

Effects of food quality and time constraints on caddisfly growth and development

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Abstract

The fitness of the non-feeding adult caddisfly, *Asynarchus nigriculus* is largely dependent on the accumulation of nutrients during its larval stage. The energy obtained during this time is then allocated into growth and development for the adult insect. This allocation of energy may be constrained by drying time of the temporary waters the larvae inhabit. We measured the effect of food quality and drying time on survivorship, time to pupation, environmental variables, larval aggression, and the sizes of adult body parts. The results suggest that low food treatments provoke higher larval aggression and cannibalism, thus lower survivorship than high food treatments. Temporary water treatments resulted in earlier pupation than permanent treatments suggesting that the larvae increased their rate of development in order to emerge as adults before their habitat dried. Surprisingly, increased food quality did not result in larger or heavier adults, but did affect male wing lengths. Allocation to certain body parts such as the wings, suggest that food quality may be important for the adults' fitness.

Introduction

An adequate understanding of life history is crucial to how biologists view fitness and hence, to predictive power in evolutionary ecology (Nylin and Gotthard, 1998). The concept of tradeoffs between traits is a central component of life history theory (Stevens et al 2002), and presumes that the allocation of resources to one trait will be at the expense of those allocated to other competing traits (Sterns, 1992).

Life history tradeoffs in organisms with complex life cycles (e.g., amphibians and many invertebrates) are often manifested across different life stages. Typically energy is obtained in immature stages (e.g. larvae) and the allocation of energy towards traits that affect adult fitness occur during metamorphosis. Although the effects of variation in the larval environment on adult fitness have been studied in terrestrial insects (e.g., Boggs, 1997), few such studies have been conducted with aquatic insects.

Caddisflies are holometabolous insects with aquatic larvae (five instars) and pupae that transform into free-flying, often non-feeding, adults (Wiggins, 1996). Many caddisfly species that live in wetlands build portable cases of organic and/or non-organic material held together with silk secreted by the larva.

The caddisfly *Asynarchus nigriculus* is common in subalpine wetlands near the Rocky Mountain Biological Laboratory. Larvae of this species typically occur in temporary habitats where drying often constrains development time (Wissinger et al. 2003). Unlike in many other types of wetlands, *Asynarchus nigriculus* habitats are typically oligotrophic and the detritus that dominates their diet is likely to be nutritionally incomplete (Wissinger et al. 1999). Thus, between-pond differences in drying time and food availability should affect patterns of allocation to growth, development, and case construction (Wissinger et al. 2004).

The purpose of this study is to determine the interactive effects of food availability and drying time on life history tradeoffs in *A. nigriculus*. Although previous research on this species has shown that a high-protein food supplement leads to faster development and increased body size (Wissinger, 2004), the interactive effects of drying time and food quality on growth and development have not been studied. This study is not only of empirical interest for understanding the ecology of this species, but of general theoretical importance because the two factors should have conflicting effects. On one hand, drying time constraints should lead to the early emergence of smaller adults (earlier-smaller hypothesis), whereas increased food availability should lead to earlier emergence of large adults (earlier-larger hypothesis) (Day and Rowe 2002).

I hypothesize that the effects of larval food supplementation on *A. nigriculus* will differ under permanent and temporary habitat conditions. Adults should be largest when larvae are in high food-permanent treatments, and smallest in low food - temporary treatments. Body size should be intermediate in low food - permanent treatments and high food - temporary treatments, but it is difficult to predict the relative adult size for these two treatments (Table 1). The results of this study might reveal which factor is more important for allocation of energy towards body size.

Table 1- Summary of predicted **adult body masses** under different food and drying conditions during larval development.

	Permanent	Temporary
Food Supplement	largest	intermediate?
Ambient Food	intermediate?	smallest

Because allometric relationships tend to differ between males and females, we only compared males with males and females with females. In addition, because of the different roles of body parts between the sexes for reproduction, we hypothesize that male wings may vary in length among the treatments. The wings may be longest in hi food - permanent treatments and shortest in low food - temporary treatments. The longer wings may be beneficial to the fitness of the males. The wing lengths may be intermediate for the other two treatments. Female abdomen masses (a positive correlation with fecundity) may be largest in high food - permanent treatments and smallest in low food - temporary treatments. The abdomen sizes should be intermediate in low food - permanent treatments and high food - temporary treatments.

I also hypothesize that the effects of food on pupation time (= development rate) should depend on drying conditions. I predict that the earliest pupation should occur in high food - temporary habitats where larvae can develop quickly and emerge before drying. The latest pupation should occur in low food - permanent habitats where larvae remain in the final instar for an extended period in order to continue to grow. Pupation should be intermediate in the other two treatments where the effects of food and drying are counteractive (Table 2). Again, the relative pupation times for these two treatments might reveal the importance of the two habitat factors on development rates in this species.

