

Comparing predator-induced changes in stream insects with varying vulnerabilities to risk of predation

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ABSTRACT

Mayflies in high-elevation streams in Colorado are more vulnerable to risk of predation based on their relative drift propensities. *Baetis bicaudatus*, a mayfly that has high drift propensity, has been shown to respond to predation risk by accelerating its metamorphosis into an adult, which results in smaller body size and a reduced fecundity. Other species of mayflies with lower drift propensities (either as a result of a fixed behavior, or a response to predation risk), as well as other aquatic insects, such as, caddisflies that do not drift may have a different response to predators. We conducted field surveys of five fish and five fishless streams to determine the effect of predation risk on aquatic insect response (i.e., body size and fecundity). Three species of mayfly were collected: *Rhithrogena robusta*, *Epeorus deceptivus*, and *Cinygmula* spp. and one species of caddisfly was collected: *A. grandis*. We also conducted a stream microcosm experiment to determine if the patterns seen in nature for *E. deceptivus* and *A. grandis* could be induced with just fish chemical cues. A general trend was seen in field surveys that showed an increase in *R. robusta* (power = 0.8264) body size in fish streams. *A. grandis* did not show a body size response to stream treatment in field surveys, but there was a higher number of eggs produced in fish streams (power = 0.0542). These results indicate that insects with high vulnerabilities but low risk to predation may not be affected by predator-induced changes in body size.

INTRODUCTION

Body size, development time, fecundity, and growth rate are important life history traits that affect the reproductive success and survival of an organism (Butler 1980). Many trade-offs occur in aquatic insects as the result of plasticity in life history traits (Nylin and Gotthard 1998). The plasticity or flexibility of these traits in different environments is closely related to the fitness of an individual in those environmental conditions. Recent studies have shown that there is a trade-off between development time and adult size or mass in insects in response to environmental conditions (Nylin and Gotthard 1998). The trade-off between development time and body size, as well as other life history trade-offs, can have far reaching implications on the fitness of an adult insect. Many insects must obtain all energy needed to reproduce as an adult while in larval form, and must also adapt to environmental constraints (McPeck and Peckarsky 1998, Wissinger *et al.* 2004).

Interactions between and within species can have an effect on the life history of an organism. These interactions make up part of the environmental conditions of the organism during ontogeny (McPeck and Peckarsky 1998). Several traits may be selected for as a result of species interactions and many of these traits may have an impact on individual fitness, and population size. McPeck and Peckarsky (1998) suggest that the population dynamics of *Baetis bicaudatus*, a mayfly, was more influenced by the changes in the life history of the insect due to non-direct species interactions than by the direct physical interaction with the species. *Baetis*, when in the presence of brook trout accelerated its metamorphosis in order to evade predation, which resulted in smaller body size at emergence. Body size in most mayflies is directly related to fecundity; thus

smaller body sized *Baetis* produce fewer eggs. The relationship between body size and fecundity shows that the interactions of these species cause a developmental shift that is more detrimental to population size than direct consumption by the predators (Peckarsky *et al.* 2002). Thus, body size is often a plastic trait that can affect the overall fitness of an organism (Nylin and Gotthard 1998).

Developmental trade-offs that appear in insects can also be affected by vulnerability to predation. The goal of this project is to better understand the varying responses of aquatic insects (i.e., body size and fecundity) with different vulnerabilities to the risk of fish predation. In a study by McIntosh *et al.* (2002), some species of mayflies are more at risk to predation because of their behavior. McIntosh *et al.* (2002) conclude that *Baetis bicaudatus* has a higher predation risk, because of its higher propensity to drift, than *Epeorus deceptivus* or *Cinygmula spp.* Drift is an important component in mayfly fitness because it is related to foraging (McIntosh *et al.* 2002). Mayflies, as hemimetabolous insects, undergo an incomplete metamorphosis meaning they allocate resources for growth and reproductive tissues gradually during their transformation into adults (Borror *et al.* 1981). A decrease in mayfly drift, and thus foraging may have far reaching implications on their reproductive success as has been observed in *Baetis bicaudatus* (Peckarsky *et al.* 2002). Other aquatic insects may respond in a different manner to predators than mayflies based on their behavior. A caddisfly, an aquatic insect that unlike the mayfly does not drift may have an even more reduced vulnerability to predation. Caddisflies instead build retreats as larvae that are used for foraging and protection from predation (Butler 1980). Here we test the generality of predator effects on

multiple species of aquatic insects that vary in morphological and behavioral attributes, which may influence their risk and vulnerability to fish predation .

