of consumed food according to Waldbauer (1968). The following are used to evaluate food processing:

Ge: Dry weight gain of the insect

Ie: Dry food consumption

Fe: Dry fecal production

Approximate digestibility (AD) measures the assimilation of the food ingested by the insect:

$$(1) \quad AD = \frac{(Ie - Fe)}{Ie}$$

Efficiency of conversion of digested material (ECD) measure the fraction of digested food which will be used by the insect for growth:

$$(2) \quad ECD = \frac{Ge}{(Ie - Fe)}$$

Efficiency of conversion of ingested material (ECI) measures the fraction of energy in the ingested food which will be used by the insect for growth:

$$(3) \quad ECI = \frac{Ge}{Ie}$$

For the gravimetric analysis, it was impossible to assign individual values for the amount of food consumed and feces produced. These variables were measured as a function of the group, over the duration of the entire larval stage. As a result, an average value for these two variables was assigned to the focal individual. However, since individuals of different sizes were randomly assigned to different dishes, the average

values of food consumption and feces production should be representative of the actual values. Gravimetric analysis was then performed using the weight gain of the focal individual and the group average values for food consumption and feces production.

Gravimetric analysis requires the use of dry weights for all of its variables. For the *Ge* and *Ie* values, wet to dry weight regressions were used to estimate the initial dry weight of the focal caterpillar and the food. To create a wet to dry weight regression, 15 newly molted second or fourth instar caterpillars were collected. They were placed in -80° C freezer for 24 hours; the caterpillars were then placed in a lyophilizer (freeze dryer) for 48 hours before they were removed and weighed. A straight-line regression was constructed with a x and y intercept of zero. This equation was used to estimate the initial dry weight of the focal individual. The final dry weight of the caterpillar was obtained using the same drying protocol.

In order to construct wet to dry weight regressions for the food, five extra cubes of diet were collected on each day that new replicates were set up, and their wet weight taken. At the end of the experiment, the extra food was placed inside an air drying oven and dried at 30° C for 48 hours. A straight-line regression, with a x and y- intercept of zero, was created using this data. As a result, it was possible to estimate the initial dry weight of the diet. The final dry weight of the diet was obtained by the same drying process used to create the regression. Using these values, the amount of food eaten by the entire group of caterpillars in a petri dish was calculated. This number was then divided by the group size and the mean amount of food eaten per individual was obtained.

The growth rate was calculated for the focal individual using the following formula:

$$(4) \quad \text{Growth Rate} = \frac{Ge}{De}$$

Where:

Ge: Dry weight gain of the insect

De: The larval instar stage duration of the insect

2.3.6 Analysis

The following statistical analyses were performed separately for second and fourth instar caterpillar data. For each larval stadium, there were six two-way factorial analysis of variances performed for the effects of trails and group size. Two-way analysis of variance were performed on: the time spent in the instar stage, the growth rate, the food consumption of the focal individual, and AD, ECD and ECI values. One ANCOVA was performed on the wet weight gain of the focal individual; the initial wet weight of the focal was used as a covariate. Appropriate transformations were performed to correct data which did not follow the normal distribution. For second instar larvae, AD values were transformed with an arcsin function, and ECI values were transformed with the natural logarithm. In fourth instar larvae, the growth rate was transformed with the square root function while the AD and ECD values were transformed with the natural logarithm. If group size had a significant effect on the variable, then a two-tailed Tukey Post Hoc Tests was used to distinguish if the group sizes had significantly different effects on the variable.

2.4 Results

2.4.1 Regressions

The wet to dry weight regression (Fig 2.1) for fourth instar larvae was used to estimate the weight gain of both second and fourth instar caterpillars. This was due to the fact that the dry weight of second instars, approximately 0.1 mg, was below the resolution of our balance. It is possible that second and fourth instar larvae differ in their ratio from wet to dry weight. However, since the same ratio was applied to all second instars, the effect of trails and group size on the weight gain of the second instar may still be detected. Fourth instar larvae of different sizes with weights encompassing the range of experimental insects, was used in this regression. For the wet to dry food regression, all of the extra diet for second and fourth instar larvae trials was pooled to create a single regression (Fig 2.2). The cubes of diet used in the regression encompassed the entire range of sizes and weights used in the experiment for second and fourth instars. The food regressions were very similar for all of the trials, therefore, all of the food regressions were pooled together.

2.4.2 Second Instar Caterpillars

Second instar caterpillars developed significantly faster in the presence of trails and in a group size of 8 (Fig 2.3, Table 2.1). Tukey Post Hoc tests indicated that individuals in groups of 8 developed significantly faster when compared to individuals reared in isolation and in groups of 3. However, there was no significant difference between individuals reared in isolation and in groups of 3 (Fig 2.3).

In addition, focal individuals grew significantly larger when reared in the presence of trails and in isolation compared to in the absence of trails and in groups of 8 (Fig 2.4, Table 2.1). Covariate analysis indicates that the initial weight of the focal individual did not have a significant effect on its weight gain (Table 2.1). Post hoc tests indicated that focal individuals grew more when reared in isolation compared to those reared in groups of 8. However, individuals reared in groups of 3 did not differ significantly from individuals reared in isolation or in groups of 8 (Fig 2.4).

FTC grew significantly more per day (Fig 2.5, Table 2.1) in the presence of trails, and in groups of 3 and 8. Although the relative consumption rate was not measured for this experiment because group members did not have the same instar stage duration, the total amount of food consumed by the entire group was measured. Therefore, it was possible to determine the average amount of food consumed by an individual caterpillar throughout the instar stage (Fig 2.6). The results indicated that there was no significant difference in the amount of food consumed in the presence of trails (Fig 2.6, Table 2.1). The results also show that the wet weight gain of the focal individual (Fig 2.4, Table 2.1) was significantly higher in the presence of trails. Group size was shown to have a significant effect on the amount of food eaten per individual (Table 2.1). Caterpillars raised in isolation ate significantly more food than those raised in groups of 3 and 8. However, there was no significant difference between the amount of food consumed between individuals reared in groups of 3 and 8 (Fig 2.6). These results, when assessed with the wet weight gain of the focal individual (Fig 2.4, Table 2.1) suggest that isolated individuals may be larger because they consumed more food.

Gravimetric analysis was then performed to examine food utilization. The AD values (Fig 2.7, Table 2.1) indicated that there was no significant difference in the percentage of non digestible material in the food. This result was expected because the same artificial diet was used across all of the treatments. ECD values show that in the presence of trails, second instar caterpillars were significantly more efficient at converting their food into body mass (Fig 2.8, Table 2.1). This may be because they are expending less energy on respiration. ECI values show that in the presence of trails, a significantly higher proportion of ingested food was converted into body weight (Fig 2.9, Table 2.1).

In terms of the group size, focal individuals raised in large groups developed faster, and were smaller, than those raised in isolation (Fig 2.3, Fig 2.4, and Table 2.1). The growth rate indicated that there was no difference in the amount of weight gained per day due to group size (Fig 2.5, Table 2.1): the significant difference in the amount of food consumed per individual over the entire instar stage results from the fact that the instar stage duration was shorter. Therefore, in larger groups second instar larvae were smaller because they develop over shorter time and hence consume less food. Gravimetric analysis, (Fig 2.8, Fig 2.9) supported this result, indicating that at the different group sizes, there was no difference in their food utilization.

2.4.3 Fourth Instar Caterpillars

The results differed between the second and fourth instar larvae. There was no significant difference in the development rate of fourth instars (Fig 2.10, Table 2.2) due to the presence of trails or at different group size. In addition, in the presence of trails and

in isolation, the weight gain was significantly higher than in the absence of trails, and in larger groups (Fig 2.11, Table 2.2). Covariate analysis indicates that the initial weight of the focal individual did not have a significant effect on its weight gain (Table 2.2).

There was no significant difference in the growth rate due to the presence of trails (Fig 2.12, Table 2.2). However, the presence of trails significantly increased the amount of dry food eaten per individual (Fig 2.13, Table 2.2). This is reflected in the increased weight gain of caterpillars reared in the presence of trails (Fig 2.10). However, in contrast to the results found in second instar larvae, gravimetric analysis demonstrated that there was no significant effect on the ECD and ECI values for fourth instar larvae (Fig 2.15, Fig 2.16, and Table 2.2). This would suggest that fourth instar caterpillars did not show any differences in their food utilization efficiency, instead, the increase in weight gain could be attributed to differences in food consumption.

There was significantly less growth occurring in larger groups (Fig 2.1, Table 2.2) for fourth instars. Post hoc Tukey tests indicated that group size of 3 and 8 differed from caterpillars raised in isolation (Fig 2.11). Results suggest that individuals in groups of 3 and 8, ate significantly less food than those reared in isolation (Fig 2.13, Table 2.2). Therefore, fourth instars reared in larger groups had significantly lower weight gain and lower growth rate because they consumed less food. Similar to the second instar results, there was no significant effect of group size on AD, ECD, and ECI values for fourth instar larvae (Fig 2.14, Fig 2.15, Fig 2.16, and Table 2.2).

Generally, in fourth instars, results for group sizes of 3 and 8 were not significantly different, but both differed from caterpillars raised in isolation. In contrast, the results for second instars were more ambiguous. A group size of 3 does not show

significant differences in development time when compared to individuals raised in isolation, but there was a significant decrease in the amount of food consumed in groups of 3. In addition, individuals in groups of 3 do not show significant differences in weight gain when compared to individuals in groups of 8 and in isolation. For second instar larvae, a group size of 3 may be an intermediate between a group size of 8 and individuals raised in isolation. A group size of 3 is sufficient to cause a decrease in food consumption when compared to isolated individuals. However, a group size of 3 is not sufficiently large to demonstrate significant differences which are detectable in terms of the weight gain and duration of the second instar.

2.5 Discussion

For second instar larvae, trails significantly decreased the development time (Fig 2.3), but significantly increased the growth of the focal individual (Fig 2.4). Gravimetric analysis indicates that this may be due to the efficiency of conversion of digested material; results suggest that in the presence of trails second instar larvae digest their food more efficiently (Fig 2.8, Table 2.1). ECD values suggest that in the presence of trails, second instar caterpillars expended less energy on respiration. In ECD values, the energy expended in respiration includes energy used by the insect for movement (Waldbauer 1968). The ECI values support this result (Fig 2.9) indicating that second instar larvae reared in the presence of trails utilized a higher percentage of food ingested for growth.

For fourth instar caterpillars, trails did not have a significant effect on the efficiency of conversion of digested material (Fig 2.15, Table 2.2). However, the presence of trails significantly increased the consumption of food over the duration of the stadium (Fig 2.13, Table 2.2) and led to significantly increased growth (Fig 2.11, Table 2.2).

There were two assumptions made concerning the presence of trails in the experimental protocol. This experiment did not directly test if pheromone was laid down in conjunction with the silk trails. Previous studies have clearly demonstrated that FTC lay down pheromone along with the silk trails during exploration (Fitzgerald 1993). It may thus be assumed that FTC placed in the petri dish before the experiment deposited pheromone and silk trails. However, it is impossible to determine the amount of pheromone which is being deposited in the petri dishes prior to the experiment. Some of replicates may have contained more pheromone trails than other dishes. In addition, it is

difficult to separate the two variables of trails and group size. As time progresses, the group members produce their own trails which will overwhelm the trails left before the experiment began. Large groups will produce more trails than individuals raised in isolation, and more quickly negate the effect of pre laid trails. Therefore, the effect of pre laid trails on isolated individuals may be more severe. If trails were only to have an effect on individuals raised in isolation, this would suggest that trails and group size could not be completely separated. This experiment was designed to try and determine if the presence of trails at the onset of the experiment was a significant predictor of FTC performance.

In trail treatments, experimental insects placed in the petri dish would immediately encounter trails. The initial presence of trails may be enough to stimulate the initial foraging bout, and help FTC locate their food sooner. For both second and fourth instar larvae, these factors may have led to a significant increase in weight gain. However, the effect of trails may have been stronger for second instars because it resulted in a significant acceleration of development. A decrease in development time may be because trails play an important role in foraging efficiency for younger larval instars. In the absence of trails, second instar caterpillars may have prolonged periods of quiescence before engaging in a foraging bout (Fitzgerald 1995). In addition, once the FTC begins to forage, it may spend time and energy laying down exploratory trails (Fitzgerald and Visscher 1996) before it is able to make initial contact with its food. These mechanisms could explain the lower efficiency of conversion of digested material (ECD) and lower efficiency of conversion of ingested (ECI) values in the absence of trails.

For fourth instar larvae, there was no significant effect of trails on the rate of development (Fig 2.10, Table 2.2); but individuals had a significant increase in weight gain associated with significantly increased food consumption in the presence of trails (Table 2.2). A significant increase in weight gain suggests that trails may help fourth instar larvae find their food sooner. Consequently, fourth instar larvae may consume more food over the duration of their larval instar.

FTC larvae have been observed to shift from highly gregarious to more solitary behaviour between second and fourth instar larvae (Despland and Hamzeh 2004). This shift in behaviour may affect the way FTC use trails during different larval stages. Since fourth instar caterpillars are more solitary, it may be argued that older instar larvae rely less on trails (Despland 2004). Fourth instar caterpillars are larger, and more mobile, therefore they may no longer require silk to maneuver over physical objects while foraging for food (Robison 1993). In addition, since fourth instar larvae no longer forage in groups, and thus do not need pheromone trails to maintain colony cohesion, they may depend less on pheromone trails. Yet fourth instar larvae continue to produce trails (Fitzgerald and Webster 1993), and this experiment demonstrated that they still benefit from them, as shown by an increase in weight gain. As fourth instars, the presence of trails may communicate the presence of other FTC. This may stimulate fourth instars to increase food consumption at a food source instead of searching for higher quality sources elsewhere. In the presence of other FTC, it may be better to stay with a known food source instead of searching for another source which may already be depleted. Under crowded conditions, this may be done to avoid possible food shortages. Forest tent caterpillar may regulate the amount of food eaten in order to regulate their weight gain. In

FTC, the weight of a caterpillar has been shown to be positively correlated to fecundity (Smith and Goyer as cited in Fitzgerald 1995). In addition, the weight gain of the individual is one of the factors which may determine the duration of the larval stadium (Muggli and Miller 1980). If an individual reaches a threshold weight then it may undergo a molt to the next larval stadium. However, if a caterpillar develops too quickly, then it may not pupate in synchrony with other FTC, and thus be unable to mate (Fitzgerald 1995). Therefore, it may not always be beneficial for the caterpillar to grow as quickly as quickly as possible.

Second instars may have demonstrated a benefit of group living: individuals in larger groups developed significantly faster (Fig 2.3, Table 2.1). However, individuals in groups of 8 had a significantly smaller weight gain (Fig 2.4). It may be argued that group sizes of 3 and 8 chosen for this experiment may not be representative of colony sizes which occur in nature. Second instar FTC have been shown to reside in colonies consisting of several hundred individuals (Muggli and Miller 1980). However, Robison (1993) found that in group sizes larger than five individuals, first instar FTC have a high rate of survival. He concluded that group sizes larger than five individuals closely resemble the population dynamics of whole colonies (Robison 1993). Therefore, a group size of 8 individuals, reared in a small petri dish may have been sufficient to mimic an actual colony.

In fourth instars, group size did not have a significant effect on the development time (Fig 2.10, Table 2.1). Fourth instar larvae may have demonstrated a cost of group living as seen by significantly lower weight gain in groups of 3 and 8 (Fig 2.4, Table 2.2), due to lower food consumption seen in both of these group sizes (Fig 2.6, Table 2.2). For

second instar larvae, when group size was found to have a significant effect on a performance variable, groups of 8 were significantly different from individuals raised in isolation. However, groups of 3 were not always significantly different from individuals reared in isolation, nor in groups of 8. According to Robison (1993) first instar larvae experienced a significant increase in the rate of development in group sizes above 5 (Robison 1993). Perhaps a group size of 3 was not large to produce significant differences in the development time for second instar larvae. In contrast, for fourth instars, when group size had an effect on performance variable, group sizes of 3 and 8 generally differed from isolated individuals. Since fourth instars have a larger body mass than second instar larvae, a group size of 3 may be sufficient to cause interference competition between group members. Reduced weight gain in fourth instars may be a cost of group living since body weight is positively correlated to fecundity in caterpillars (Smith and Goyer as cited in Fitzgerald 1995).

At larger group size, second and fourth instar larvae are likely to experience crowding. The exact effect of crowding on gregarious insects remains unclear: *Mamestra brassicae* caterpillars develop faster in groups at the expense of pupal size. *Plagioderma versicolora*, the willow leaf beetle has been shown to be larger when reared in groups (Applebaum and Heifetz 1999, Breden and Wade 1987). *Epirrita autumnata* caterpillars develop faster under crowded conditions but are of similar mass as individuals reared under solitary conditions (Haukioja et al. 1988). Smits (2002) found that in *Bupalus piniarius*, crowding in later larval stages resulted in enhanced larval performance: the growth rate was higher and the development time shorter. Crowded individuals pupated faster but were of the same weight as those raised in isolation (Smits 2002). Several

insects have been shown speed up development under crowded conditions but the effect on growth can differ between insect species. Some insects had increased growth under crowded conditions, while other insects had reduced growth. Therefore, it is possible that forest tent caterpillars also increase their rate of development at the expense of weight gain under crowded conditions. An increased rate in development may be a benefit of group living because FTC may spend less time in a vulnerable stage where they can be killed by predation or suffer food shortages (Fitzgerald 1995).