Table 2- Summary of predicted **pupation times** under different food and drying conditions during larval development.

	Permanent	Temporary
Food Supplement	intermediate?	earliest
Ambient Food	latest	intermediate?

Because aggression may be pertinent to survivorship of the larvae, it is important to analyze the levels of aggression in the various treatments. I predict that aggression should be highest in the low food - temporary treatments where the larvae are most highly stressed by time and food quality, and lowest in the high food - permanent treatments where they are most comfortable. Aggression should be intermediate in high food - temporary treatments and low food - permanent treatments (Table 3). The relative aggression levels for these two treatments may reveal the importance of drying on aggression in this species.

Table 3- Summary of predicted **aggression levels** under different food and drying conditions during larval development.

	Permanent	Temporary
Food Supplement	lowest	intermediate
Ambient Food	intermediate	highest

Although my primary goal is to focus on growth and development tradeoffs, there are other response variables that might provide other clues to strategies for energy allocation in this species. For example, patterns of mortality before emergence might provide corroborative evidence for how low food quality and drying affect the propensity for cannibalism, although desiccation might complicate the interpretations. I predict that cannibalism should be highest (and survival lowest) in low food - temporary conditions where the need for a high-energy food supplement would be the most important for escaping drying habitats. In contrast, cannibalism should be lowest (and survival highest) in high food - permanent treatments where the risks associated with cannibalism might not outweigh the benefits (Table 4).

Table 4- Summary of predicted larval **survival** under different food and drying conditions during larval development.

	Permanent	Temporary
Food Supplement	highest	Intermediate?
Ambient Food	Intermediate?	lowest

Methods

This research project was conducted in Gothic, Colorado, USA at the Rocky Mountain Biological Laboratory (RMBL) from June through August of 2004. Fourth and fifth instar *Asynarchus nigriculus* larvae were collected from a pond at Cottonwood Pass (12,126 ft. elevation) using D-net sweeps on July 5, 2004. The larvae were separated by size and distributed into various treatments. Fourteen fifth instar larvae and two fourth instar larvae were used for each treatment. To obtain starting population statistics, sixteen larvae similar to those used in the experiment were measured for total length and head length to determine instar. They were dried for 24 hours at 50°C and then weighed on a M-220D electric microbalance (Denver Instrument) to the nearest 0.001g. The results were consistent with allometric relationships determined by Wissinger et al. 2003.

Larvae were randomly assigned to microcosms (0.25m² sweater boxes) with the following four treatments: 1) High food/ permanent water 2) High food/ temporary water 3) Low food / permanent water 4) Low food/temporary water. Each treatment was repeated six times, with a total of 24 microcosms. The microcosms contained natural detritus collected from ponds 11, 12, and 36 of the Mexican Cut Nature Reserve located in Gunnison County, Colorado, USA. Detritus was collected using D-net sweeps on July 2, 2004 and was cleaned of all macroinvertebrates. Three rocks were also added to the microcosms as pupation substrata. The microcosms were then filled to 5cm with water. The sorted caddis fly larvae were added to the microcosm on July 5, 2004, and each microcosm was labeled and color-coded to identify its treatment on July 6, 2004.

For treatments with high food, 0.225g of Dried Tubifex Worms (50% crude protein, 15% crude fat, fiber, and moisture) were weighed, crumbled, and dissolved in 140mL of water for at least 8 hours before being added every three days. Treatments with low food were left with only detritus. In permanent treatments, water levels were maintained at 5cm by adding distilled water to prevent changing the salinity of the treatment. In temporary treatments, the water level was then allowed to naturally evaporate to a depth of 2.5 cm by July 7, 2004. If in any of the temporary replicates water

had evaporated and no pupation occurred, the water level was returned to 2.5cm with tap water and allowed to evaporate again.

The experiment was conducted in a weather port near the Willey Laboratory at RMBL. The microcosms were arranged twelve on each side of the weather port, three quadrants per side. The four treatments were then assigned in a stratified manner so that each replicate was located in a different position in the weather port. Temperature was taken in each corner of the weather port on July 1, 2004 at 11:30 am. All windows and entrance to weather port were left open to allow circulation and natural evaporation. Morning and evening temperatures were taken in all the treatments on July 23rd and 26th. Salinity was also measured in each treatment with a TDS 3 Testr on July 23rd.

Daily observations were made for every microcosm to check for pupation and to observe the activity of the larvae and to check for any empty cases or carcasses. Upon pupation, the pupae were removed and transferred to an emergence chamber (100.3 cm²; 500ml water; natural detritus; one rock) with an overhead funnel. Eight days after the caddisfly larvae were added to the treatments, a census was taken to determine the stages of the larvae in the various treatments. The number of pupae, pre-pupae, fifth instar, and fourth instar larvae were counted and recorded. This treatment census was repeated one week later to update these data.