The objectives of this paper were to test if there are different phenotypic and developmental responses to predators of insects (mayflies and caddisflies) with varying relative vulnerabilities to predation. We hypothesized that in the presence of fish predators, there will be accelerated development (i.e., earlier emergence time) that will result in smaller body size of all mayfly species. However, because it is unknown whether a reduced drift propensity is a fixed behavior or a predator response, it is possible that there will be an even greater developmental response with other mayfly species. Furthermore, since caddisflies do not drift, we hypothesized that they will not respond to the presence of a predator. If a response is seen, however, we hypothesized that fecundity will not be reduced based on the possibility for resource allocation to eggs during pupation. We tested these hypotheses using field observations and artificial stream channel experiments with the mayfly species', *Epeorus deceptivus* (Ephemeroptera: Heptageniidae), *Rhythrogena robusta* (Ephemeroptera: Heptageniidae), and *Cinygmula spp.* (Ephemeroptera: Heptageniidae) and the caddisfly, *Arctopsyche grandis* (Trichoptera: Hydropsychidae).

MATERIALS & METHODS

Field Survey

In order to estimate the effects of predation risk on body size, fecundity, and emergence times of species of stream insects with different vulnerabilities in nature, field surveys were conducted in five naturally fishless streams and five streams containing reproducing populations of brook trout (*Salvelinus fontinalis*) in the upper East River

Watershed near the Rocky Mountain Biological Laboratory in Western Colorado. At weekly intervals, we collected three different species of mayfly larvae, *Rhithrogena robusta*, *Epeorus deceptivus*, and *Cinygmula* spp. We collected mature larval mayflies with black wing pads (BWP) indicating that they will metamorphosize into adults within 48 h. Head capsule width measurements were taken from each mayfly collected, as well as dry body weight measurements for *R. robusta*. All dry weights were obtained by drying the individuals (or eggs) in a drying oven at 60 °C for 24 h and weighing them on a Cahn microbalance. Body weights for *E. deceptivus* and *Cinygmula* spp. were obtained using regression equations developed by Peter Ode and Jonas Dahl (*E. deceptivus*: $y = 0.0101e^{1.661x}$, $r^2 = 1$, and *Cinygmula* male: $y = 0.0236e^{1.9554x}$, $r^2 = 0.8902$, female: $y = 0.145x^{3.4507}$, $r^2 = 0.4294$) while a regression equation was developed for *R. robusta* males and females separately (male: $y = -1.143709 + 0.2969367e^x$, $r^2 = 0.8618$ and female: $y = -1.233809 + 0.3486643 e^x$, $r^2 = 0.7191$). While we were unable to estimate the number of eggs produced by *R. robusta* females, an estimate of eggs produced by *E. deceptivus* and *Cinygmula* spp. were obtained using regression equations developed by Peter Ode and Jonas Dahl based on their HCW measurements and body size (*E. deceptivus*: $y = 0.3089x^{1.3067}$, $r^2 = 0.5672$, and *Cinygmula*: $y = 1074.1x - 1527$, $r^2 = 0.7521$).

To establish if a significant amount of variance in size of *R. robusta* could be explained by time of emergence in either fish or fishless streams, we regressed body size of mayflies of mature larvae over time in each stream. Since there was no change in size of *R. robusta* with time of emergence, we used a nested ANOVA to compare the mean size at emergence of each species of mayfly in fish streams to fishless streams to

determine if there is a response by *R. robusta* to presence of a predator. Nesting stream within type (fish or fishless) considers the variance between streams as well as the variance between treatments since each stream is a replicate and the streams are physically different. As soon as the data collection of *E. deceptivus* and *Cinygmula spp.* is completed, we will test similar hypotheses for these species. As an alternative explanation for variance in mayfly size at emergence, temperature data were obtained from each stream using Onset tidbit data loggers (Onset Incorporated, Boston, MA) over the time of the field survey. We will test whether mean size at emergence of each mayfly species varied with stream temperature using a correlation analysis with degree-days per day on a per stream basis.

Since caddisflies do not produce eggs until pupation, *Arctopsyche grandis* pupae collected in each stream were allowed to pupate in their respective streams until emergence into adult form. Rocks containing pupae were placed in five gallon buckets that allowed for stream flow, but were covered in mesh to retain the adult insects. Upon emergence, adult caddisflies were collected from the buckets and dry body weights were measured. Eggs from female caddisflies were harvested and counted, plus a dry weight measurement was established for 20 eggs to estimate size per egg. A one-way ANOVA was used to determine a treatment response of body size to the presence of a predator; males and females were analyzed separately. An ANCOVA was used to establish a relationship between female size and fecundity under the presence of a predator to test whether females were allocating more resources to eggs in fish or fishless streams, and a t-test was used to determine the effect of fish on the mean size of eggs produced by each female collected.