The results in this experiment suggest that FTC second instar larvae develop faster at the expense of size when reared in large groups. However, it is possible that FTC will compensate for this size deficiency by undergoing extra molts and pupating sooner such as seen in *Spodoptera exempta* under crowded conditions (Applebaum and Heifetz 1999). The growth rate of second instar caterpillars indicates that there is no effect of group size on the growth of the individual per day (Fig 2.5, Table 2.1); however, the instar stage duration is shorter (Fig 2.3, Table 2.1). Therefore, second instar larvae may be smaller in larger groups because they had less time to consume food and increase in body mass. A higher development rate may be due to a competition effect which can result in FTC larvae pooling all of their energetic resources into developing faster in order to prevent food shortages (Iwao 1968). In this experiment, there is an abundant amount of food, however, it is possible that FTC evaluate the presence of colony mates through visual, chemosensory, or tactile cues (Costa and Pierce 1997). The exact mechanism by which FTC sense colony mates remains unclear, but many other insects rely upon tactile cues (Applebaum and Heifetz 1999). Since food was abundant in this

study, this experiment suggests that group size may be sufficient to induce a competition response from larval FTC.

Fourth instar larvae did not show a significant effect group size on development time. However, there was a significant decrease in the weight gain of the focal individual in larger group size which may be cost of group living. In large groups, the growth rate decreased, indicating that fourth instar larvae grew less per day. A decrease in weight gain in larger groups may be due to a decrease in food consumption. In contrast to second instars, where only a group size of 8 had a significant effect on the weight gain, fourth instars showed effects even in groups of 3. As mentioned previously, fourth instar caterpillars are more solitary; consequently, even the presence of two individuals could result in interference competition (Iwao 1968). In this experiment, fourth instar caterpillars reared in groups may have experienced interference competition and thus consumed less food. This study suggests that second instar larvae may have compensated for competition by increasing their rate of development, but fourth instars were unable to compensate in this manner. Instead, fourth instars may have suffered the effects of reduced weight gain without the benefit of faster development time.

FTC colonies disperse between the second and fourth instar larvae (Fitzgerald 1995), and there is evidence that an actual ontogenetic shift between second and fourth instars (Despland and Hamzeh 2004). However, it is evident that second and fourth instars differ greatly in size: fourth instars have approximately ten times the biomass of second instar larvae (Personal Observation). The size of an organism often determines its energetic requirements and places constraints on the way an organism interacts with its environment (Werner 1984). Larval FTC experience an increase in energetic needs as

they develop from second to fourth instar caterpillars. Although the larval FTC does not undergo an extensive ontogenetic shift in food or habitat use (Fitzgerald 1995), caterpillars may change the manner in which they interact with colony members. As a second instar larvae, the presence of colony mates may improve foraging efficiency, but as a fourth instar, colony mates may be competitors for food (Costa 1997).

This experiment suggests that the balance between costs and benefits may change as the size of the insect increases. This experiment suggests that second and fourth instar FTC respond differently to trails and group size. For fourth instars, due to increased food requirements, the cost of group living may now outweigh the benefits.

Table 2.1: ANOVA table for second instar larvae. T and G refers to the presence of trails and group size respectively. The ANCOVA covariate refers to the initial weight of the focal individual. The rate development time is the duration of the second instar; the growth is the amount of weight gained by the individual over the duration of the instar; the growth rate is the weight gain of the individual per day. AD values were arc sign transformed, and ECI values were transformed with the natural logarithm. Bold values were found to be statistically significant

Dependent Variable	Independent Variable	F value	Degrees of Freedom	Probability (P value)
Development time	Trails	7.99	1, 58	0.007
	Group	18.68	2, 58	<0.0005
	G X T	3.04	2, 58	0.056
Growth	Trails	17.62	1, 58	<0.0005
	Group	7.23	2, 58	0.002
	G X T	0.89	2, 58	0.42
	Covariate	0.95	1, 58	0.34
Growth rate	Trails	17.60	1, 58	< 0.0005
	Group	7.23	2, 58	0.002
	G X T	0.89	2, 58	0.42
Food eaten	Trails	1.13	1, 58	0.29
	Group	6.26	2, 58	0.004
	G X T	1.53	2, 58	0.23
AD	Trails	2.85	1, 58	0.097
	Group	3.03	2, 58	0.057
	G X T	0.44	2, 58	0.645
ECD	Trails	10.54	1, 58	0.002
	Group	0.60	2, 58	0.56
	G x T	0.04	2, 58	0.65
ECI	Trails	9.01	1, 58	0.004
	Group	0.04	2, 58	0.96
	G X T	1.24	2, 59	0.30

Table 2.2: ANCOVA table for fourth instar larvae. T and G refers to trails and group size respectively. The ANCOVA covariate refers to the initial weight of the focal individual. The rate development time is the duration of the fourth instar; the growth is the amount of weight gained by the individual over the duration of the instar; the growth rate is the weight gain of the individual per day. The growth rate was transformed with the square root function, AD and ECD values were transformed with the natural logarithm. Bold values were found to be statistically significant.

Dependent Variable	Independent Variable	F value	Degrees of freedom	Probability (p value)
Development time	Trails	0.86	1, 57	0.36
	Group	0.25	2, 57	0.78
	G X T	0.08	2, 57	0.92
Growth	Trails	5.58	1, 57	0.022
	Group	8.40	2, 57	0.001
	G X T	0.72	2, 57	0.49
	Covariate	1.10	1, 57	0.25
Growth rate	Trails	2.54	1, 57	0.12
	Group	6.58	2, 57	<0.0005
	G X T	0.37	2, 57	0.70
Dry food eaten	Trails	15.99	1, 57	<0.0005
	Group	20.89	2, 57	<0.0005
	G X T	0.27	2, 57	0.77
AD value	Trails	1.51	1, 57	0.23
	Group	2.32	2, 57	0.11
	G X T	0.41	2, 57	0.67
ECD	Trails	0.40	1, 57	0.53
	Group	2.54	2, 57	0.09
	G X T	0.21	2, 57	0.81
ECI	Trails	0.002	1, 57	0.96
	Group	0.69	2, 57	0.51
	G X T	0.50	2, 57	0.61

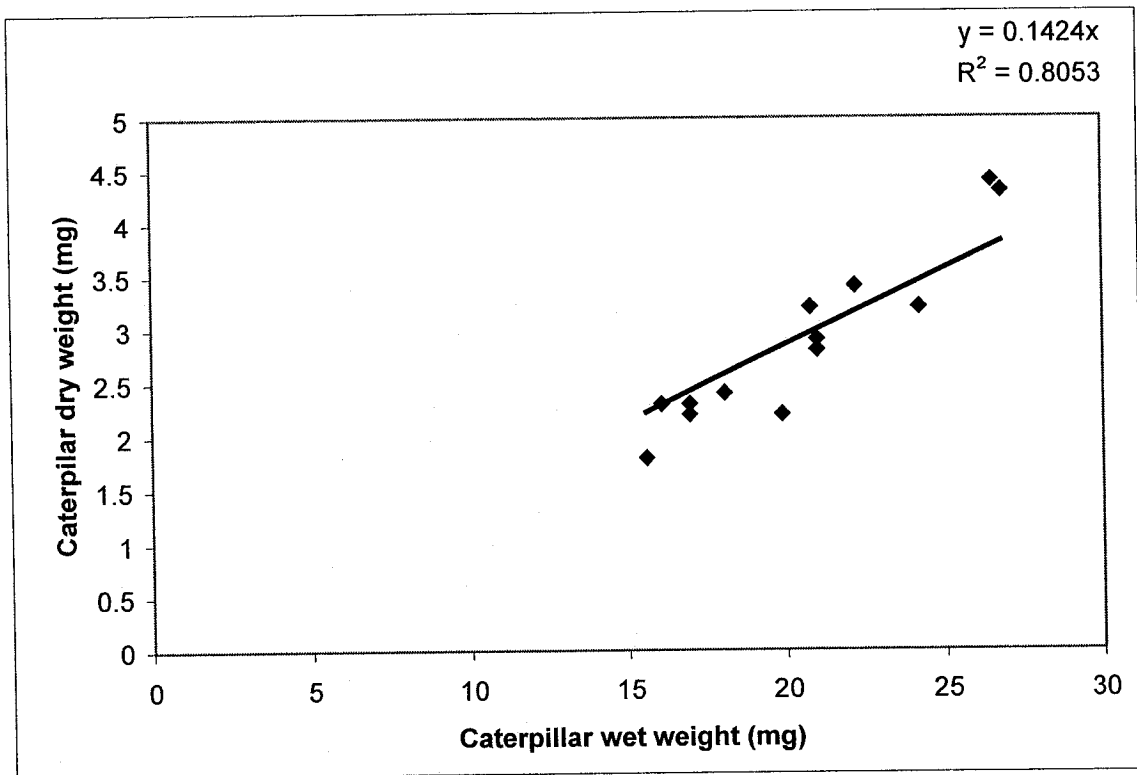


Figure 2.1: Wet to dry weight regression (fourth instar). The straight line regression was given a y and x intercept of 0.

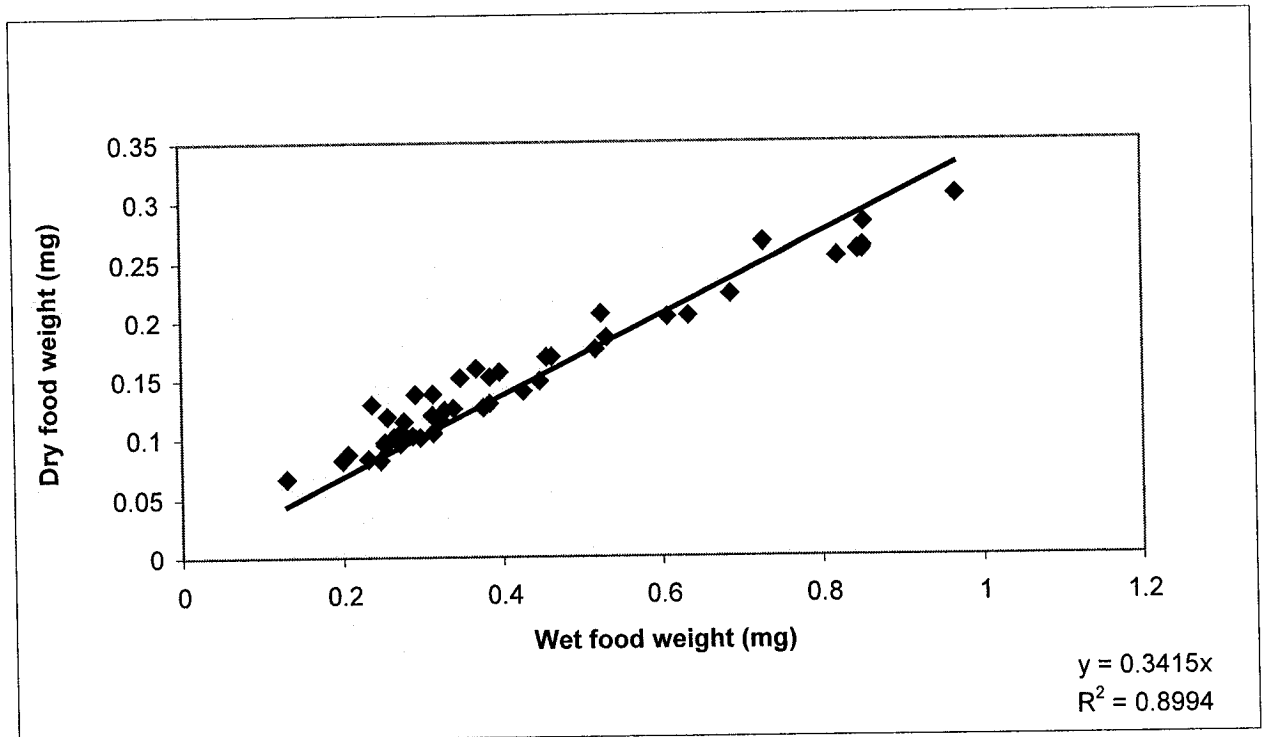


Figure 2.2: Compilation of wet to dry food weight regressions. All of the wet to dry food regression data was compiled and a straight line regression was created. The straight line was given a y and x intercept of 0.

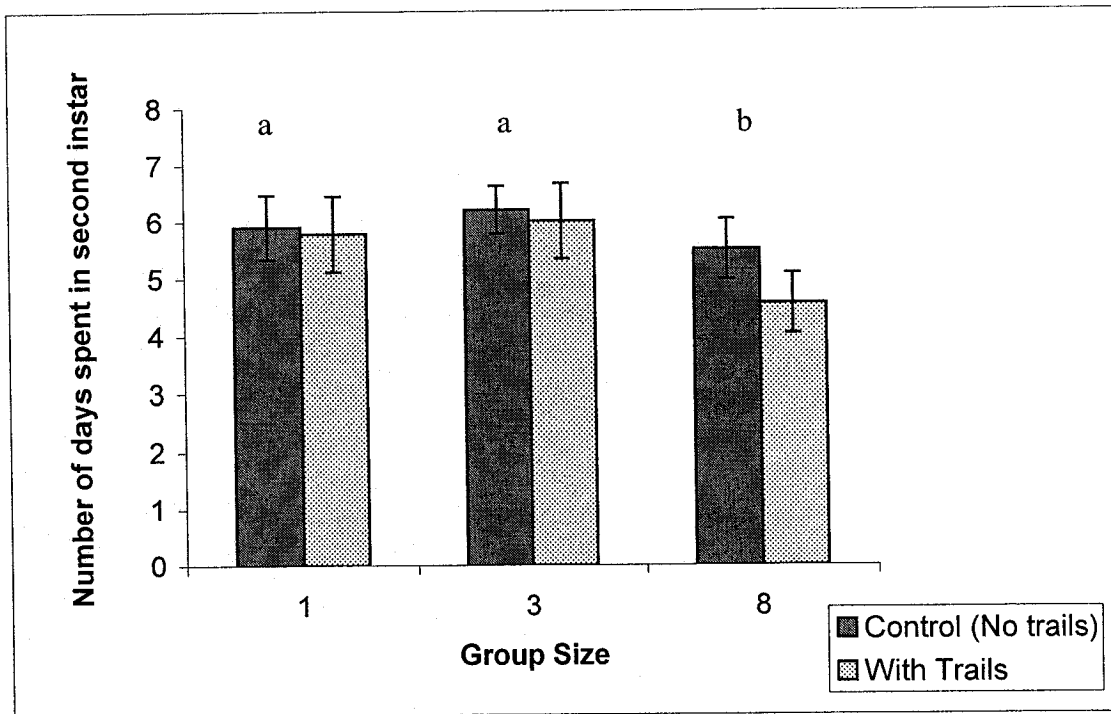


Figure 2.3: The effect of trails and group size on the development time of the second instar larvae focal individual. Mean values are shown here with standard error. Post Hoc Tukey tests indicated that there was a significant decrease in the number of days spent in second instar stage between focal individuals reared in groups of 8 when compared to individuals reared in groups of 3 and in isolation ($P < 0.00005$, and $P < 0.00005$ respectively). There was no significant difference between focal individuals reared in groups of 3 or in isolation ($P=0.34$). The letters a and b above the bars indicate statistically different groups.

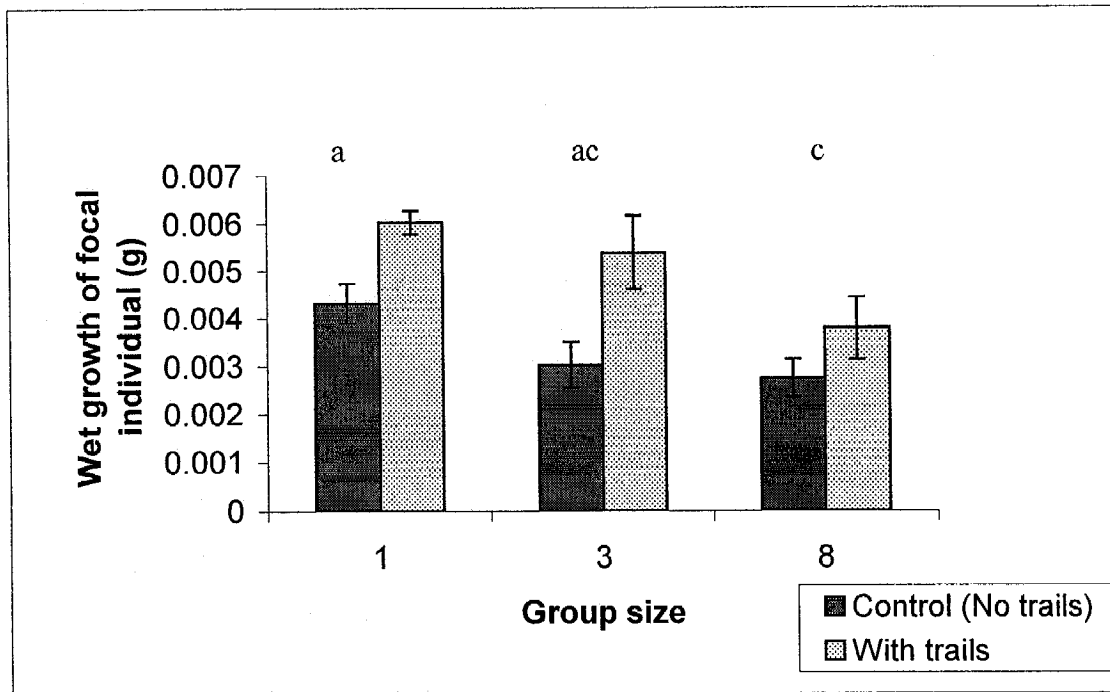


Figure 2.4: The effect of trails and group size on the wet growth of the focal individual. Mean values are shown here with standard error. Post Hoc Tukey tests indicated that focal individuals reared in groups of 8 were significantly smaller than those reared in isolation 1 ($P=0.001$). However, there was no significant difference between individuals raised in groups of 3, and those raised in groups of 8 ($P=0.133$). There was also no significant difference between individuals reared in groups of 3 and in isolation ($P=0.160$). The letters a and c above the bars indicate statistically significant groups.