Behavioral observations were taken a few weeks after initiating the experiment. 10-min. trials were used to observe the aggressive behavior of the larvae from each of the 24 microcosms. Five individuals from the same microcosm were removed and placed inside a small plastic storage container (base of the emerging chamber described above). One of the individuals was chosen as a control and every encounter associated with aggression: foreleg wrestling, biting, and case shaking with the control larva was recorded with a laboratory counter.

To determine the effects of the treatments on allocation to different body parts, adult caddisflies were dissected and then dried for weighing. The sex of the adult was also determined. The animals' fore wings and hind wings were removed and the abdomen was detached from the thorax.

The animals' left fore and hind wings were then measured to the nearest 0.01mm along the longest axis using a Wild M-8 dissecting microscope. The animal's fore and hind wings, thorax, and abdomen were separated into pre-weighed foil containers and dried at 50 °C for 24 hours. The separate parts were weighed on a CAHN C-31 microbalance to the nearest 0.001mg.

Results

Environmental Data

Temperature did not vary among treatments (Figure 1), but there was a significant difference in salinity among treatments (Figure 2). Temporary treatments had a much higher salinity than permanent treatments (ANOVA $F_{1,20} = 46.565$, $P = <.0001$). There was no effect of food on salinity (ANOVA $F_{1,20} = 1.67$, $P = 0.21$), neither was there an interaction between drying and food (ANOVA $F_{1,20} = 0.306$, $P = 0.59$).

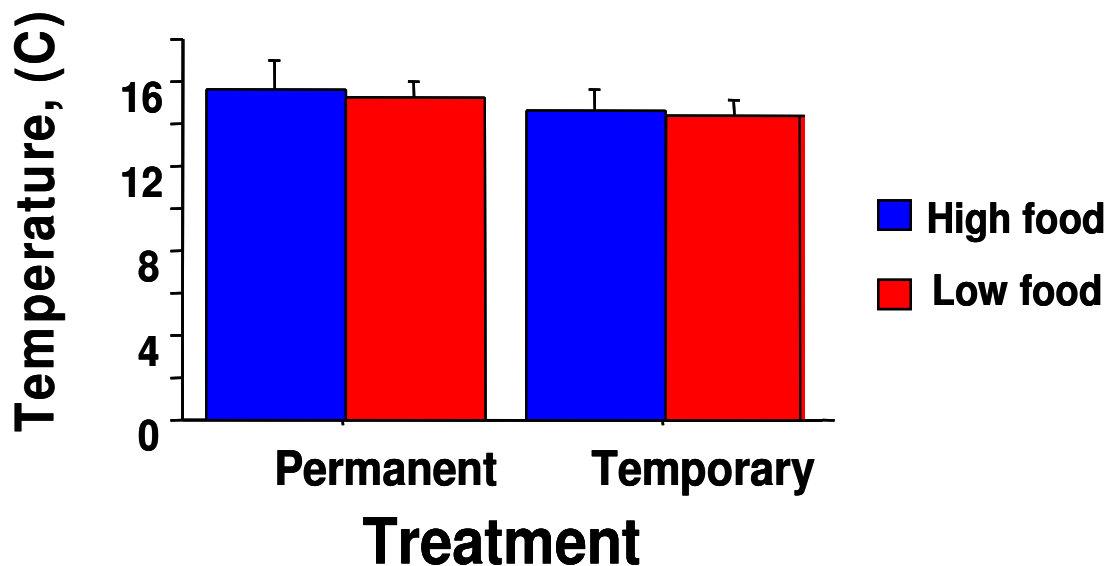


Figure 1. Temperature as a function of drying and food treatments. Error bars are 1 S.E.