Field Experiment

While patterns may exist in nature concerning body size, emergence time, and fecundity of insects suggesting a response to risk of predation, the numbers of variables that may cause this variation are high and need to be controlled for to examine the effects of predators (brook trout) on these differences. To test whether these life history traits can be induced by fish predators, *E. deceptivus* and *A. grandis* were collected from various naturally fishless streams near the Rocky Mountain Biological Laboratory and placed in small circular artificial flow-through microcosms (Peckarsky *et al.* 1993). Two treatments were used for each species. One treatment consisted of water containing no fish chemical cues, while the other treatment contained fish chemical cues originating from two small brook trout (size yet to be determined because experiment is not yet complete) that were captured via electro-fishing. Drip rate of chemical cues as well as velocity in tanks will be calculated upon completion of experiment. For each species, there were 10 replicates per treatment (fish or no fish) to provide enough statistical power to detect differences if they do exist (avoid type II error). We added eight *E. deceptivus* and four *A. grandis* to each replicate. The insects were collected in early life cycle stages (early stage III mayflies and instar III or IV caddisflies) to allow for sufficient development to occur with and without predator cues. (average starting size *E. deceptivus*: 0.202 ± 0.013 mg, HCW: 1.758 ± 0.036 mm, *A. grandis*: 5.318 ± 0.597 mg, HCW: 1.985 ± 0.095 mm). *E. deceptivus* individuals were removed from microcosms upon development of BWP, and a HCW measurement was obtained to estimate body size and egg mass based on regression equations used in the field survey methods. A t-test

was used to determine if the presence of predator chemical cues affects mayfly body size or fecundity at maturation.

Unfortunately, *A. grandis* did not have sufficient time or adequate conditions to pupate in the microcosm; thus an estimate of fecundity for females cannot be obtained. However, growth of the caddisflies was estimated in each treatment by comparing size estimates of each caddisfly after five weeks of treatment exposure to starting size estimates; a t-test was used to test whether predator cues affected larval growth. Males and females were analyzed separately.

RESULTS

Field Survey

Rhythrogena robusta mayflies emerging from streams containing fish populations tended to have a larger body size than those emerging from fishless streams. Male *R. robusta* on average were ~35% larger in fish streams ($F_{1,6} = 2.56$, $P = 0.16$, power = 0.86) and females were 40 % larger ($F_{1,6} = 5.16$, $P = 0.05$, power = 1.00, Fig. 1). The different trend was observed for *E. deceptivus*, in that individuals emerging from fish streams were ~15% larger ($F_{1,8} = 1.75$, $P = 0.22$, power = 0.99, Fig. 2), and in females, the egg mass, which is closely related to body size, was similarly ~ 20% larger ($F_{1,7} = 1.83$, $P = 0.22$, power = 0.99, Fig. 3). In addition, *Cinygmula* spp. males were ~ 35% larger in fish streams ($F_{1,6} = 6.07$, $P = 0.05$, power = 0.99), and females were ~ 25% larger ($F_{1,7} = 2.35$, $P = 0.17$, power = 0.39, Fig. 4). This size difference resulted in a fecundity increase of ~ 20% in fish streams ($F_{1,6} = 2.16$, $P = 0.19$, power = 0.39, Fig. 5). It should be noted that the sampling has not yet been completed for either *E. deceptivus* or *Cinygmula* spp. thus the results stated for these species are preliminary.

Adult caddisflies emerged from fish and fishless streams at approximately the same size. There is a difference of ~5% in male *A. grandis* emerging from various streams, which was not significant ($F_{1,11} = 0.044$, $P = .84$, power = 0.05) and a difference of ~ 10% in females ($F_{1,8} = 6.28$, $P = 0.42$, power = 0.12, Fig. 6). There was no difference in the slope between fish and fishless streams when female body size of *A. grandis* was compared to number of eggs produced (Fig. 7) indicating a similar egg number per body unit relationship in both treatments. However, females from fish streams had ~ 30% more eggs than females from fishless streams ($F_{1,8} = 1.37$, $P = 0.05$, power = 0.53, Fig. 8). Egg size did not vary between streams ($F_{1,8} = 0.014$, $P = .91$, power = 0.05, Fig. 9).

Field Experiment

Due to time constraints, data collection for this portion of this study has not yet been completed. As a result, there is no data to discuss in this section. An updated version of this paper will be submitted to RMBL upon completion of the project.