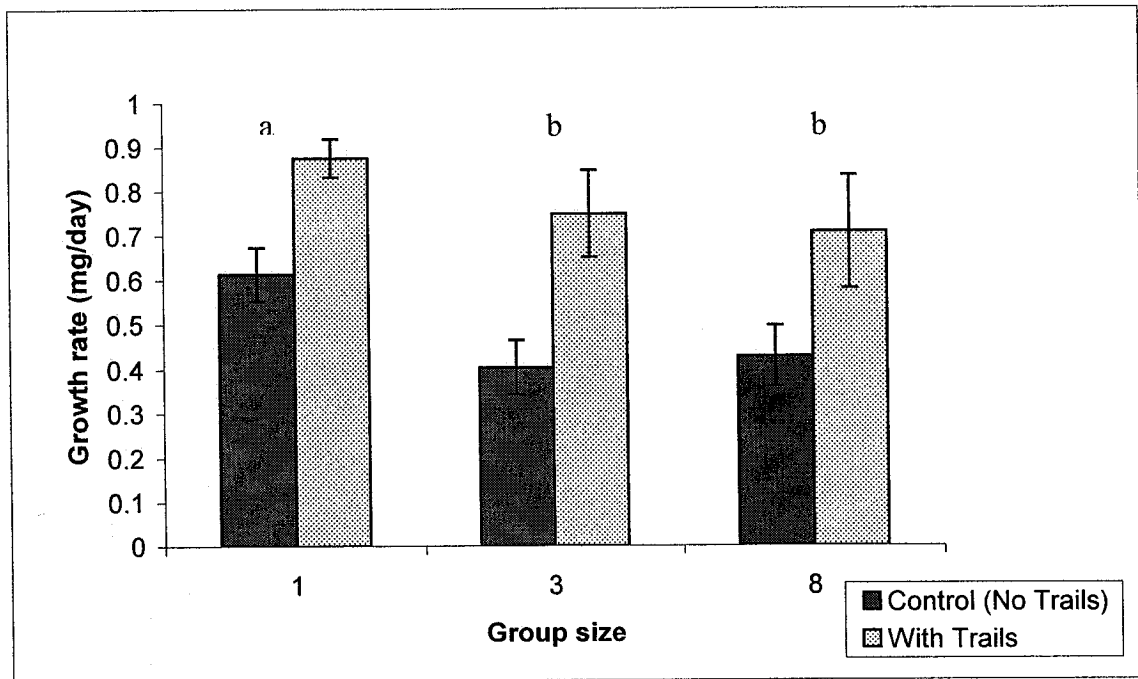


Figure 2.5: Growth rate of second instar larvae. Mean values are shown with standard error. Post Hoc tests indicated that groups of 3 and 8 were significantly different from individuals raised in isolation ($P=0.003$). However, groups of 3 were not significantly different from groups of 8 ($P=0.421$). The letters a and b above bars indicate statistically different groups.

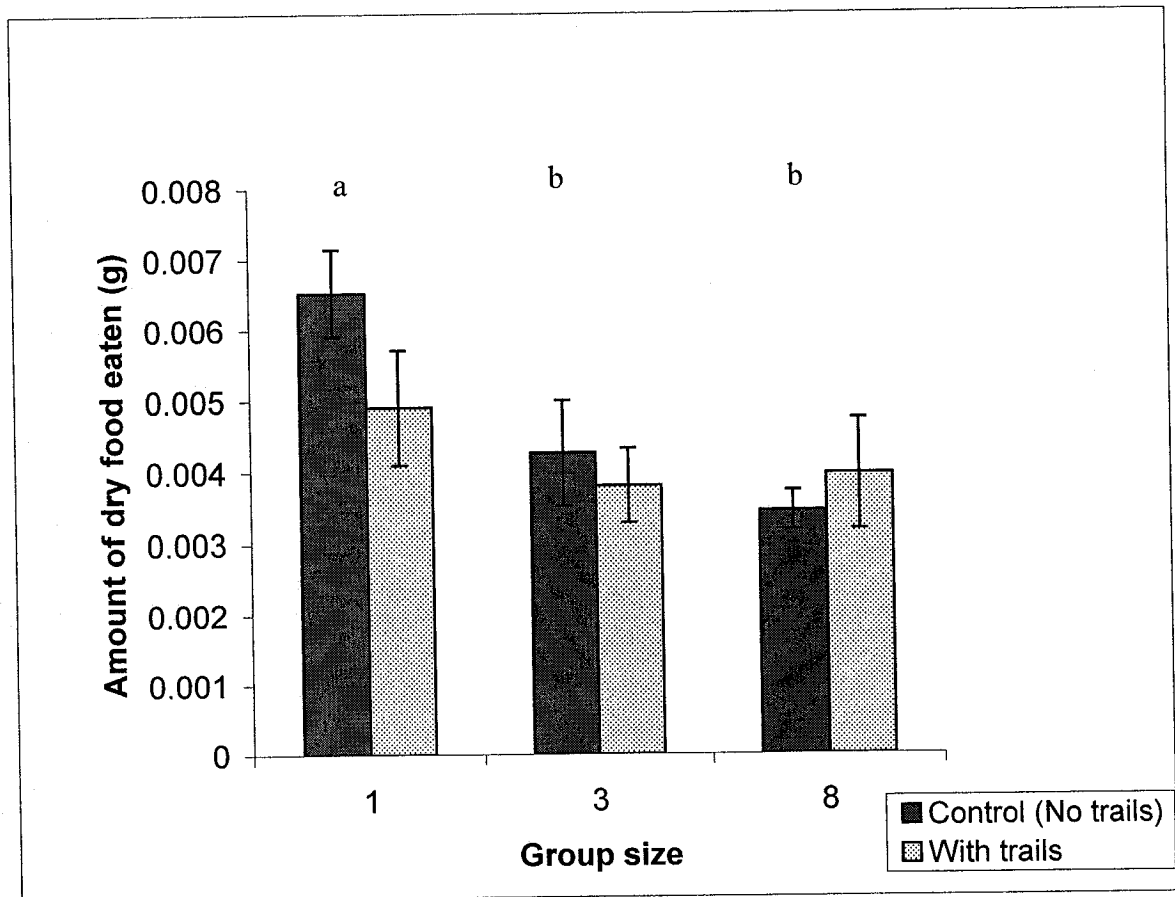


Figure 2.6: Effect of trails and group size on the amount of dry food eaten per individual caterpillar (second instar larvae). Mean values are shown here with standard error. A Tukey post hoc test indicated that there was a significant difference in the amount of food eaten between individuals raised in isolation and in groups of 3 ($P=0.16$), and between individuals raised in isolation and in groups of 8 ($P=0.04$). However, there was no significant difference between individuals raised in groups of 3 and 8 ($P=0.839$). The letters a and b above the bars indicate statistically different groups.

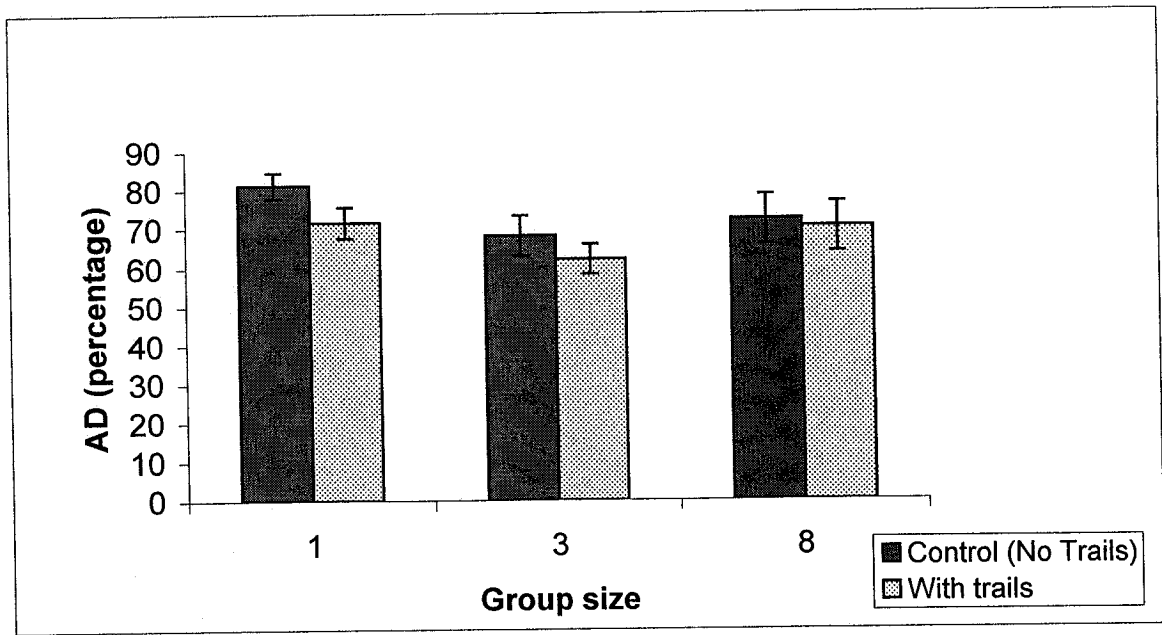


Figure 2.7: Percent AD (second instar larvae). Mean values shown here with standard error.

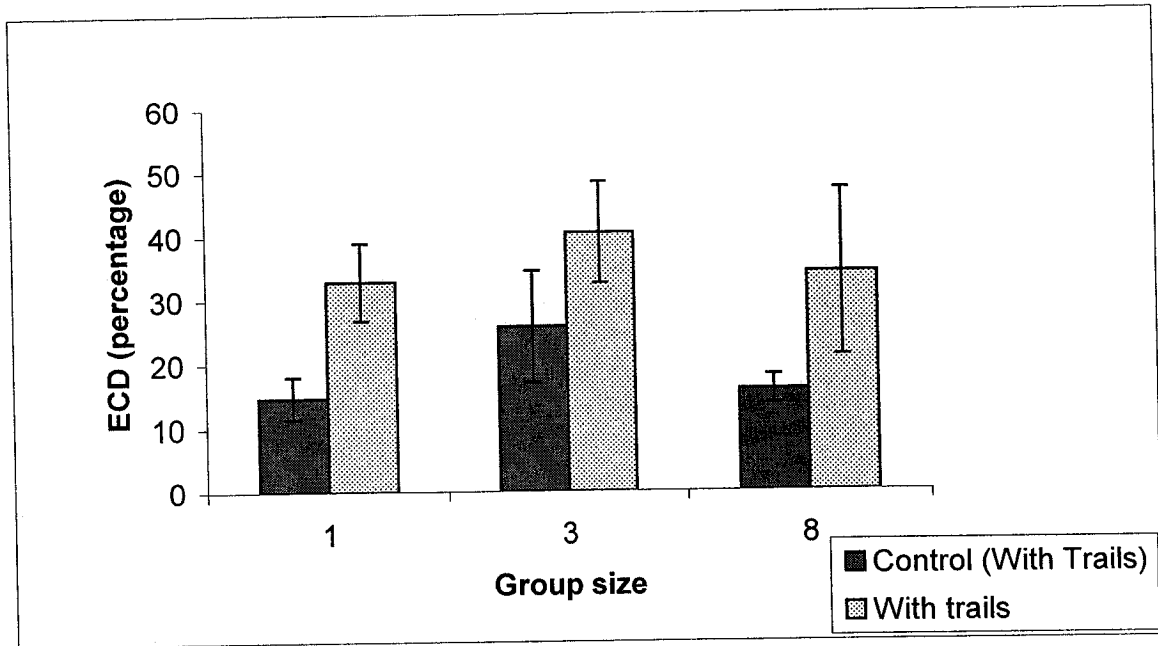


Figure 2.8: Effect of trails and group size on ECD (second instar larvae). ECD is the efficiency of conversion of digested material. Mean values are shown here with standard error.

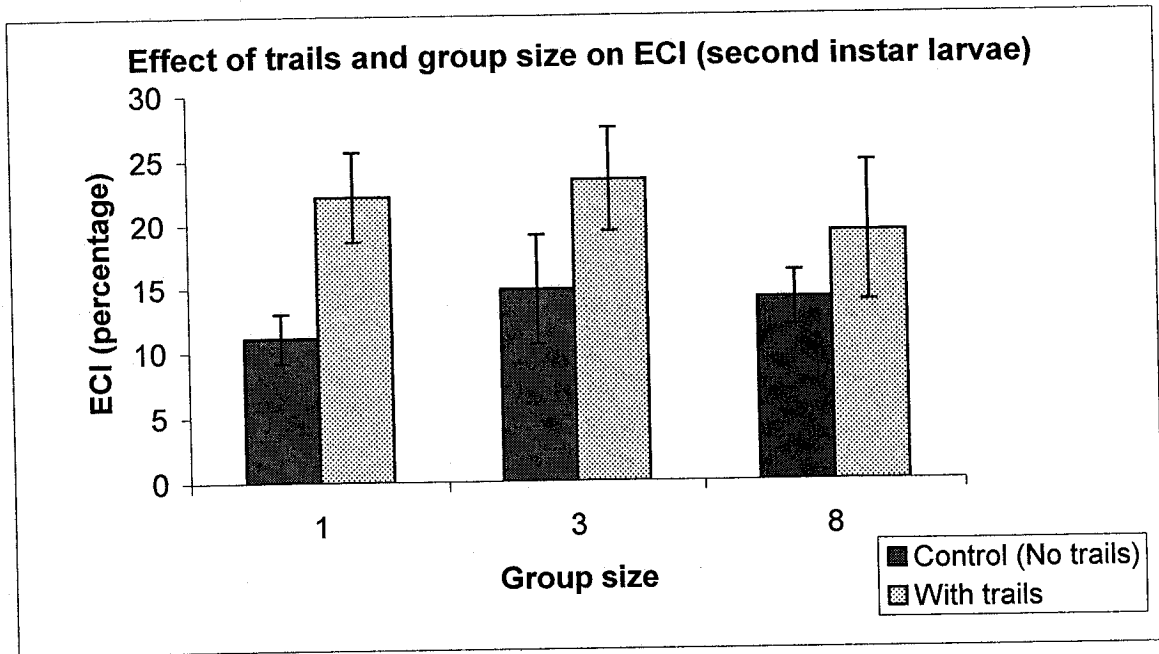


Figure 2.9: Effect of trails and group size on ECI (second instar larvae). ECI is the efficiency of conversion of ingested material. Mean values shown here with standard error.

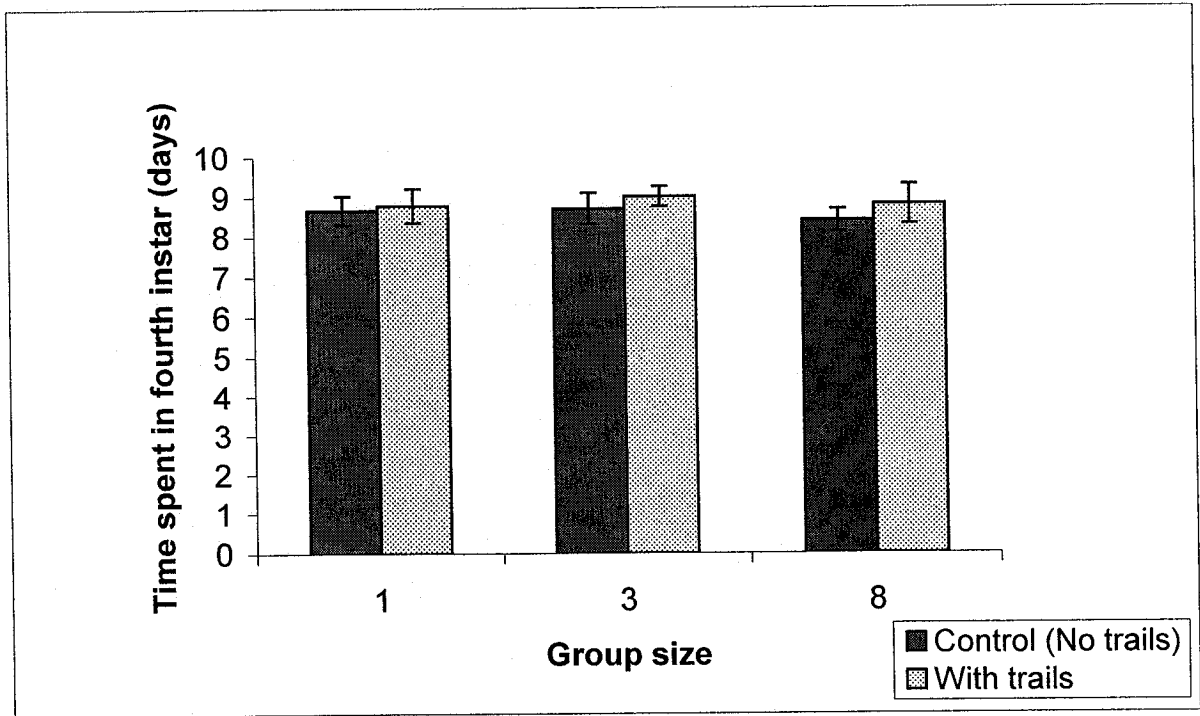


Figure 2.10: Effect of trails and group size on the time spent in the fourth instar. Mean values are shown here with standard error.