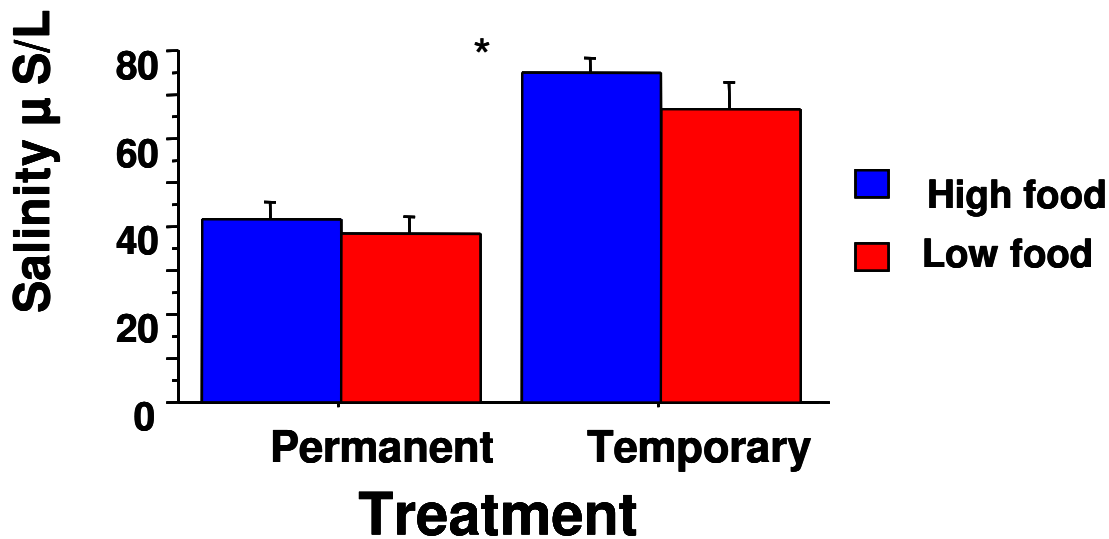


Figure 2. Salinity among different treatments. Error bars are 1 S.E

Larval and Pupal Data

Time to pupation differed slightly among treatments (Figure 3). Larvae pupated at later dates in permanent treatments than in temporary treatments (ANOVA $F_{1,20} = 5.03$, $P = 0.029$). There was no effect of food (ANOVA $F_{1,20} = 0.017$, $P = 0.90$), and no interaction between drying and food (ANOVA $F_{1,20} = 0.21$, $P = 0.65$). I conducted a protected one-way ANOVA on the high food treatments only, and found that drying had a significant effect on pupation time (Figure 4). Larvae pupated at later dates in the permanent treatments than in temporary treatments (ANOVA $F_{1,20} = 13.79$, $P = 0.0005$).

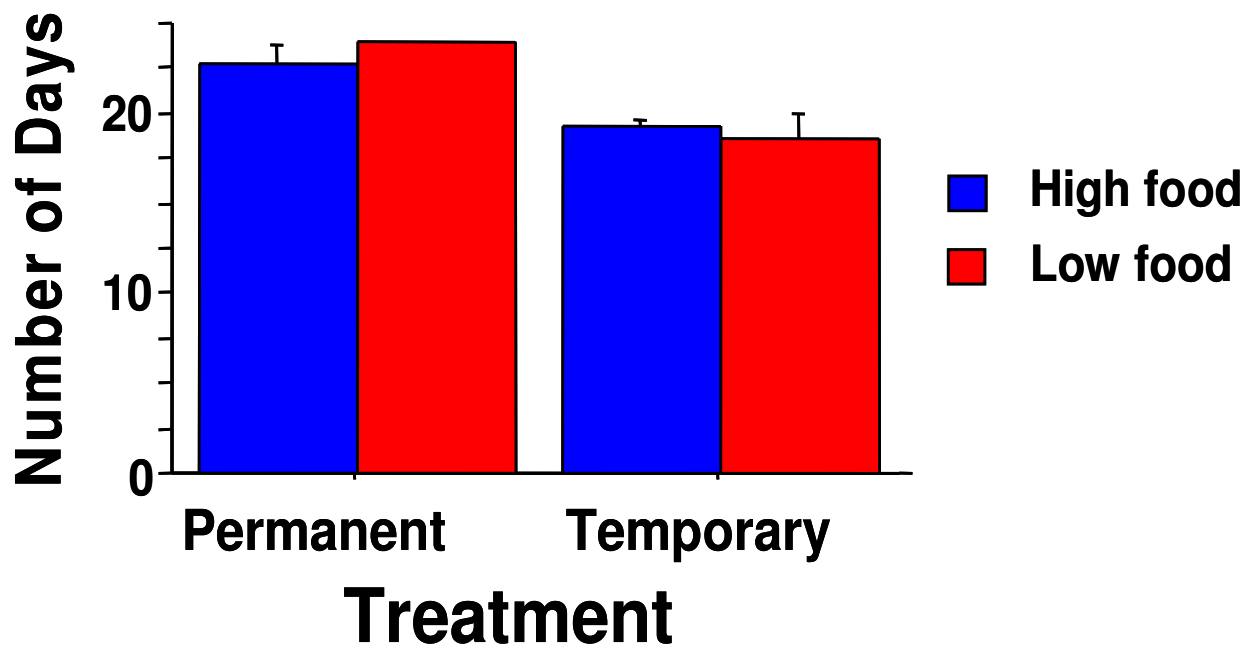


Figure 3. Time to pupation as a function of drying and food treatments. Error bars are 1 S.E.