DISCUSSION

The results of the field survey helped to answer the question of how insect behavior (i.e., drift) relates to the responses (i.e., body size and fecundity) of these aquatic insects to predators. When behavior is fixed, the relative risk of predation that mayflies face could be less than that of *Baetis*, thus resulting in a reduced developmental response to the presence of a behavior. At the same time, if the behavior is the result of predation, then the developmental response should be greater than that of *Baetis* when faced with the risk of predation (McIntosh *et al.* 2002). The recent study by McIntosh *et al.* (2002), showed that other species of mayflies, like *E. decptivus* and *Cinygmula spp.*, have a

greater risk of predation when drifting than compared to *Baetis*, and concluded that it was based primarily on their morphology. Heptageniids are larger, as well as, have dorsoventrally flattened bodies that make swimming and settling out of the water column both difficult and energetically wasteful. Since the results of this study have shown that in most cases insects are actually larger in fish streams, it would seem to reason that the drift propensity behavior of heptageniid mayflies is fixed. As a result of this fixed behavior, the mayflies may be indifferent to the presence of predators in the water. It is also possible that there is a propensity for trout to prey upon *Baetis*, which results in a life history shift in *Baetis* (Peckarsky *et al.* 2001) thus releasing other species of mayflies from predation pressures. This interaction could explain the increased size of other mayfly species in fish streams and not fishless streams. Alternative explanations for this difference in size could be water temperature, or food availability. Unfortunately, the temperature data for this project has not yet been downloaded; however, previous studies done in these same streams have concluded that neither temperature nor food availability were a factor in size differences of insects between streams (Peckarsky *et al.* 2001).

Since there is no size difference between *A. grandis* emerging from fish streams and fishless streams, we conclude that these insects are indifferent to the presence of predators and are less vulnerable to predation risk than mayflies. However, it should be noted that the low sample size in these surveys resulted in a low power in the analysis of these treatments, thus more insects may be needed to see a pattern. An interesting result of this study, however, is that there were significantly more eggs produced (~ 100 more eggs) in fish streams than in fishless streams. Again, the small sample size resulted in a low power in the analysis of these treatments, but it is possible that *A. grandis* is

allocating more resources while pupating to egg production in fish streams than in fishless streams as a means by which increase fitness in adverse conditions. A study done by Nijhout and Emlen (1998) showed that beetles of the species, *Onthophagus taurus* and *Onthophagus acuminatus*, allocated resources while pupating from eye production to horn production as a response to adverse conditions (limited resources). Beetle horns are used in intrasexual competition in order to access females and it is assumed that it would be advantageous for this insect to allocate resources to body parts that increase the individual's fitness. It is theorized that the evolutionary shift to complete metamorphosis (i.e., holometabolous) of pupating insects was driven by the ability to separate resources (e.g., non-feeding adult) obtained at different life stages (Truman and Riddiford 1999).

Alternative explanations for this trend may also be temperature and food availability. Correlates to adult fitness, like fecundity, are known to be related to the diet of many insects including caddisflies, so it is plausible that the increased fecundity may have been related to the stream diet (Wissinger *et al.* 2004). However, the positive relationship between size and number of eggs produced determined in this study should seemingly result in a similar number of eggs produced between treatments. In addition, previous studies based on caddisfly diet and fecundity found there to be no relationship between egg size and diet (Wissinger *et al.* 2004). Once the data is analyzed for the field experiment, which controls for physical stream differences, like water temperature, a better understanding of these responses (i.e., body size and fecundity) to risk of predation can be obtained.

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Table 1.

Species		Fish	Fishless
<i>R. robusta</i>			
	male	6.707 ($\pm$ 1.20)	4.355 ($\pm$ 0.14)
	female	10.647 ($\pm$ 1.85)	6.224 ($\pm$ 0.40)
<i>E. deceptivus</i>			
		0.542 ($\pm$ 0.05)	0.450 ($\pm$ 0.02)
<i>Cinygmula spp.</i>			
	male	1.108 ($\pm$ 0.10)	0.811 ($\pm$ 0.08)
	female	1.967 ($\pm$ 0.20)	1.474 ($\pm$ 0.17)
<i>A. grandis</i>			
	male	7.920 ($\pm$ 1.08)	8.424 ($\pm$ 2.80)
	female	13.294 ($\pm$ 1.84)	11.376 ($\pm$ 1.27)

Table 2.

Species	Fish	Fishless
<i>E. deceptivus</i>	0.1558 ($\pm$ 0.02)	0.1202 ($\pm$ 0.01)
<i>Cinygmula spp.</i>	654 ($\pm$ 36.9)	523 ($\pm$ 60.8)
<i>A. grandis</i>	487 ($\pm$ 38.7)	350 ($\pm$ 0.73)

* *E. deceptivus* is egg mass, NOT fecundity

FIGURE LEGEND

Table 1. Mean body Sizes (mg) of insects obtained from fish and fishless streams, listed by species. Standard error is in parentheses.

Table 2. Fecundity of insects obtained from fish and fishless streams, listed by species. Standard Error is in parentheses.