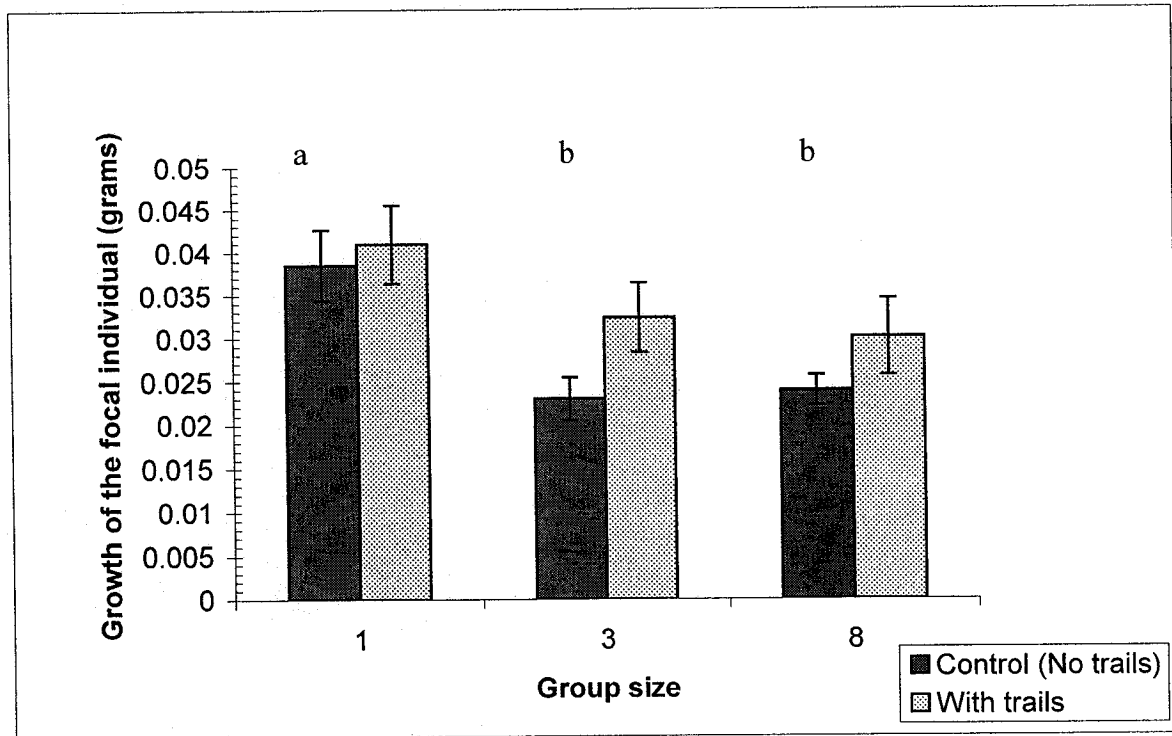


Figure 2.11: Effect of trails and group size on the wet growth of the focal individual (fourth instar larvae). Mean values are shown here with standard error bars. Post Hoc tests indicated that caterpillars reared in isolation were significantly different from caterpillars reared in groups of 3 and 8 ($P=0.01233$ and $P=0.012741$ respectively). Letters a and b above the bars indicate statistically significant groups.

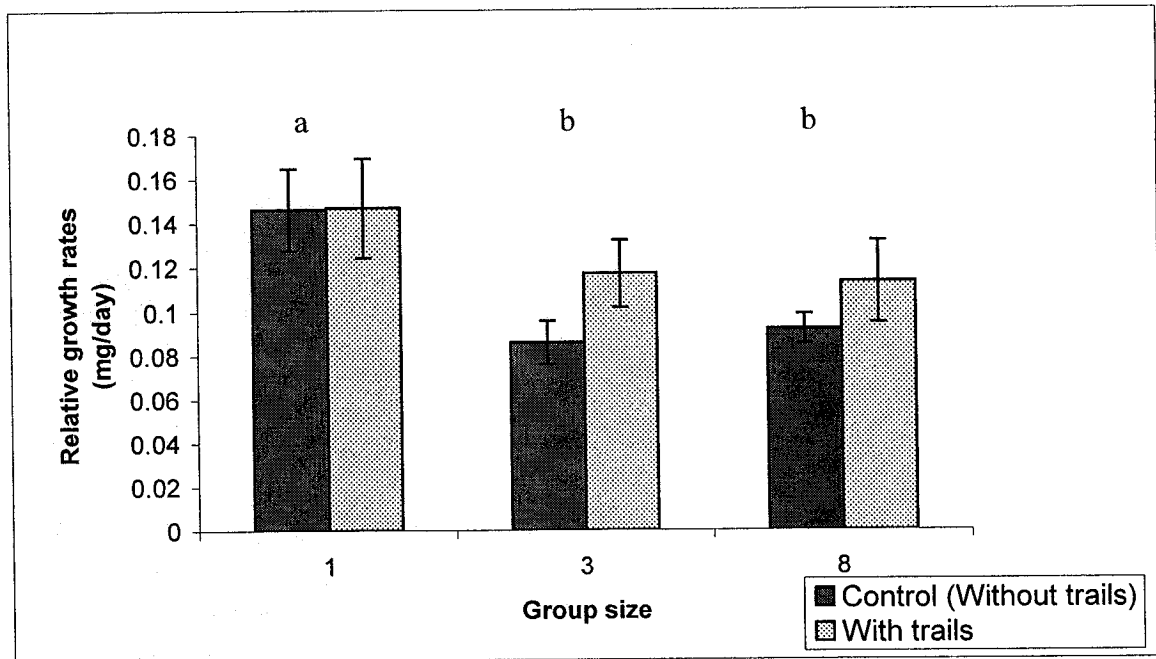


Figure 2.12: Growth rate of fourth instar larvae. Mean values are shown with standard error. Tukey Post Hoc tests indicated that group size of 3 and 8 differed from caterpillars raised in isolations ($p=0.045$ and $p=0.0423$). Letters a and b above the bars indicate statistically different groups.

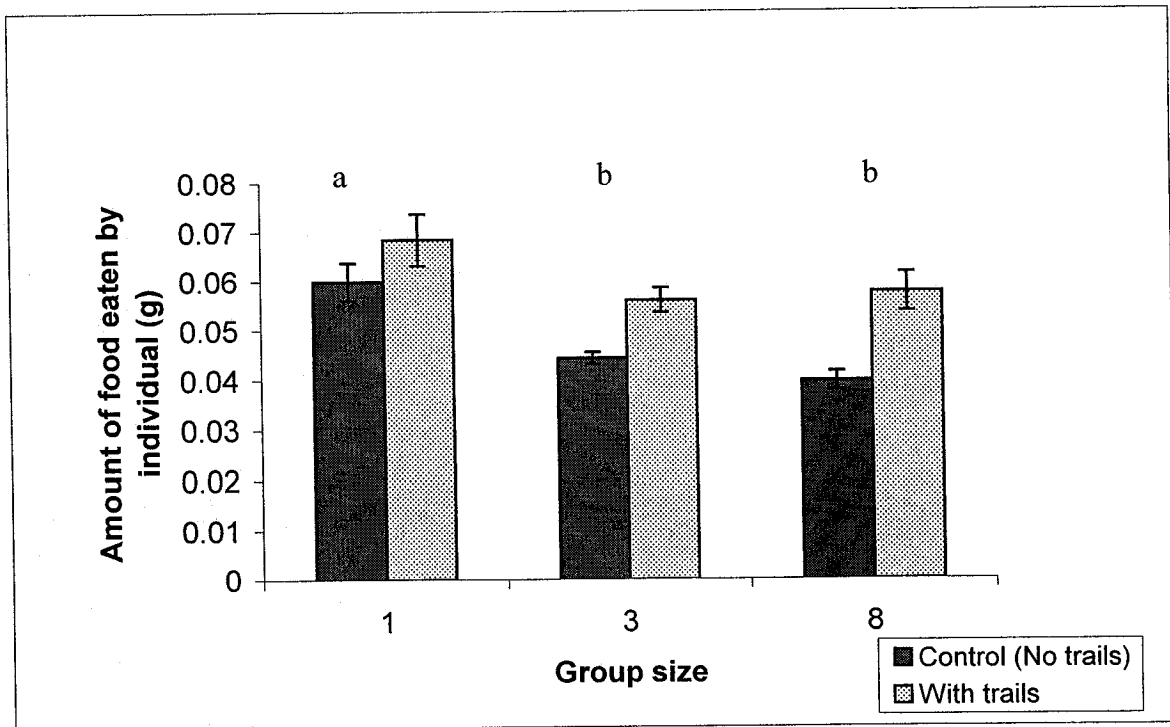


Figure 2.13. Effect of trails and group size on the amount of dry food eaten by individual caterpillar (fourth instar larvae). Mean values are shown here with standard error bars. Post Hoc tests indicated that there was a significant decrease in food consumed between caterpillars reared in isolation and caterpillars with a groups of 3 and 8 ($P < 0.00005$ and $P < 0.00005$ respectively). Letters a and b above the bars indicates statistically different groups.

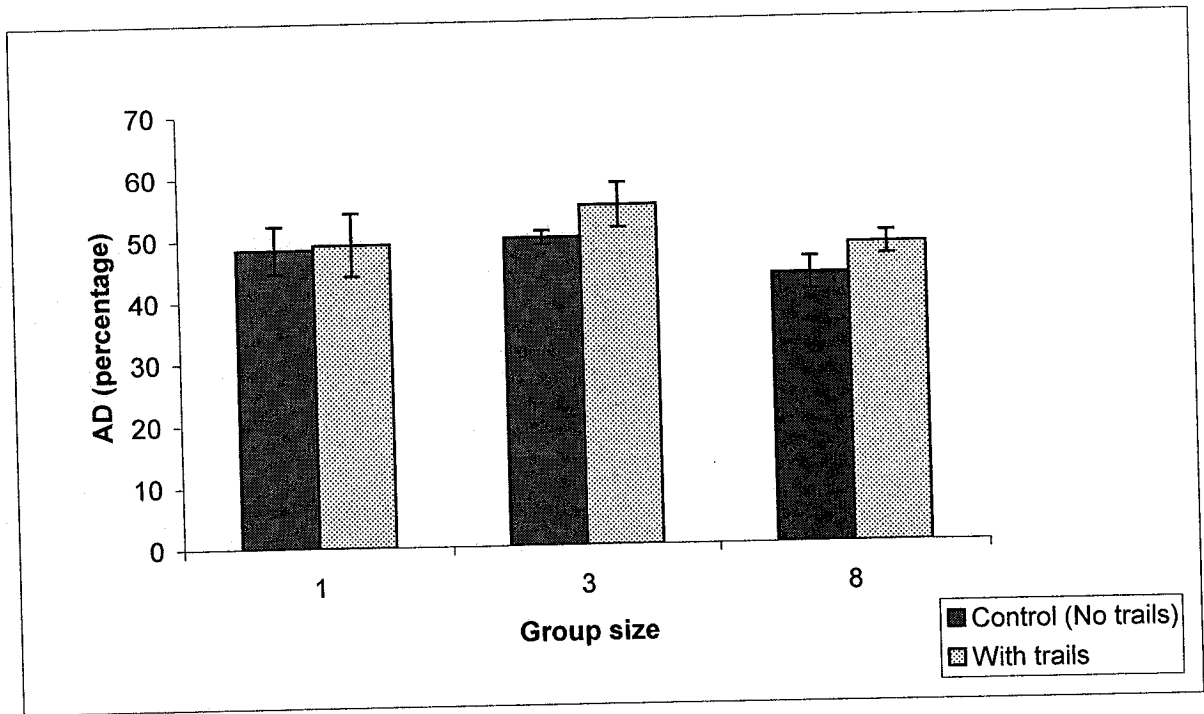


Figure 2.14: Percent AD (fourth instar larvae). AD is the approximate digestibility of the food. Mean values are shown here with standard error.

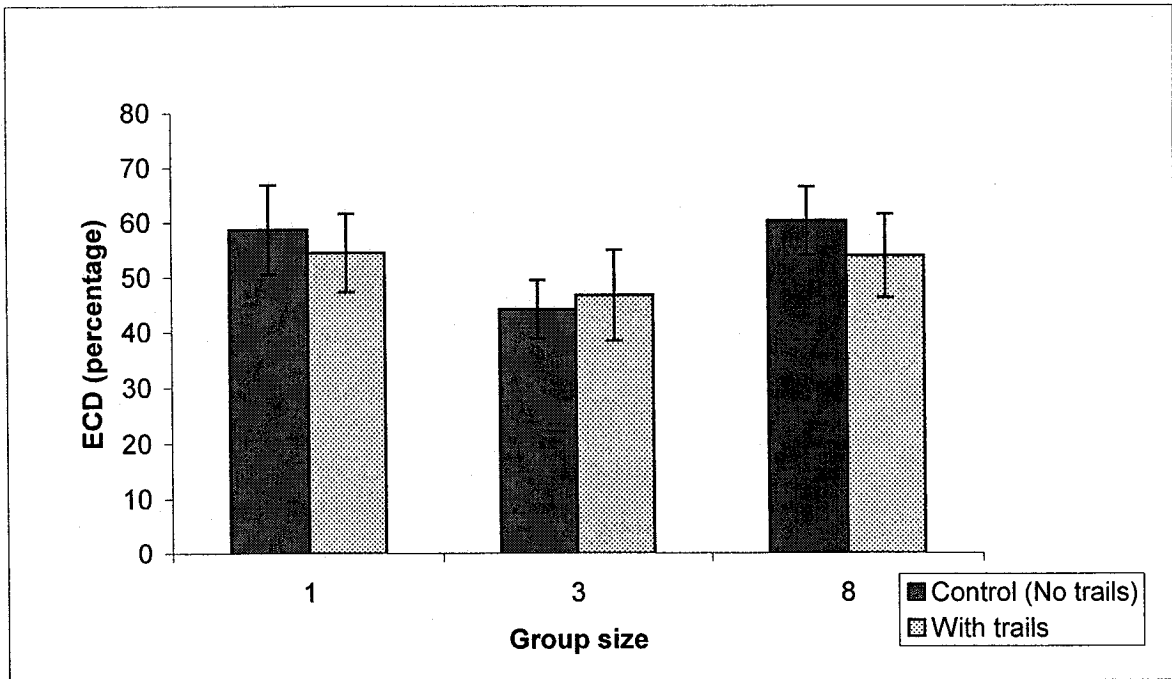


Figure 2.15: Effect of trails and group size on the ECD for the fourth instar larvae. ECD is the efficiency of conversion of digested material. Mean values are shown here with standard error.