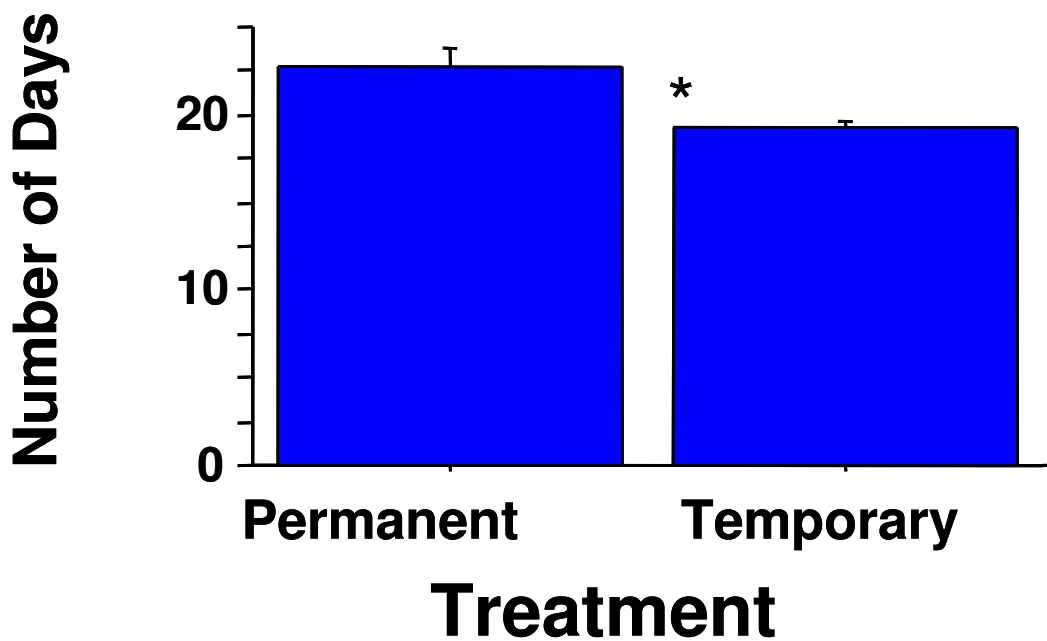


Figure 4. Time to pupation in high food treatments. Error bars are 1 S.E.

Food had a significant effect on larval aggression (Figure 5). Aggression was much higher in treatments with low food than treatments with high food (ANOVA $F_{1,20} = 14.34$, $P = 0.002$). There was no effect from drying (ANOVA $F_{1,20} = 0.753$, $P = 0.40$), or an interaction between drying and food (ANOVA $F_{1,20} = 0.1$, $P = 0.76$).

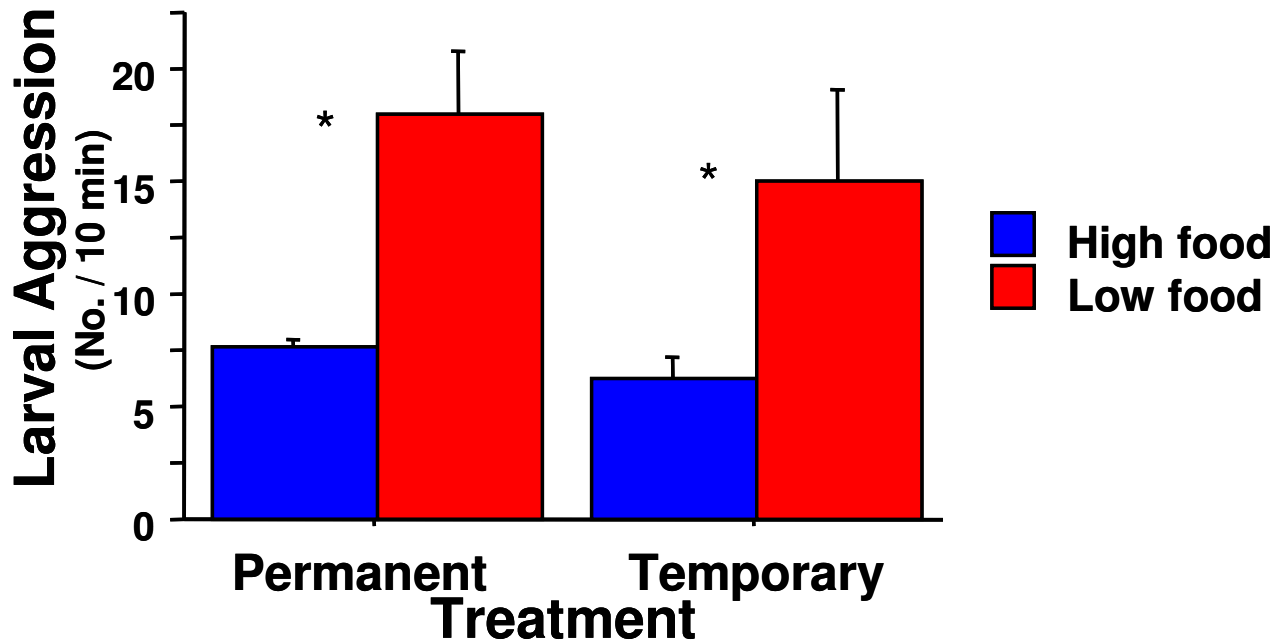


Figure 5. Larval aggression as a function of drying and food treatments. Error bars are 1 S. E.