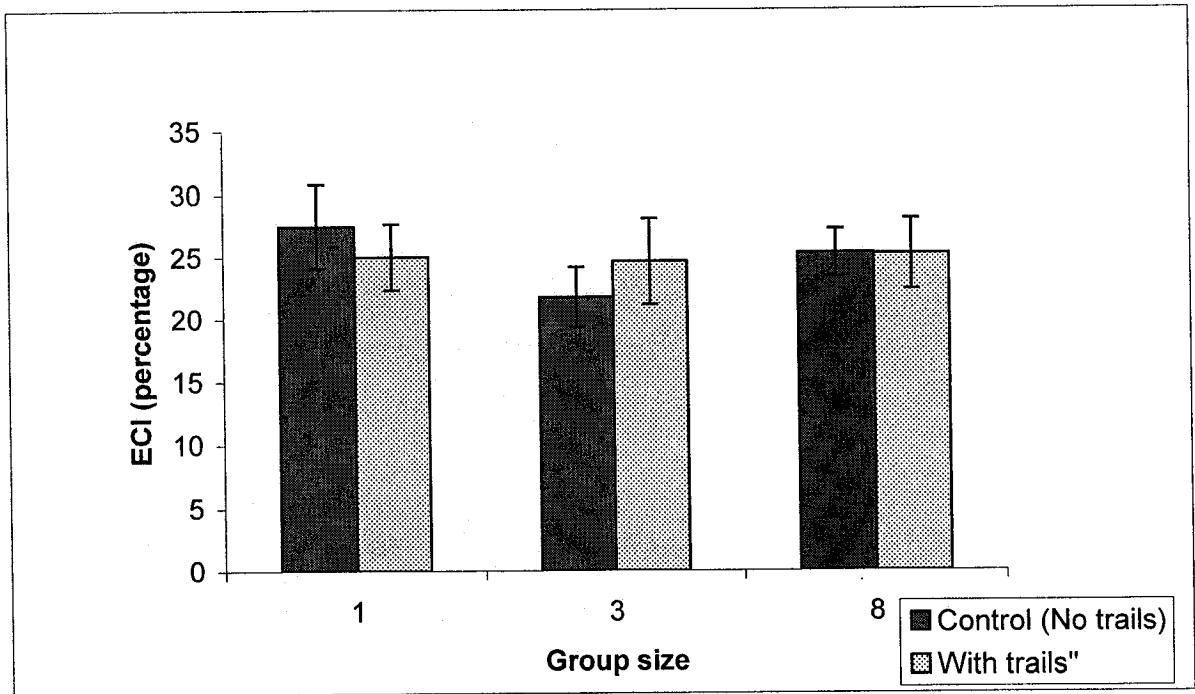


Figure 2.16: Effect of trails and group size on the ECI of fourth instar larvae. ECI is the efficiency of conversion of ingested material. Mean values are shown here with standard error.

Chapter 3

The Effect of Trails and Group Size on the Behaviour and Foraging Success of the Forest Tent Caterpillar

3. 1 Abstract

Young forest tent caterpillars (*Malacosoma disstria* Hübner) are social insects that use a complex system of pheromone trails to engage in large, synchronous foraging bouts. However, as they mature, fourth instar larvae become more solitary and often abandon their colony mates. Consequently, this study was designed to examine the effect of trails, group size, and instar on the behaviour and foraging success of forest tent caterpillars. Second and fourth instar caterpillars were placed in environments with and without pre-laid trails and of different group sizes. Their behaviour was monitored for a duration of four hours to assess the frequency and duration of six behaviours: resting, searching, feeding, contact with food, spinning, and walking. The latency to contact with food was recorded as a measure of foraging success.

Generally group size did not have a significant effect on behaviour of second and fourth instar caterpillars. However, group size may play a role in the foraging success of second instar larvae. In the presence of trails, second instar larvae searched significantly more and found their food significantly sooner than in the absence of trails. This effect appeared to diminish in older instars. A decrease in reliance on trails and group size for foraging success may reflect an ontogenetic change from a colonial to a more solitary insect.

3.2 Introduction

Young larval FTC are highly gregarious, colonial insects. However, as they mature, caterpillars become much more solitary and colonies tend to fragment (Fitzgerald 1993, Costa and Ross 2003). This change from colonial to solitary living may be the result of a delicate balance between costs and benefits associated with each larval stage.

Results from the previous experiment suggest that the effect of trails and group size on the performance of caterpillars differs between second and fourth instars. For second instars, the presence of trails and large group size resulted in accelerated development. In contrast, the rate of development for fourth instars was unaffected by these two variables. In the first experiment, it is hypothesized that the mechanism behind the effect of trails and group size on FTC performance may be linked to foraging success.

In this experiment, foraging success, which is defined as the amount of time required for the caterpillar to come in contact with food, was studied. Fitzgerald (1993) suggests that trails increase recruitment from food to silk mat; however, the use of trails to increase foraging success has not been conclusively shown (Fitzgerald 1993). Forest tent caterpillars have not been shown to recruit colony mates to profitable food sources (Fitzgerald and Webster 1993), however, trails may still play an important role in foraging success. This experiment will examine the time for individuals to reach the food as a measure of foraging success. If caterpillars reach their food sooner in the presence of trails, this may indicate that trails play an important role in foraging behaviour.

In this experiment, the behaviour of the forest tent caterpillar was also observed to establish time budgets for second and fourth instar caterpillars in the presence of food. Time budgets may provide evidence that an ontogenetic shift occurs between second and

fourth instars. As seen in Despland and Hamzeh (2004), the presence of trails increased the locomotion in second instars while fourth instars were less affected (Despland and Hamzeh 2004). In addition, this experiment will determine the effect of trails and group size on the behaviour of the caterpillar in the presence of food. The length and duration of feeding bouts in order to try and establish costs and benefits of group living.

3.3 Material and Methods

3.3.1. Experimental Insects and Design

Rearing of the experimental insects was done as in chapter two. For this experiment, a three way factorial design was used to determine the effect of trails, group size, and instar stage, on the behaviour of second and fourth instar caterpillars. For each trail and group size treatment there were 10 replicates.

3.3.2 Experimental Protocol

For this experiment, newly molted second and fourth instar larvae were removed from their culture and starved for four hours prior to the experiment. This was done to prevent prolonged periods of inactivity during which larval FTC are digesting their food. A focal individual was identified by painting its abdominal setae; this was the individual that would be monitored for the different behaviours. For the experiment, a focal individual was placed in a petri dish at the point furthest from the location of the diet. Consequently, at the beginning of the experiment, the focal individual and the diet were located at opposite ends of the petri dish. Second instars were placed within petri dishes with a diameter of 9 cm, while fourth instars were placed in dishes with a diameter of 15 cm. Larger petri dishes were used for fourth instars to try and control for their larger body sizes and increased mobility compared to second instars. If fourth instars were placed in 9 cm petri dishes, they may have experienced more crowding than second instars in similar sized dishes.

Trail treatments were created as in the first experiment with the exception that food was placed in the dish along with the insects. Once the experimental insects were placed in the Petri dish, second and fourth instar focal individuals were observed live for a duration of 4 hours. Throughout the experiment, the observer scanned the Petri dishes every 30 seconds and recorded the behavioural state which the focal individual was in by typing a letter code into event recorder software.

3.3.3 Behaviours

The behaviour of the caterpillar was monitored for these actions: resting, searching, eating food, in contact with food, spinning silk, and walking. The behaviours were defined by the following description:

1. Resting: the caterpillar is immobile
2. Searching: the abdominal prolegs of the caterpillar remain stationary while the anterior portion of the body moves from side to side.
3. Eating food: the caterpillar is seen to be ingesting food.
4. In contact with food: the caterpillar is in contact with the food, touching it with its legs, or resting, searching, and walking on the diet. However, the caterpillar is not ingesting food.
5. Spinning silk: the caterpillar is laying down silk in order to form a silk mat which can be used as a resting place. The caterpillar moves its head from side to side, drawing silk from the spinnerets and anchors the silk at both ends to the substrate (Fitzgerald 1993).
6. Walking: the individual is moving at a constant rate of one body length per

second (Despland and Hamzeh 2004).

Although the caterpillars are confined to a petri dish for this experiment, these six behaviours are not exhaustive of its entire repertoire of behaviours. Walking behaviour may further be defined as walking on a trail or following another individual (Fitzgerald 1995). In addition, individuals may walk and deposit pheromone by rubbing their abdomen against the substrate (Fitzgerald and Costa 1986). However, for the purpose of this experiment, these six behaviours were sufficient to establish locomotory time budgets and foraging success.

3.3.4 Analysis

The data was then transformed with Markov chain analysis and then analyzed with a three-way factorial ANOVA. Continuous Markov chain analysis was used to calculate the initiation and termination of the different behaviours. This type of analysis can be used to model systems that move between a defined number of mutually exclusive states in terms of the duration of each state, and its transition to the states.

Markov analysis uses the number and duration of behaviour bouts to calculate two parameters: the termination tendency and transition tendency.

The termination tendency of a , λ_a , is the chance per unit time, given that the state is a , that a will be left and another process will begin. It is calculated by the following formula:

$$(1) \quad \lambda_a = 1/\bar{x}_a$$

The transition tendency from a to b , α_{ab} , is the chance per unit time that the state b , will be entered once the state a has begun. It is calculated by the following formula:

$$(2) \quad \alpha_{ab} = N_{ab}/N_a \times 1/\bar{x}_a$$

Where \bar{x}_a is the mean bout duration for behaviour a . N_a is the number of bouts of behaviour a and N_{ab} is the number of transitions from a to b (Haccou and Meelis 1992). The continuous Markov chain analysis assumes that the tendency to enter or leave a state is constant. This implies the following: that the behaviour does not change from the beginning to the end of the record (Despland and Hamzeh 2004) and that it is a first order process, in the sense that each transition depends only on the state in which it occurs. The transition tendency will thus give the frequency of a particular behaviour, while the termination tendency is a measure of the duration of each behaviour bout.

To test the Markov assumptions, a linear regression on the log survivor plot was used. If the Markov model was an approximation of the data, the bout distribution for each behavioural category would be exponential and the log survivor plot would be a straight line through the origin. If the Markov assumptions were met, then the transition and termination tendencies for the different variables are asymptotically independent. The observations can then be used in analysis of variance, and multiple hypotheses about behavioural transitions can be tested independently (Haccou and Meelis 1992). The termination tendency (λ_a) and the transition tendency (α_{ab}) was calculated for each behaviour. Three-way factorial analysis of variances was then used to evaluate the effect of trails and groups size on the termination and transition tendency of each behaviour. If three-way factorial analysis showed an interaction between two variables, then two-way analysis of variance were performed to elucidate the effects.

The mean time for larval FTC to reach the food was recorded and analyzed using three-way factorial analysis of variance. These data were also analyzed with survival analysis. Traditional survival analysis tracks the survival of animals over the course of an experiment to determine if a treatment has a detrimental effect on the number of individuals dying per unit time. The Wilcoxon (Gehan) test then compares the survival distribution between the different groups. For this experiment, the contact with food was used as the survival variable. If the individual reached the food, its survival time was considered over.

Survival analysis was performed 1) For the effect of group size on the proportion of individuals that reach the food in the presence and absence of trails. This was done for each instar. 2) For the effect of trails on the proportion of individuals that contact food in different group sizes. This was done for each instar. 3) For the effect of the instar stage on the proportion of individuals that reach their food. This survival analysis will determine if a greater proportion of second or fourth instars reach their food per unit of time. This survival analysis pools both trails and group size treatments together and only takes into account the instar stage.

3.4 Results

3.4.1. Spinning and Search Behaviour

The results indicated that there was a significant effect of trails, group size, and larval instar, on transitions to spinning bouts. The results show that there is a significant interaction between trails and larval stage, as well as an interaction between group size and larval stage (Fig 3.1, Table 3.1). Two-way analysis of variance further elucidated these effects. The incidence of transitions to spinning bouts was significantly higher in the presence of trails than it was in the absence of trails for second instar caterpillars (Fig 3.1, Table 3.2). The interaction term suggests that relationship between group size and transitions to spinning bouts depends on the presence of trails (Table 3.2). Fourth instar larvae were not significantly affected by the presence or absence of trails (Table 3.2). Tukey Post Hoc tests performed on group size for second instar larvae showed that a group size of 8 had a significantly more transitions to spinning bouts than groups of 1 and 3 (Fig 3.1). In contrast, group size had a significantly different effect on fourth instar larvae (Fig 3.1): transitions to spinning bouts for older larvae were not significantly affected by group size (Table 3.2). For both second and fourth larvae, there was no significant effect of trails and group size on the termination of the spinning bouts (Fig 3.2, Table 3.1).

The results show that there was a significant effect of the presence of trails, group size, and larval instar on the frequency of transitions to searching bouts. There were two significant interactions: between trails and larval instar, as well as between group size and larval stage (Fig 3.3, Table 3.1). Two-way analysis of variance suggests that, for second

instar larvae, there were significantly more transitions to searching bouts in the presence of trails than in the absence of trails. This result differed significantly from fourth instar larvae (Fig 3.3, Table 3.1) whose transitions to searching were not significantly affected by the presence of trails and group size (Table 3.2). For second instar larvae, a group size of 3 did not differ from a group size of 1 (Fig 3.3). For both second and fourth instar caterpillars, there was no significant effect of trails, group size, or instar stage on the termination of searching bouts (Fig 3.4, Table 3.1).

3.4.2 Resting Behaviour

For second instar larvae, there was not a significant effect of trails or instar stage on transitions to resting bouts. In a similar manner, for fourth instars, there was not a significant effect of trails or group size to on transitions to resting bouts. In contrast, for both larval instars, a group size of 1 had a significantly more transitions to resting bouts than groups of 3 or 8 (Fig 3.5, Table 3.1). Groups of 3 and 8 did not differ significantly from one another (Fig 3.5). There was no significant effect of trails, group size, or instar stage on the termination tendency of the resting bouts (Fig 3.6, Table 3.1).

3.4.3 Eating Behaviour

The results suggest that there is a significant effect of instar stage on transitions to eating bouts. There is also an interaction between instar stage and the presence of trails (Fig 3.7, Table 3.1). Two-way analysis of variance indicated that, for second instar caterpillars, the incidence of transitions to eating bouts was significantly higher in the

presence of trails than in the absence of trails (Fig 3.7, Table 3.2). This effect was significantly different from fourth instar larvae, where transitions to eating bouts was not significantly affected by the presence of trails (Table 3.2). The results show that there was not a significant effect of trails on the termination of eating bouts for second and fourth instar caterpillars (Fig 3.8, Table 3.1). However, group size and instar stage was found to have a significant effect on the termination of eating bouts. There was also an interaction between group size and instar larval stage (Fig 3.8, Table 3.1). Two-way analysis of variance was then performed to clarify the interaction effect. For second instars, there was not a significant effect of group size on the duration of eating bouts (Table 3.2). In contrast, for fourth instar caterpillars, large group size had a highly significant effect on the termination of eating bouts (Table 3.2).

3.4.4. Foraging Success

As mentioned previously, the foraging success is defined as the amount of time for the focal individual to come into contact with its food. The results show a significant effect of trails and instar on the time to reach food. In addition, there was a significant interaction between trails and instar stage (Fig 3.9, Table 3.1). Two-way analysis of variance indicated that in the presence of trails, second instar caterpillars reach their food sooner (Fig 3.9, Table 3.2). In contrast, for fourth instars, there was not a significant effect of trails on the amount of time needed for them to reach their food (Fig 3.9, Table 3.2). For both second and fourth instar caterpillars, group size did not have a significant effect on foraging success (Fig 3.9, Table 3.1).

3.4.5. Latency to contact with food

Survival analysis was first performed for the effect of group size on time to contact with food in the presence and absence of trails. This analysis was performed for second and fourth instar larvae. For both instars, in the presence of trails, there was no significant effect of group size on the proportion of individuals reaching the food per interval of time (Table 3.3, Fig 3.11, Fig 3.13). In a similar manner, in the absence of trails, there was no significant effect of group size on the proportion of individuals reaching the food per interval of time (Fig 3.10, Fig 3.12, Table 3.3).

Survival analysis was then performed to determine the effect of trails on time to contact with food between each individual group size. For second instar larvae, there was no significant effect of trails on the proportion of isolated individuals reaching their food per interval of time, compared to isolated individuals in the absence of trails (Fig 3.10, Fig 3.11, Table 3.3). In contrast, the proportion of individuals in groups of 3 reaching their food in the presence of trails was significantly higher than in the absence of trails (Fig 3.10, Fig 3.11, Table 3.3). A similar result occurred in groups of 8: in the presence of trails a higher proportion of individuals reached their food than in the absence of trails (Fig 3.10, Fig 3.11, Table 3.3). For fourth instar larvae, there was not a significant effect of trails on the proportion of individuals reaching their food. For each group size, the proportion of individuals reaching their food in the presence of trails was not significantly different than in the absence of trails (Fig 3.12, Fig 3.13, Table 3.3). Finally, survival analysis was then performed to determine the effect of instar stage on the

proportion of individuals reaching the food. Fourth instars had a significantly higher proportion of individuals that reached the food per interval of time compared to second instar caterpillars (Fig 3.14, Table 3.3).

3.4 Discussion

The results suggest that trails and group size had a significant effect on transitions to spinning and searching behaviour of second instars but fourth instars were unaffected (Fig 3.1, Fig 3.2, and Table 3.1, Table 3.2). For second instars, an increase in spinning behaviour in the presence of trails may be related to pheromones. Trails may communicate the presence of colony mates to individual FTC. Consequently, in the presence of trails larval FTC may be stimulated to engage in colonial behaviour such as spinning a silk mat. Building a silk mat usually entails the work of several FTC (Fitzgerald 1995). Since silk may be energetically costly to produce in caterpillars (Costa and Pierce 1997), it may be wasteful to spin mats in the absence of colony mates.

Trails significantly increased transitions to searching behaviour in second instar larvae (Fig 3.3, Table 3.2). This may be because FTC often forage in synchrony, and move in large groups from one resource to another. In large groups, there may be an increase trails. The increase in pheromone may stimulate second instars to engage in search behaviour. If trails increase the amount of time FTC spend searching rather than resting, then this will increase the likelihood that second instar larvae will encounter food. Isolated larval FTC have been shown to move very slowly over unmarked substrates, presumably due to the absence of pheromone trails (Fitzgerald and Costa 1986). Therefore, in the absence of trails, second instars may perceive themselves to be alone and remain quiescent for long periods of time before they begin to engage in searching behaviour. Isolated individuals may not engage in foraging behaviour because they may have difficulty maneuvering over obstacles in on their way to food sources (Robison 1993, Fitzgerald 1995). In addition, isolated caterpillars which are foraging

alone may be more vulnerable to predation and parasites, and this may cause them to wander extensively searching for colony mates (Fitzgerald 1995).