Survival to pupation differed significantly among treatments (Figure 6). More animals survived and successfully pupated in high food treatments than in low food treatments (ANOVA $F_{1,20} = 19.6$, $P = 0.003$), but there was no effect of drying (ANOVA $F_{1,20} = 1.82$, $P = 0.19$), and no interaction between drying and food (ANOVA $F_{1,20} = 0.333$, $P = 0.57$).

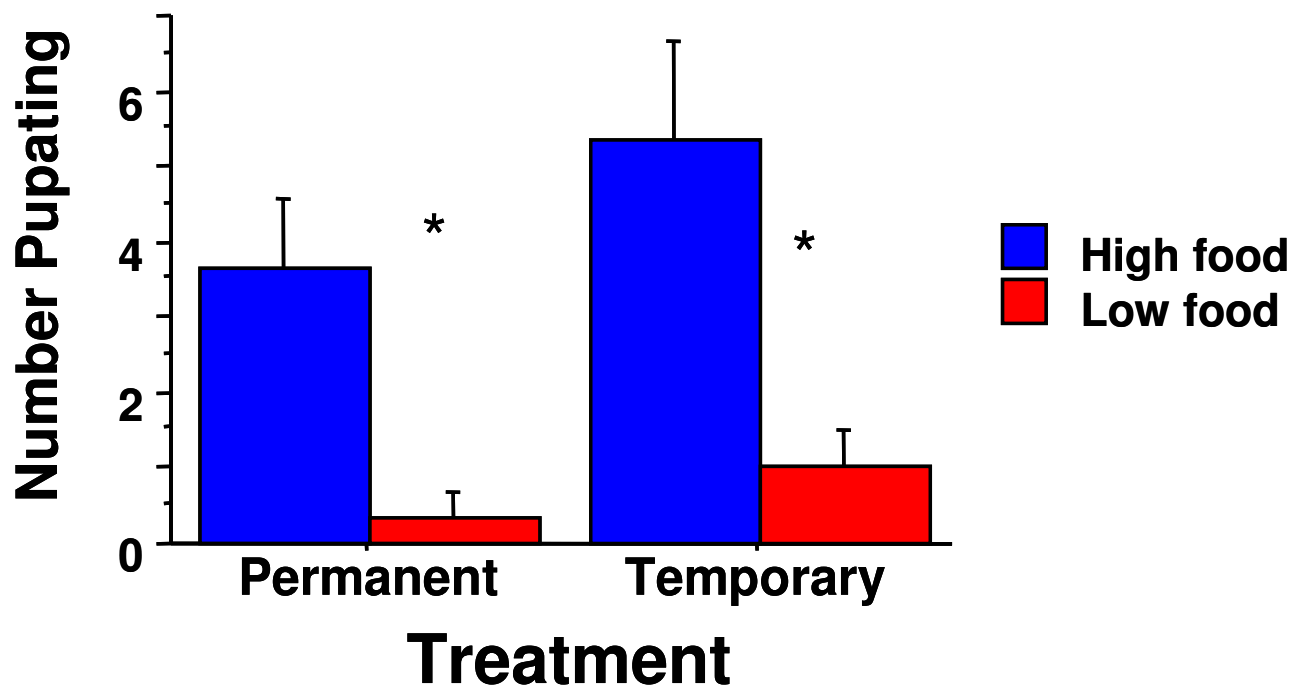


Figure 6. Survival to pupation as a function of drying and food treatments. Error bars are 1 S. E.

Adult Data

There was no effect of drying (ANOVA $F_{1,20} = 0.167$, $P = 0.70$), and /or food (ANOVA $F_{1,20} = 0.179$, $P = 0.69$) on male body mass (Figure 7). There was a drying and food effect on male fore wing length (Figure 8). Fore wing lengths were longer in treatments with high food than in treatments with low food (ANOVA $F_{1,20} = 22.159$, $P = 0.005$). Fore wing and hind wing lengths were also longer in permanent treatments than in temporary treatments. There was a food effect on male wing mass. The wing masses were higher in treatments with high food than in treatments with low food (ANOVA $F_{1,20} = 8.14$, $P = 0.036$). There was no change among the male thorax or abdomen masses among the treatments.

Because of high mortality rates in low food treatments, there is insufficient data to support any conclusions of adult female body mass or wing length.

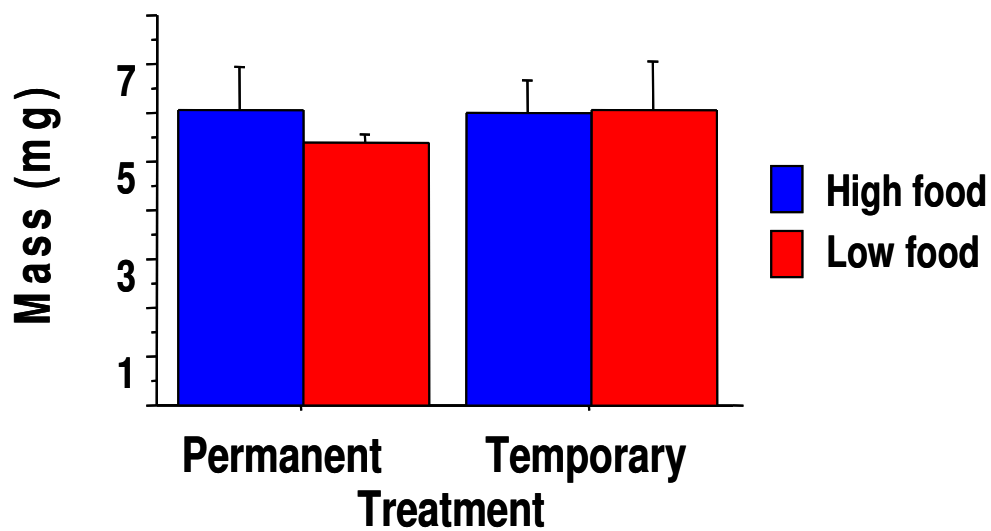


Figure 7. Male body mass as a function of drying and food treatments. Error bars

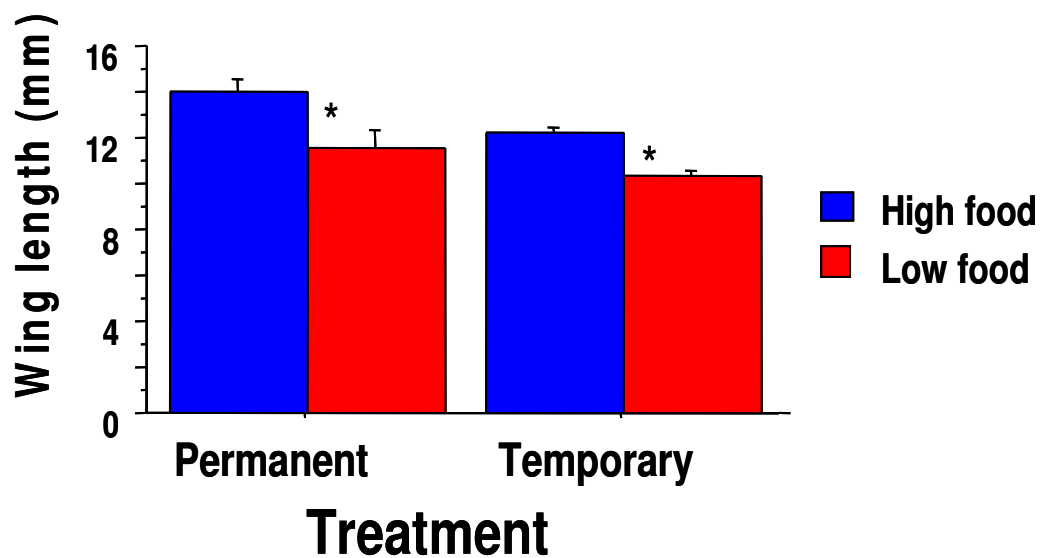


Figure 8. Male fore wing length as a function of drying and food treatments. Error bars are 1 S.E.

Discussion

Adult fitness

Many aquatic insects do not feed after pupation and, thus all energy and nutrients required by adults must be obtained during larval development (Wissinger et al. 2004). In this study, surprisingly there was no effect by food or drying on body mass of the male adult *A. nigriculus*. Instead, it seems that dietary supplementation increases development rates in *Asynarchus nigriculus* (Wissinger et al. 2004). There was however, a drying and food effect on the fore wing and hind wing lengths of the male adults. This suggests that the longer the growth and development time and the higher the quality of food, the longer the wings will be. Since increased food quality has an effect on certain adult body parts and not the whole body, it is suggestive that this allocation strongly effects fitness. Thus, the increased fore and hind wing length in males may be related to the mating success in this species. Understanding how wing length affects the mating success of males of *A. nigriculus* will require additional study of the mating system (Wissinger et al. 2004). The wing mass in adult males from high food treatments was higher which may be a length effect. Future research may utilize a length-mass relationship to better understand this phenomenon.

Larval growth and development

As a generally accepted guideline, increased temperature results in higher growth rates, shorter development times, and smaller adult size in insects (Nylin and Gotthard, 1998). Acknowledging this theory, it was certain that we did not want temperatures to interfere with this study. Accordingly, temperature did not differ in any of the treatments.