Larger group size was also found to significantly increase transitions to spinning and searching in second instar larvae (Fig 3.1, Fig 3.3, and Table 3.2). An increase in group size may have resulted in more pheromone trails over the duration of the experiment. Consequently, this would mean that it is the increase in pheromone trails, and not the group size that stimulates behaviours which require more than one individual caterpillar. This experiment did not verify if the strength of pheromone trails is the method used by FTC to determine the presence of other caterpillars. Insects may use strength and frequency of pheromone trails, or of body contact incidents, to evaluate the presence of colony mates (Costa and Pierce 1997). The use of pheromones to assess the presence of colony mates is seen in ants. Devigne et al. (2004) found that although group density is assessed through contact and interaction, during foraging ants leave home range marks. These marks reflect the distribution of ants and allow ant scouts to assess the density and activity of nestmates (Devigne et al. 2004). For second instar caterpillars, both trails and group size encourages spinning and searching behaviour. Consequently, it may be a combination of pheromone trails and interaction between colony mates that allows second instar larvae to assess the presence of other caterpillars.

In contrast, the spinning and searching behaviour of fourth instar larvae was not significantly affected by the presence of trails and large group size (Fig 3.1, Fig 3.3, and Table 3.2). Since fourth instar caterpillars rarely rest together on silk mats and often forage alone, this result was expected. Their solitary behaviour may decrease their dependence on pheromone trails for locomotion. This result is in accordance with the

findings of Despland and Hamzeh (2004), who found that trails influenced locomotion less in fourth instar larvae than in second instars.

For fourth instar caterpillars there was a significant increase in the termination tendency of eating bouts, while second instars were not significantly affected (Fig 3.1, Table 3.2) This may have been due to competition among fourth instar larvae which was absent in second instar larvae, possibly because second instars are much smaller and fit easily fit on the food used in this experiment. The decrease in length of eating bouts for fourth instars may be evidence of interference competition. Fourth instar caterpillars, due to their large body mass, may be able to physically interfere with other individuals during feeding bouts.

In the presence of trails, second instar caterpillars reached their food significantly faster than in the absence of trails, however, fourth instars were not significantly affected (Fig 3.9, Table 3.2). This would suggest that second instars are more dependent on trails for foraging success. Since food was present in the arena while pre-experimental insects were laying down trails, if any of these insects fed on the diet, they would leave strong pheromone trails. These trails would have incited trail following behaviour, which would help experimental insects locate the food. A previous study has demonstrated that young FTC preferentially follow trails from sated caterpillars over exploratory trails (Fitzgerald and Webster 1993). In addition, social caterpillars including the Eastern tent caterpillar, and *Malacosoma neustria*, have been shown to prefer the trails of sated over hungry caterpillars (Fitzgerald and Peterson 1983; Peterson 1988). FTC have not been specifically shown to recruit other colony members to profitable food sources, but they have been shown to mark trails more heavily once they have fed (Fitzgerald and Costa

1986). In addition, they have been shown to prefer trails laid by well fed Eastern tent caterpillars (Fitzgerald and Edgerly 1979). In addition, FTC have been shown to use trails as guides to new silk mats (Fitzgerald and Webster 1993). Therefore, FTC may also be able to use the trail strength to enhance their foraging success.

Group size was not found to have a significant effect on the time to reach food, for second instars (Fig 3.9, Table 3.2). However, survival analysis for the effect of trails on the individual group sizes, suggests that second instar larvae may need both trails and group size to increase foraging success. For isolated individuals, the presence or absence of trails had no effect on the proportion of individuals reaching their food. In contrast, in the presence of trails, a higher proportion of individuals raised in groups of 3 reached their food; a similar result was seen in groups of 8 (Fig 3.10, Fig 3.11). In the presence of trails, isolated individuals may lack body contact interactions with other colony mates. In contrast, in the presence of trails, grouped caterpillars have both pheromone trails and interaction with colony mates. This suggests that for second instars, the presence of trails alone is not sufficient for foraging success.

The results suggest that fourth instar caterpillars are less dependent on trails for foraging success. Although it may be argued that this experimental decrease is the result of fourth instars being constrained in a 15 cm dish, other studies have shown that fourth instars are willing to leave trails while foraging for food (Fitzgerald and Costa 1986). Other social caterpillars including *Eriogaster lanestri*, *Malacosoma pluviale* have also been shown to abandon trails during their later instars (Ruf et al. 2001, Iwao 1968). Since fourth instar FTC are much more mobile, they may rely instead on plant chemicals to help them locate food sources (Fitzgerald 1995). However, it has been shown that late

instar larvae still produce pheromones (Fitzgerald and Costa 1986). This suggests that fourth instar larvae still follow trails, although they are no longer crucial for foraging success. For fourth instars, trails may still serve to mark their foraging arena and help them return to profitable food source which they have already located. Despland and Hamzeh (2004) also found that fourth instar FTC rely less on silk trails and are more likely to engage in exploratory behaviour than second instar larvae. Unlike second instars, survival analysis for fourth instars suggests that the different group sizes were equally able to find their food in the presence or absence of trails (Fig 3.12, Fig 3.13, and Table 3.3).

These results suggest that there may be a change in larval FTC's dependence on trails between the second and fourth instar caterpillars. Second instar larvae may rely more heavily on trails due to their colonial lifestyle. The pheromone component of trails may be important in maintaining colony cohesion during synchronous foraging bouts (Fitzgerald 1995). The silk component of trails may be important to second instar larvae who need silk to navigate obstacles in order to reach food resources. As caterpillars pass over obstacles they lay down silk which may increase purchase for caterpillars that are following behind (Robison 1993). In contrast, trail following may be less essential to fourth instar larvae. They do not forage in groups, and thus do not depend on trails to locate colony mates. In addition, fourth instar FTC occur in late spring, when suitable food resources are scarcer; consequently, they travel further for resources (Fitzgerald and Costa 1986). For fourth instar FTC, trails may not necessarily lead to a nutritious resource. Trails may lead to a resource that has already been depleted by other FTC. Therefore, they cannot be highly dependent on trails for foraging success. Behavioural

assays of FTC seem to support this conclusion as late instar larvae are more willing to leave chemical trails to explore new substrates (Fitzgerald and Webster 1993). Although second instar larvae must explore new areas as well, young FTC colonies have been shown to alternate leaders who are venturing onto unexplored substrates. A single individual caterpillar is not consistently exploring unmarked territory. Therefore, second instar caterpillars explore unmarked substrates less often than fourth instars.

For young caterpillars, aggregation may occur for protection against predators, increased foraging efficiency, and rapid shelter construction. However, exhaustion of food resources may cause late instar caterpillars to abandon their groups and forage independently (Costa 1997). In FTC an ontogenetic shift from colonial to solitary behaviour in FTC is believed to occur between the second and fourth instar. In this experiment, there were differences in the time budget of second and fourth instar caterpillars. For younger FTC, increases in spinning and searching behaviour in the presence of trails and in large groups may reflect a colonial lifestyle. As larval FTC grow older, spinning and searching behaviour is no longer affected by trails and group size. In addition, this experiment demonstrated that second instar larvae rely significantly on trails for foraging success while fourth instar larvae do not. Dependence on trails during foraging would only be useful if colony mates were present to engage in this system of communication. Since both instars were subject to similar experimental conditions, differences in second and fourth instars may reflect an ontogenetic change from gregarious to solitary behaviour which is thought to occur under natural conditions.

In larval FTC, an ontogenetic shift from gregarious to solitary individual may be linked to an increase in body size throughout the different instars (Despland and Hamzeh

2004). As FTC grow in body size, intraspecific competition becomes more intense, and fourth instar caterpillars must forage independently in order to meet their nutritional needs (Hebert 1993). These results suggest that intraspecific competition does indeed become increasingly intense as demonstrated by shorter eating bouts in fourth instars. Consequently, the results suggest that an ontogenetic shift may occur between second and fourth instar caterpillars, and that this change may be due to increased intraspecific competition attributed to larger body size.

Table 3.1: ANOVA table for the effect of trails and group size on the behaviour of second and fourth instar larvae. T, G, I refers to trails, group size, and instar respectively.

Dependent Variable	Independent Variable	F Value	Degrees of Freedom	Probability (p value)
Transitions to spinning	Trails	12.08	1, 543	0.0005
	Group	4.72	2, 543	0.0093
	Instar	23.75	1, 543	< 0.0005
	T X I	11.61	1, 543	0.024
	T X G	3.75	2, 543	0.063
	I X G	2.66	2, 543	0.04
	T X I X G	2.17	2, 543	0.067
Termination of spinning	Trails	0.39	1, 57	0.54
	Group	0.03	2, 57	0.97
	Instar	60.03	1, 57	0.09
	T X I	3.01	1, 57	0.43
	T X G	0.85	2, 57	0.91
	I X G	0.09	2, 57	0.93
	T X I X G	0.07	2, 57	0.93
Transitions to searching	Trails	3.41	1, 543	0.048
	Group	32.16	2, 543	< 0.0005
	Instar	26.41	1, 543	< 0.0005
	T X I	3.06	1, 543	0.05
	T X G	1.89	2, 543	0.15
	I X G	6.41	2, 543	0.001
	T X I X G	0.47	2, 543	0.62
Termination of searching	Trails	0.31	1, 97	0.57
	Group	2.04	2, 97	0.14
	Instar	0.89	1, 97	0.35
	T X I	0.18	1, 97	0.67
	T X G	0.99	2, 97	0.38
	I X G	1.09	2, 97	0.34
	T X I X G	0.89	2, 97	0.41
Transitions to resting	Trails	0.38	1, 543	0.56
	Group	9.48	2, 543	< 0.0005
	Instar	0.0001	1, 543	0.97
	T X I	0.75	1, 543	0.38
	T X G	1.23	2, 543	0.29
	I X G	4.30	2, 543	0.08
	T X I X G	1.52	2, 543	0.21
Termination of resting	Trails	0.186	1, 99	0.67
	Group	2.66	2, 99	0.07
	Instar	2.66	1, 99	0.07
	T X I	0.24	1, 99	0.63
	T X G	0.49	2, 99	0.61
	I X G	2.11	2, 99	0.13
	T X I X G	0.97	2, 99	0.38
Transitions to eating	Trails	0.06	1, 543	0.80
	Group	0.17	2, 543	0.84
	Instar	7.87	1, 543	0.0005
	T X I	6.19	1, 543	0.01
	T X G	1.29	2, 543	0.28
	I X G	0.27	2, 543	0.77
	T X I X G	0.51	2, 543	0.60
Termination of eating	Trails	0.0009	1, 78	0.97
	Group	15.24	2, 78	< 0.0005
	Instar	5.8	1, 78	0.025
	T X I	0.46	1, 78	0.49
	T X G	0.91	2, 78	0.41
	I X G	7.28	2, 78	0.001
	T X I X G	3.75	2, 78	0.09
Time to reach food	Trails	13.75	1, 107	< 0.0005
	Group	2.93	2, 107	0.06
	Instar	147.2	1, 107	< 0.0005
	T X I	7.44	1, 107	0.007
	T X G	1.99	2, 107	0.14
	I X G	0.208	2, 107	0.812
	T X I X G	1.60	2, 107	0.20

Table 3.2: Two Way ANOVA table for the effect of trails and group size on the behaviour of second and fourth instar larvae. L2 and L4 refers to second and fourth instar larvae respectively

Dependent Variable	Independent Variable	F value	Degrees of freedom	Probability (p value)
Transitions to spinning (L2)	Trails	14.0	1, 282	<0.005
	Group	4.16	2, 282	0.017
	T X G	3.61	2, 282	0.028
Transitions to spinning (L4)	Trails	0.04	1, 249	0.83
	Group	2.65	2, 249	0.07
	T X G	1.04	2, 249	0.35
Transitions to searching (L2)	Trails	6.83	1, 285	0.009
	Group	37.18	2, 285	< 0.0005
	T X G	1.52	2, 285	0.2197
Transitions to searching (L4)	Trails	0.0037	1, 249	0.95
	Group	2.65	2, 249	0.08
	T X G	1.035	2, 249	0.356
Transitions to eating (L2)	Trails	9.37	1, 282	0.0024
	Group	0.627	2, 282	0.534
	T X G	2.65	2, 282	0.07
Transitions to eating (L4)	Trails	1.92	1, 249	0.167
	Group	0.13	2, 249	0.871
	T X G	0.97	2, 249	0.626
Termination of eating (L2)	Trails	0.17	1, 40	0.682
	Group	2.48	2, 40	0.10
	T X G	2.65	2, 40	0.08
Termination of eating (L4)	Trails	0.32	1, 38	0.057
	Group	34.84	2, 38	< 0.0005
	T X G	0.59	2, 38	0.559
Time to reach food (L2)	Trails	13.07	1, 54	0.001
	Group	1.34	2, 54	0.270
	T X G	2.197	2, 54	0.121
Time to reach food (L4)	Trails	3.427	1, 68	0.069
	Group	2.967	2, 68	0.059
	T X G	1.48	2, 68	0.236

Table 3.3: Latency to contact with food.

Effect of group size (second instar)	Wilcoxon (Gehan) Statistic	Degrees of Freedom	Probability
Control (No trails)	2.05	2	0.37
With trails	5.19	2	0.08
Effect of group size (fourth instar)			
Control (No trails)	3.10	2	0.21
With trails	3.99	2	0.13
Effect of trails (Second instar)			
Group size 1	0.007	1	0.932
Group size 3	5.871	1	0.015
Group size 8	7.002	1	0.008
Effect of trails (Fourth instar)			
Group size 1	2.247	1	0.134
Group size 3	0.082	1	0.774
Group size 8	1.947	1	0.169
Effect of instar	15.28	1	< 0.0005

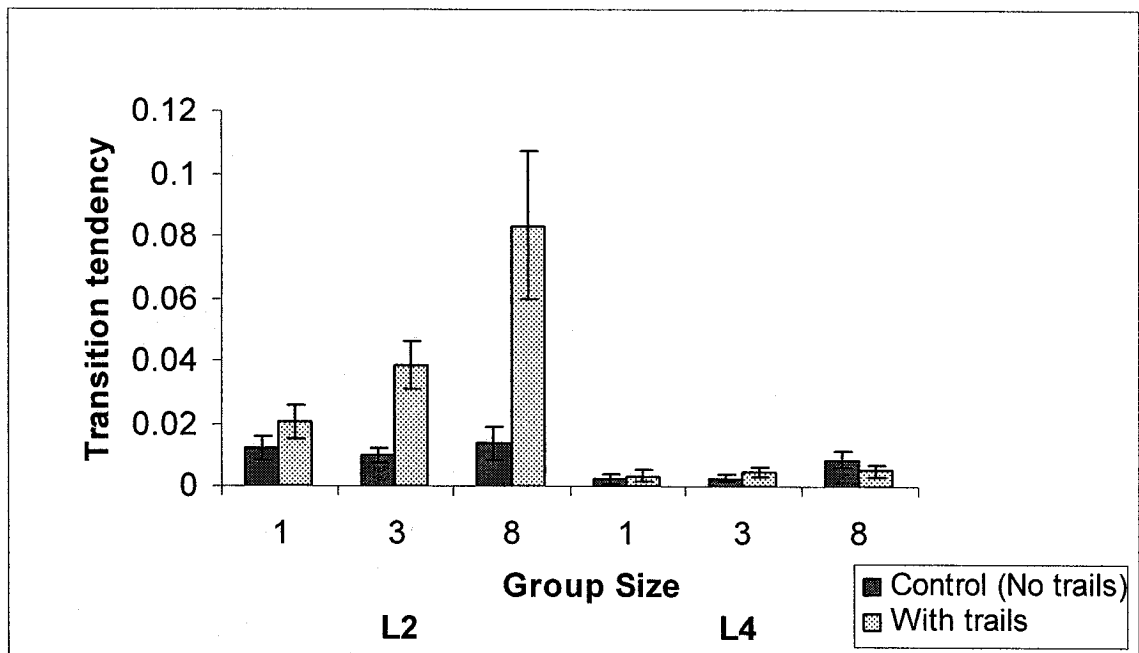


Figure 3.1: Transitions to spinning bouts. Mean values shown here with standard error. L2 and L4 represent second and fourth instar larvae respectively. For second instars, Post Hoc Tukey test indicated that there were more transitions to spinning bouts in groups of 8 compared to groups of 1 ($P=0.01089$) and groups of 3 ($P=0.041$). Groups of 3 did not differ from groups of 1 ($P=0.843$). For fourth instars, there was no significant effect found on transitions to spinning bouts due to group size.

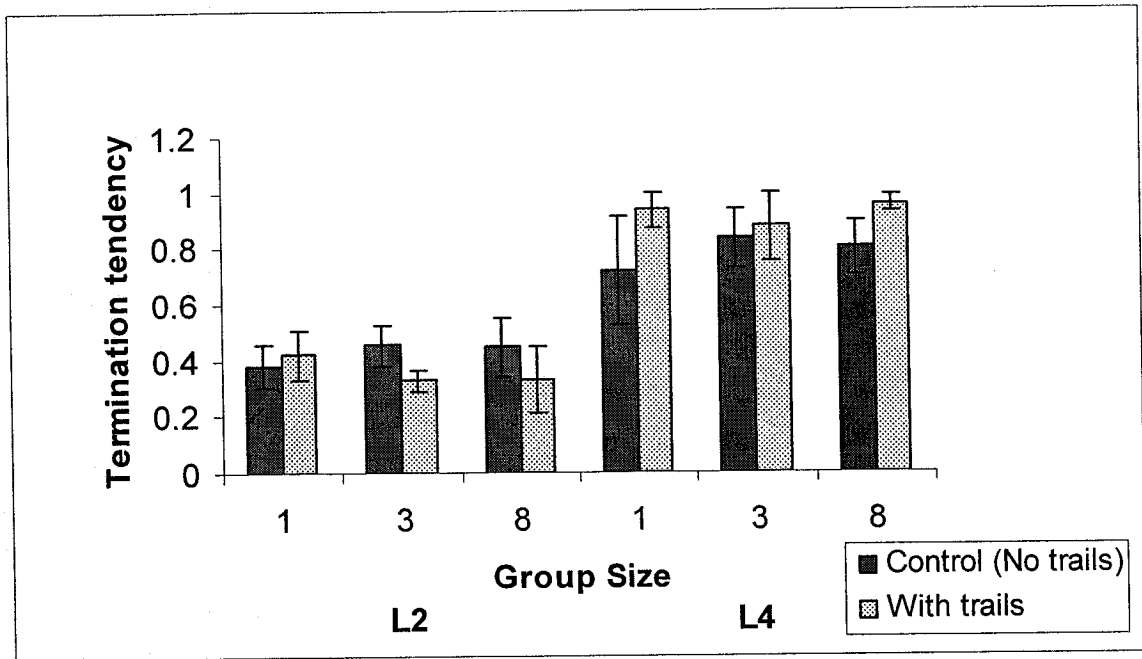


Figure 3.2: Termination tendency of spinning bouts. Mean values shown here with standard error. L2 and L4 represent second and fourth instar larvae respectively.

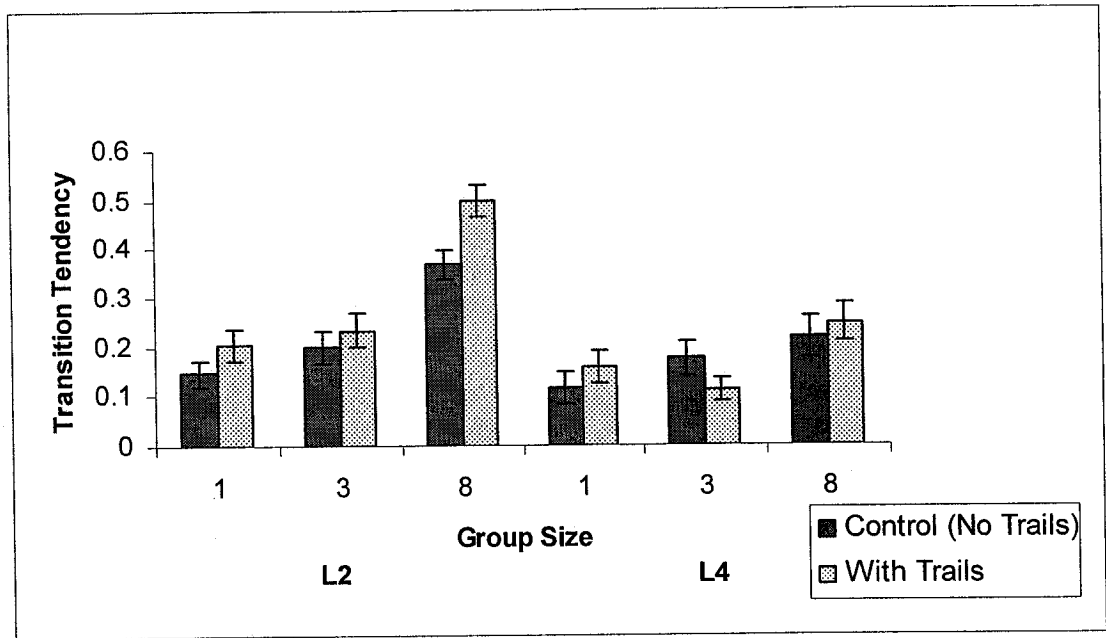


Figure 3.3: Transition to searching bouts. Mean values shown here with standard error. L2 and L4 represent second and fourth instar larvae respectively. For second instars, a Post Hoc Tukey test indicated that there were more transitions to spinning bouts in groups of 8 compared to groups of 1 ($P < 0.0005$) and groups of 3 ($P < 0.0005$). Groups of 1 and 3 did not differ significantly ($P = 0.432$).

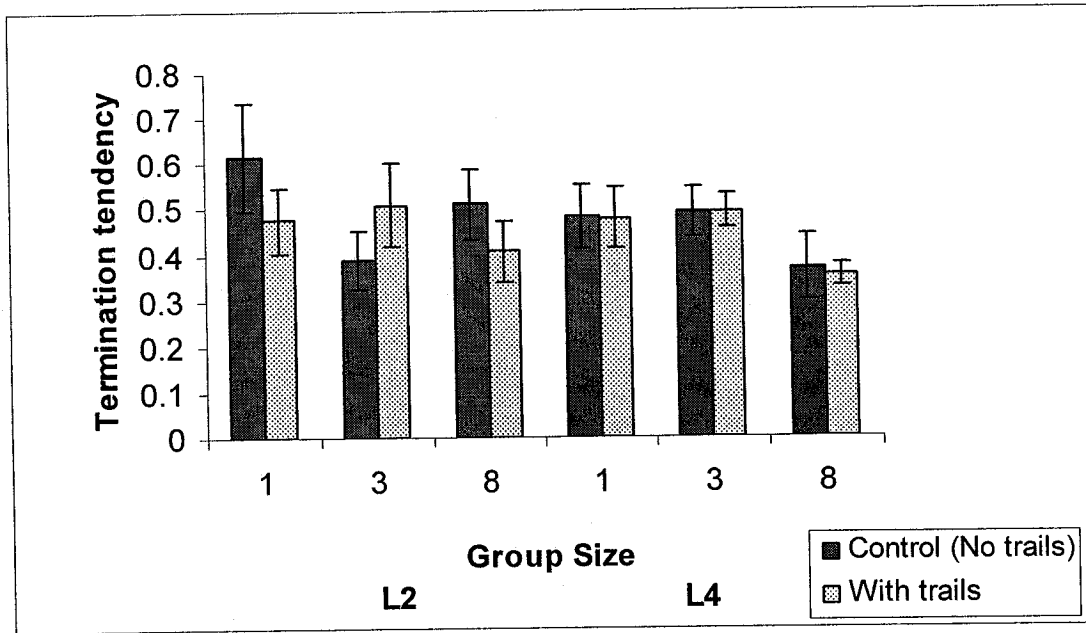


Figure 3.4: Termination tendency of searching bouts. Mean values shown here with standard error. L2 and L4 represent second and fourth instar larvae respectively.

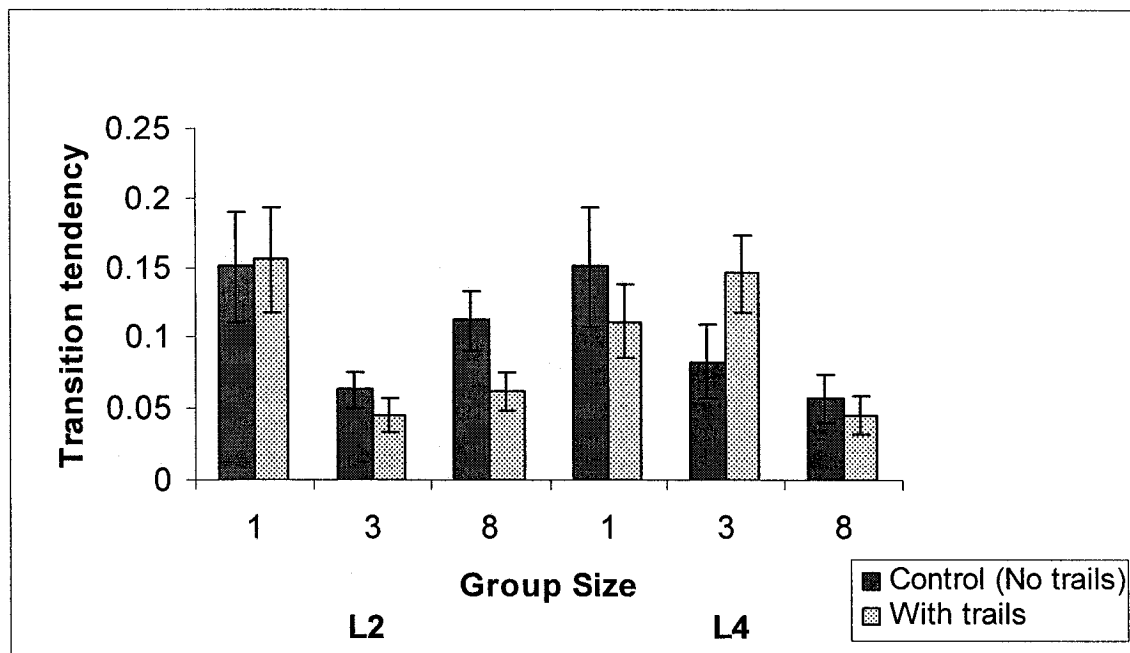


Figure 3.5: Transitions to resting bouts. Mean values shown here with standard error. L2 and L4 represent second and fourth instar larvae respectively. For both second and fourth instars, a Post Hoc Tukey test indicated that groups of 1 had significantly more transitions to resting than groups of 3 ($P < 0.0009$) and groups of 8 ($P < 0.0005$). However, groups of 3 and 8 did not differ significantly ($P = 0.931$)

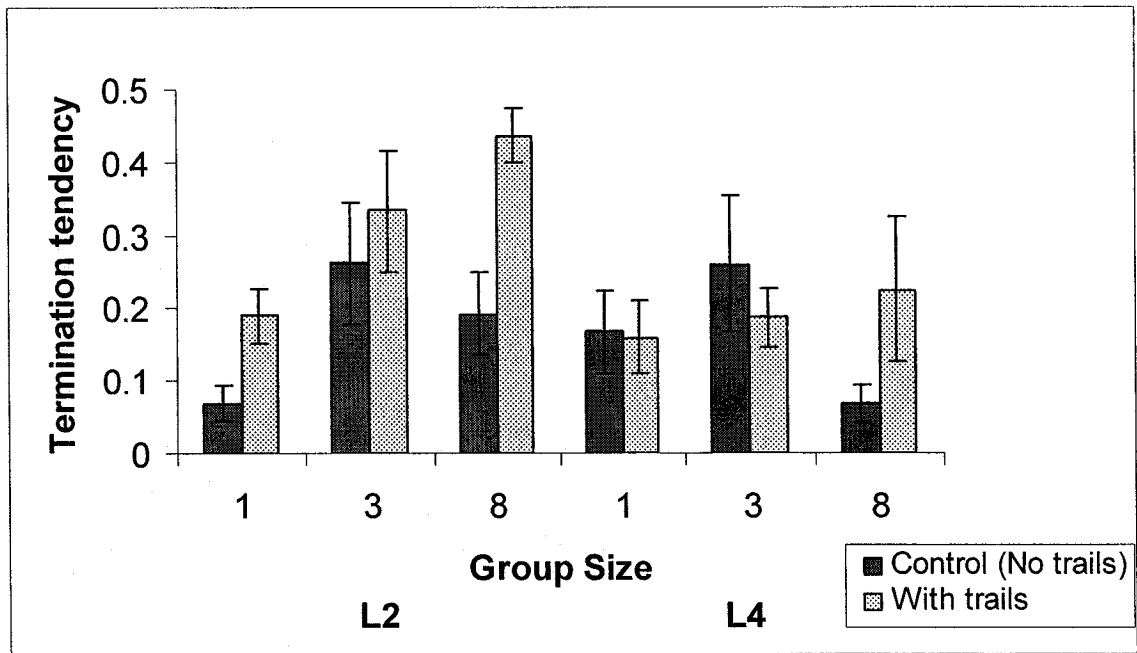


Figure 3.6: Termination of resting bouts. Mean values shown here with standard error. L2 and L4 represent second and fourth instar larvae respectively.

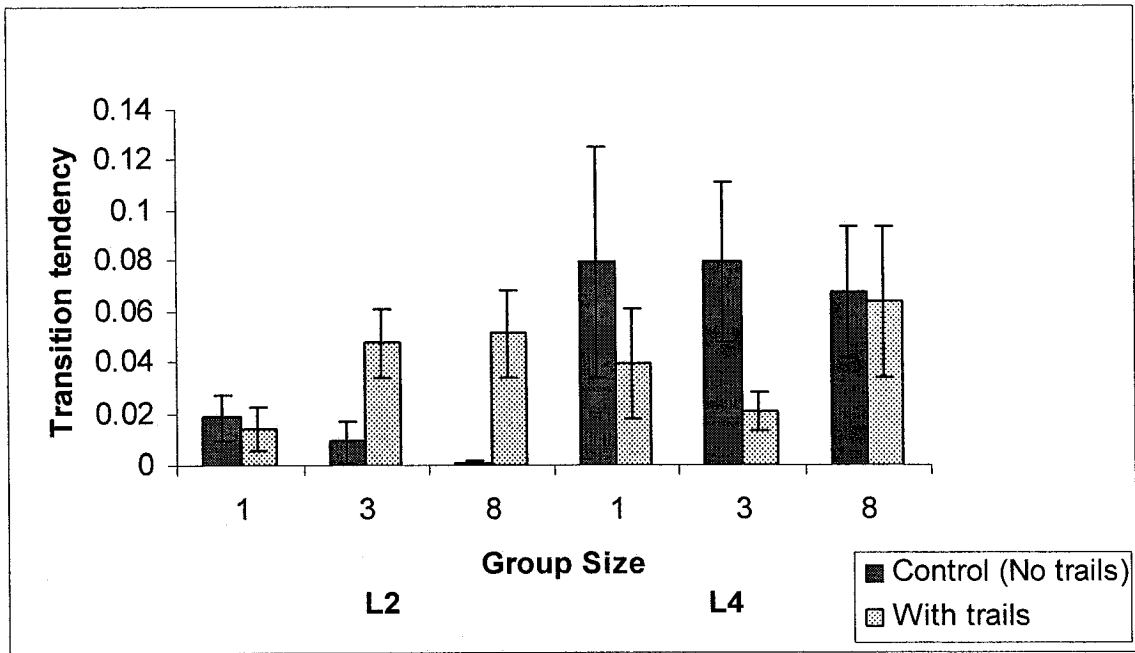


Figure 3.7: Transitions to eating bouts. Mean values shown here with standard error bars. L2 and L4 represent second and fourth instar larvae respectively.