As we suspected, drying time did have an effect on the time of pupation. The larvae in temporary treatments were able to develop quickly and emerge before drying, earlier than those in permanent treatments. Because larvae in permanent treatments, which were replenished with distilled water, pupated later than those in temporary treatments, we concluded that these individuals had no

cues implying that they should rush their development. The cue that we suspect as being an indicator of drying in the temporary treatments is salinity, which was significantly higher in temporary treatments than in permanent treatments. It may be this cue and others, presently unknown, that leave *A. nigriculus* the only species that can complete larval and pupal development before vernal pools dry in early summer (Wissinger et al 2004). Life history plasticity influenced by environmental factors such as salinity allows *A. nigriculus* to survive in time constraining environments. Further study will be required to discover how *Asynarchus nigriculus* detect these cues. Contrary to our predictions, there was no food effect on pupation, thus no intermediate pupation times.

Predictions for aggression were correct suggesting that when less food is available the larvae become more aggressive. There was no interaction with drying that affected the aggression on the larvae. Although we did not witness cannibalism, the high levels of aggression indicate that it may have taken place in the low food treatments. Moreover, no dead larvae were seen in the treatments, suggesting consumption after death. Cannibalism in *A. nigriculus* differs from that observed in most populations because it is a result of mobbing behavior among same-sized larvae in synchronously developing cohorts (Wissinger et al. 2003). If an individual is injured, a mob (5-15) of conspecifics attacks and devours the injured animal (Wissinger et al. 1996). With animals in low food treatments, injured individuals become potential food for the healthy individuals who become more aggressive and competitive with other healthy individuals. Consequently, the attackers usually become secondary victims (Wissinger et al. 2004). The trade-offs of cannibalism put the larvae at risk of being eaten versus being the eaters in resource low habitats. Drying had no effect on aggression, and therefore resulted in no intermediate aggression levels as predicted.

Asynarchus nigriculus who have enhanced food, have a better probability of survival. Correctly predicting this outcome, larvae in high food treatments were better able to complete development and had the highest survival to pupation. Most larvae in low food treatments were not able to complete

development and had low survival to pupation. There was no intermediate survival as predicted and there was no effect from drying.

Initially this project was designed to investigate not only the empirical interest for understanding the ecology of the caddisfly *A. nigriculus*, but the theoretical importance of their growth and development in conditions that have conflicting effects; drying and food. We did find that drying alone had an effect on time to pupation with pupation occurring sooner in temporary treatments than in permanent treatments. Food only had an effect on larval aggression. Larvae in low food treatment were much more aggressive than those in high food treatments. Food also was a sole factor in survival with high food treatment surviving at a significant level higher than those in low food treatments. Wing lengths of adult males were affected by food alone also. Our investigation did conclude aspects of the two factors separately, but not combined for all factors.

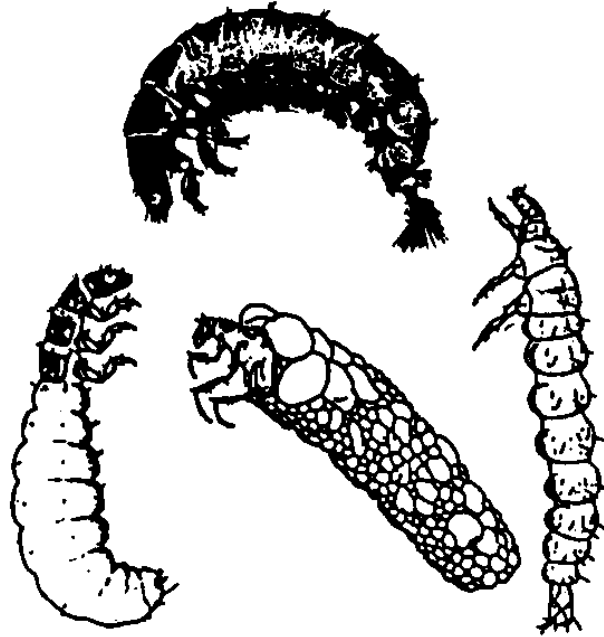
Future research of these inquiries may choose to design treatments that better represent high food, low food, temporary, and permanent environments. This could be accomplished by using different food supplements and amounts fed to the larvae in high food treatments. Low food treatments might be more effective if all other invertebrates were removed leaving the larvae with only plant material. Maintaining the water level at lower than 2.5cm in temporary treatments and higher than 5cm in permanent treatments will provide better cues of drying or permanence. Also, a round or tapered microcosm would give the larvae additional cues of drying. These changes and others may produce more combined results for these conflicting factors and contribute to the knowledge of *A. nigriculus* and their larval allocation of energy to growth and development.

Cannibalism is a factor that disrupts the investigation of drying and food quality. To prevent this from happening in future investigations, larvae could be separated from each other while still in the same microcosm and experiencing the same treatment. With the no cannibalism, there will be clearer interpretations of food quality and drying effects alone on growth and development.

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