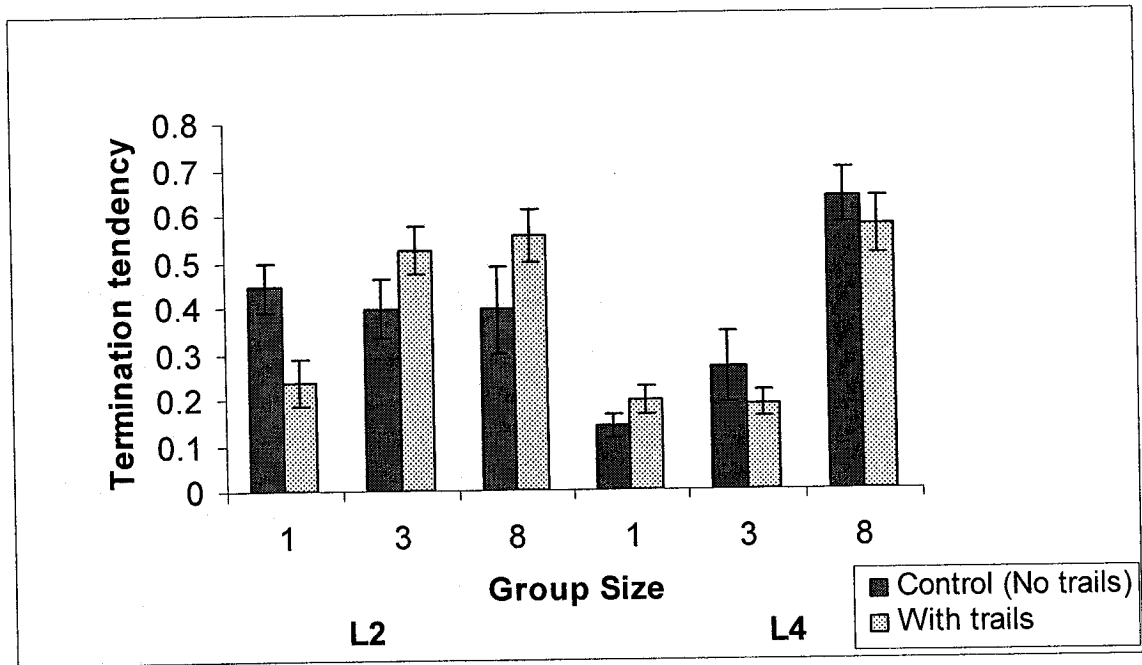


Figure 3.8: Termination tendency of eating bouts. L2 and L4 represent second and fourth instar larvae respectively. For second and fourth instars, a Post Hoc Tukey test indicated that groups of 8 had higher termination tendency than groups of 1 ($P < 0.0005$) and 3 ($P < 0.0005$). Groups of 1 and 3 were not significantly different from one another ($P = 0.693$)

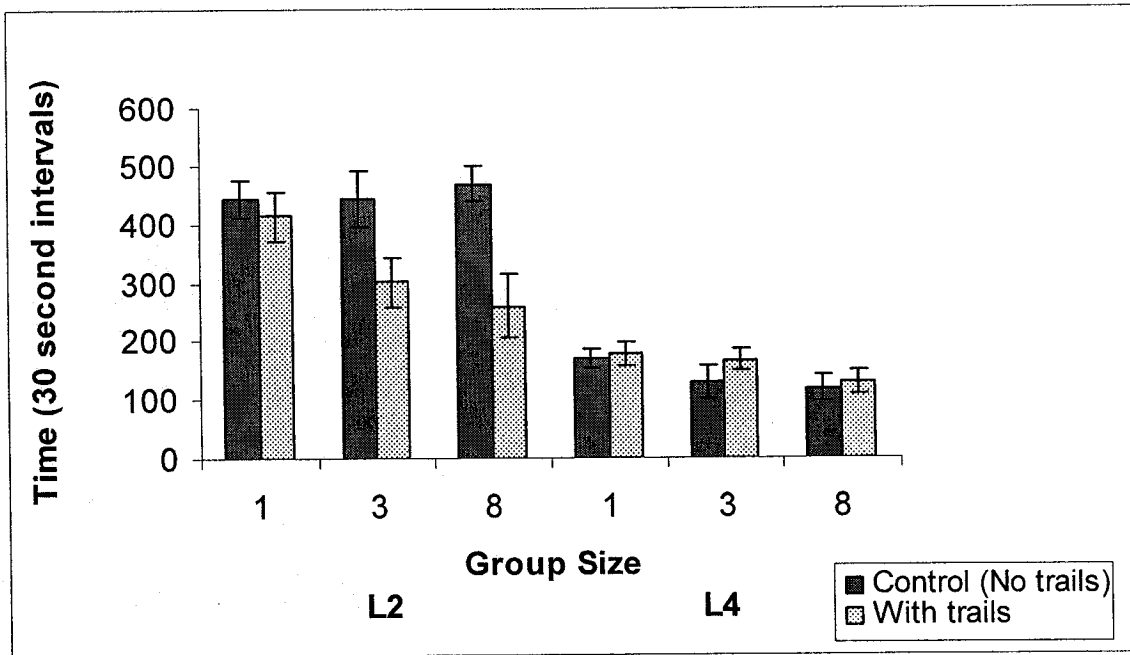


Figure 3.9: Time to reach food (second and fourth instar larvae). Mean values shown here with standard error. L2 and L4 represent second and fourth instar larvae respectively.

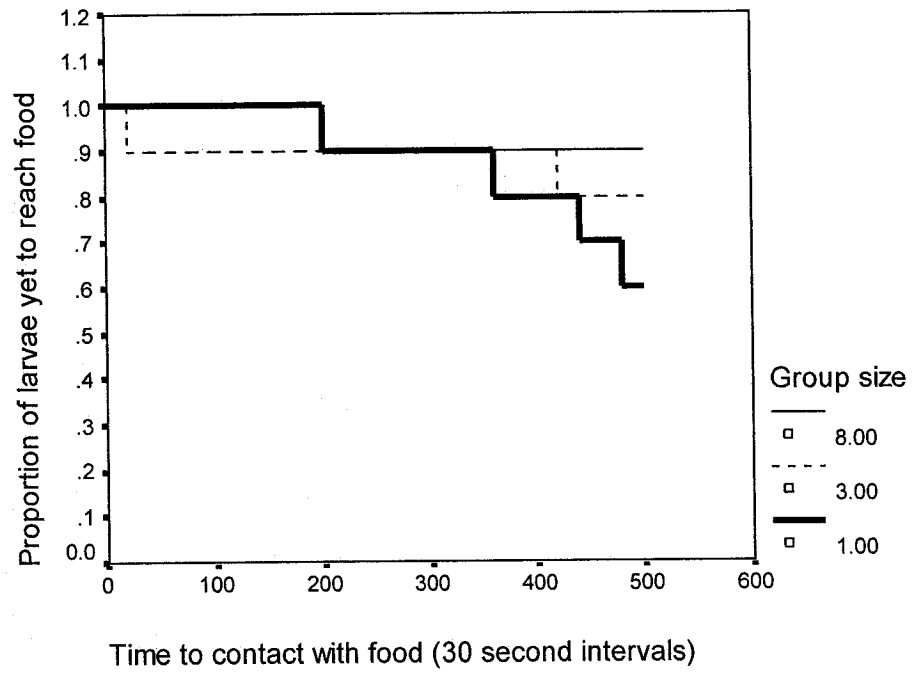


Figure 3.10: Proportion of second instars reaching the food (Control: No trails).

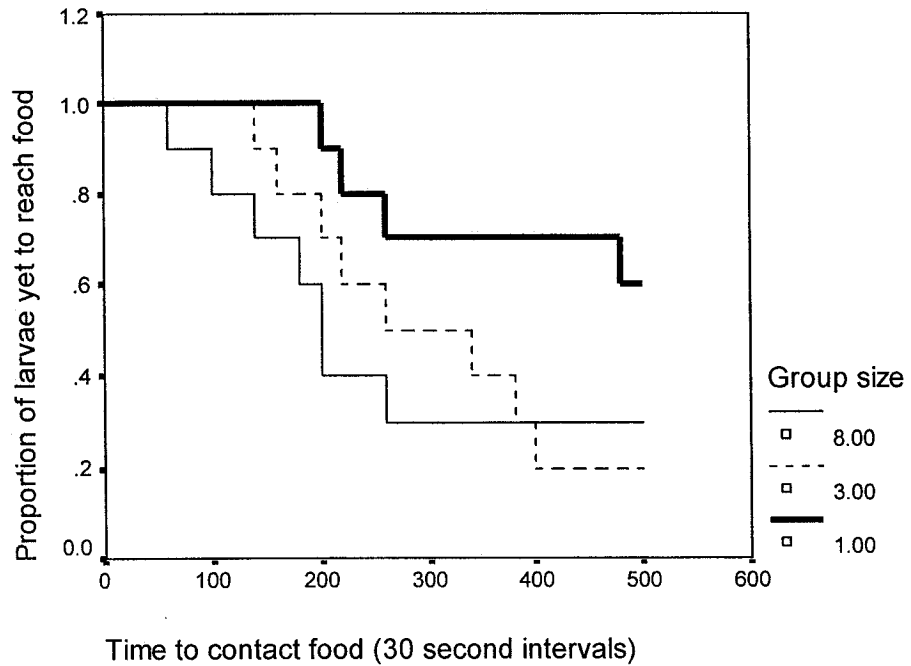


Figure 3.11: Proportion of second instars reaching the food (With trails)

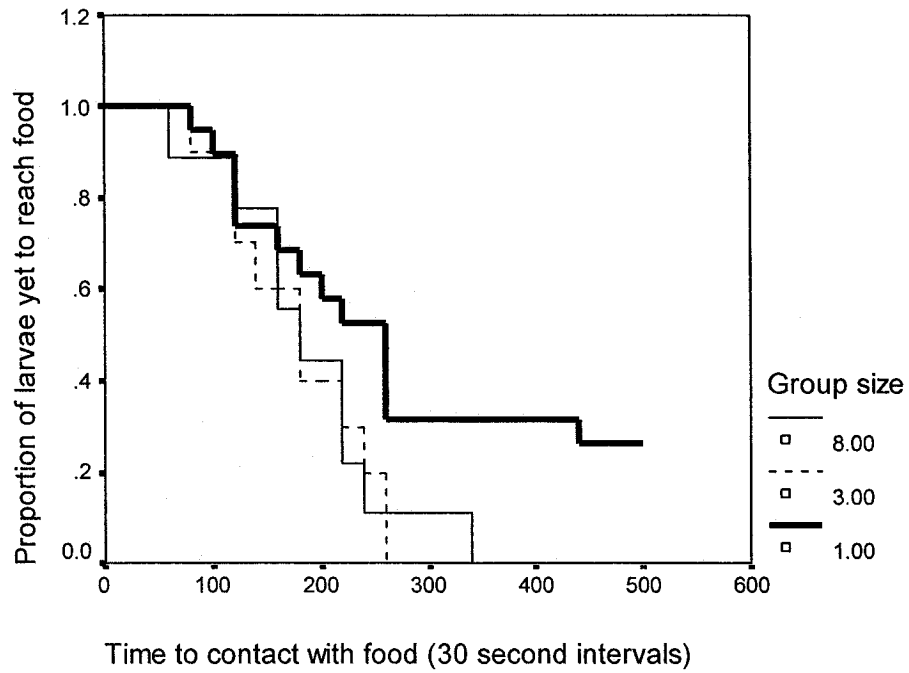


Figure 3.12: Proportion of fourth instars reaching the food (Control: No trails)

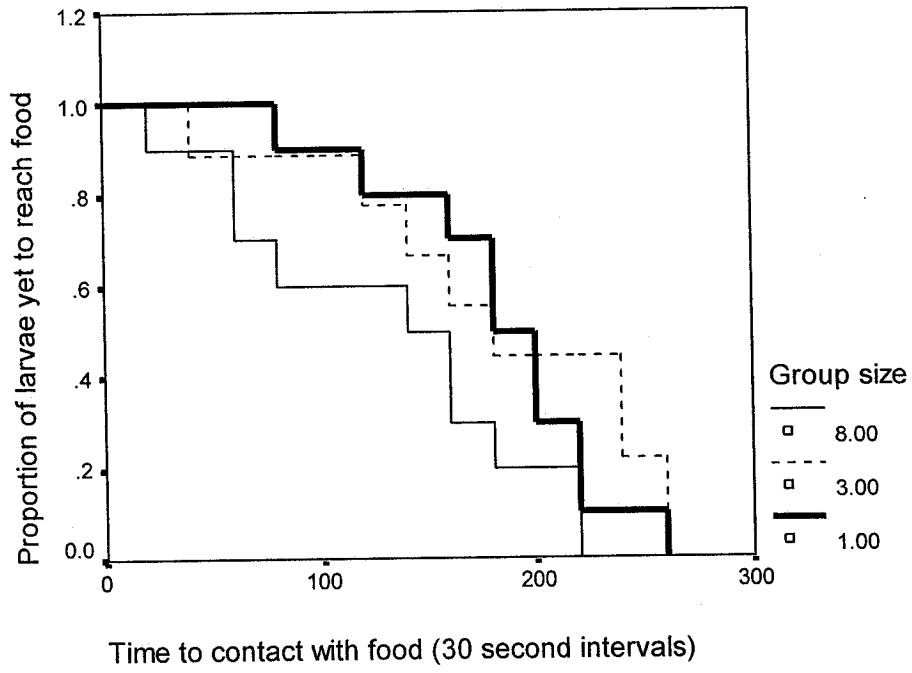


Figure 3.13: Proportion of fourth instars reaching the food (With trails)

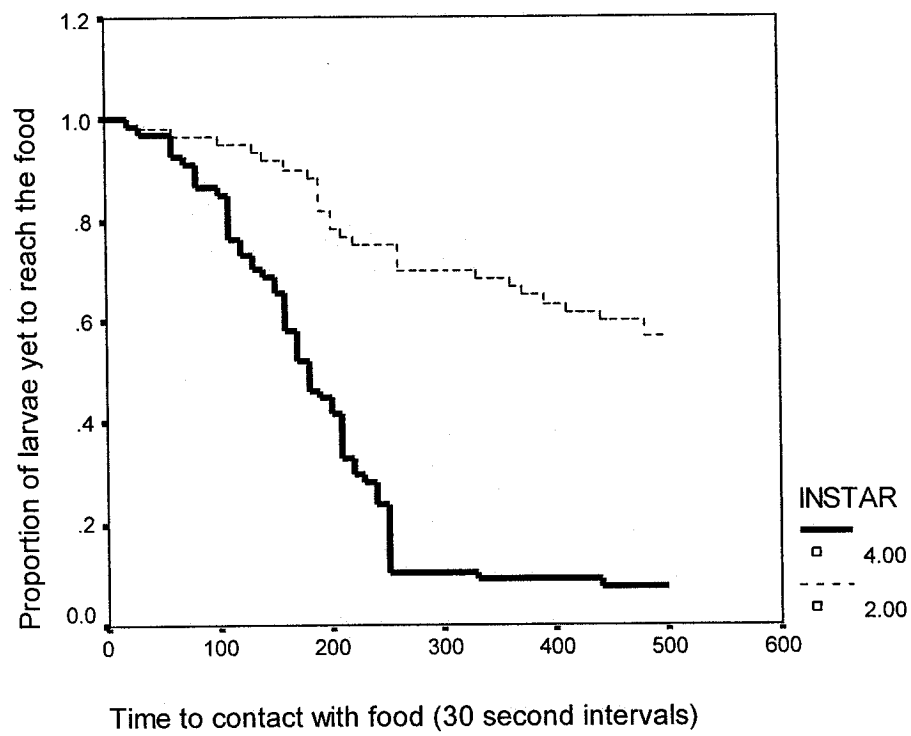


Figure 3.14: Proportion of second and fourth instar larvae to reach the food.

Chapter 4

General Discussion

Both trails and group size were shown to play an important role in forest tent caterpillar performance and behaviour. In the first experiment, trails and group size significantly increased the performance of second instars. For fourth instars, trails significantly increased the performance of caterpillar by increasing its weight gain over the duration of the instar stage. In contrast, group size decreased the performance of fourth instars by significantly decreasing its weight gain over the larval stadium. The second experiment suggests that the effect of trails and group size on the behaviour and foraging success of second and fourth instars was different. These differences may be a reflection of a change from colonial to solitary living.

The results suggest that the presence of trails at the onset of the experiment can ultimately have an effect on the rate of development and weight gain of second instar larvae. In addition, gravimetric analysis indicates that second instar larvae incorporate a significantly higher percentage of digested material into body weight in the presence of trails. Consequently, these results suggest that trails are an integral component of the second instar biology, affecting both growth and development. This is a particularly strong effect considering that trails were present only at the onset of the experiment, at the beginning of the larval instar.

The second experiment suggests that the mechanism behind the accelerated development may be increased foraging success in the presence of trails. This study provides evidence that second instar larvae depend heavily on trails to locate their food within a defined search area. In the absence of trails, very few second instars were able to

come into contact with their food within the duration of four hours. If the absence of trails imposes a significant delay in the time to reach the food, this may result in a prolonged development time. In addition, the latency to contact with food may explain the increase in ECD values in the presence of trails. If second instar FTC reach their food sooner, they may expend a lower proportion of digested material on respiration as they exert themselves searching for food.

The effect of trails appears to decrease in fourth instars. The rate of development and efficiency of food use of these older larvae was not significantly affected by the presence of trails. In addition, the second experiment suggests that fourth instar larvae no longer rely on trails for foraging success. There was not a significant difference in the time to reach their food in the presence or absence of trails. However, the first experiment indicated that fourth instars gained significantly more weight over the duration of the instar in the presence of trails. This suggests that although trails do not have an effect on foraging success, they may still play a role in stimulating feeding .

Group size was also found to have a significant effect on the performance of second instar larvae. In the largest groups, these young larvae developed significantly faster but were significantly smaller than those raised in isolation. Results from the first experiment suggest that second instar larvae may be directing their energetic resources to accelerated development in order to avoid food shortages (Iwao 1968). The second experiment did not directly test the mechanism behind this effect. However, results do suggest that the accelerated growth in large groups may be related to enhanced foraging success. A greater proportion of second instar larvae reached their food in the presence of trails and in large groups.

Results from the first experiment suggest that larger group size decreases the weight gain of fourth instars, but the rate of development was not significantly affected. Behavioural observations suggest that this may be due to interference competition between colony mates. Fourth instar larvae had shorter eating bouts in the presence of colony mates. This may be because individuals are interfering with one another during feeding behaviour.

Generally, the results of both experiments suggest that second and fourth instars are different. This may reflect a shift from a gregarious to solitary lifestyle as the caterpillar grows (Fitzgerald 1993). As the FTC increases in body size, many of the benefits it incurred from group living may be surpassed by the costs of intraspecific competition. Results from the second experiment suggest that interference competition does increase between second and fourth instar larvae. Consequently, fourth instar FTC may engage in solitary living to avoid competition associated with group living.

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