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Does living in streams with fish involve a cost of induced morphological defences?

Jonas Dahl and Barbara L. Peckarsky

Abstract: Previous studies have shown that chemical cues from brook trout (*Salvelinus fontinalis*) induce relatively longer caudal filaments and heavier exoskeletons in the mayfly *Drunella coloradensis*. These characters constitute morphological defences that reduce larval mortality from brook trout predation. There is also a potential fitness cost of living in streams with trout, as *D. coloradensis* females emerge at smaller sizes from streams with fish compared with females in streams without fish. In this study, we obtained additional data to evaluate the hypothesis that these costs of living in streams with fish could be attributed to inducible defences. A field survey of seven different streams showed that mature (black wing pad) female larvae from streams with fish invested a smaller proportion of their body mass in eggs than females maturing in streams without fish. Furthermore, a negative relationship between female allocation to eggs and to morphological defence characters (relative length of the caudal filament) provides evidence of a cost of inducible defences in this species.

Résumé : Des études antérieures ont démontré que des signaux chimiques provenant de l'omble de fontaine (*Salvelinus fontinalis*) induisent la formation de filaments caudaux relativement plus longs et d'exosquelettes relativement plus lourds chez l'éphémère *Drunella coloradensis*. Ces caractéristiques constituent des défenses morphologiques pour réduire la mortalité des larves due à la prédation par les ombles de fontaine. Il y a aussi un coût potentiel en ce qui concerne le fitness, car les *D. coloradensis* femelles qui émergent des cours d'eau à ombles sont de taille inférieure à celles qui sortent de cours d'eau sans poissons. Notre étude présente des données additionnelles pour évaluer l'hypothèse selon laquelle de tels coûts supplémentaires dans les cours d'eau où il y a des ombles sont attribuables à l'induction des moyens de défense. Un inventaire de sept cours d'eau différents montre que les larves femelles arrivées à maturité (avec ptérothèques noires) dans les cours d'eau à poissons investissent une plus petite proportion de leur masse corporelle dans la production d'œufs que les femelles qui se développent dans des cours d'eau sans poissons. De plus, l'existence d'une corrélation négative entre l'allocation de la femelle dans la production d'œufs et les structures de défense morphologiques (longueur relative des filaments caudaux) fournit un indice qu'il y a un coût à l'induction de ces défenses chez cette espèce.

[Traduit par la Rédaction]

Introduction

Prey species have evolved different ways to reduce mortality when exposed to predation, including behavioural, morphological, and chemical defences (Edmunds 1974; Endler 1986). Whereas behavioural antipredator defences are often flexible (Lima and Dill 1990), most morphological defences are permanent (Edmunds 1974). Nevertheless, an increasing number of studies have reported predator-induced phenotypic plasticity in morphological defences (for a review see Tollrian and Harvell 1999). Most conceptual models of inducible defences assume that there is a fitness cost associated with the defence that can be recouped when predators are absent; otherwise, permanent defences should be favoured (Lively 1986). Potential costs include costs of construction and maintenance, plasticity, and lost opportunity, i.e., when developmental canalization into one phenotype constrains other options (Tollrian and Harvell 1999). There are, however, a number of studies that have not demonstrated fitness costs associated with inducible defences (e.g., Spitze 1992; Karban 1993), and theoretical work has suggested that such costs may not always exist (Adler and Karban 1994). Moreover, the cost hypothesis has been questioned on the grounds that natural selection can be expected to minimize costs of inducible defences over evolutionary time (DeWitt et al. 1998).

Drunella coloradensis (Ephemerellidae) is a mayfly common in high-altitude streams in western Colorado. In the East River drainage basin in western Colorado, *D. coloradensis* larvae emerge to the adult stage and reproduce in late August and September (Dahl and Peckarsky 2002). The main predators of mayfly larvae are brook trout (*Salvelinus fontinalis*) and predatory stoneflies (Peckarsky 1985; McPeek and Peckarsky 1998). Although stonefly distributions overlap with those of *D. coloradensis*, the ability of

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trout to disperse is limited in some streams by waterfalls or other physical obstacles, resulting in environmental heterogeneity and the presence or absence of fish in different stream reaches (Peckarsky et al. 2000, 2001). Most larval growth occurs during the summer when stream water temperature is warmer and trout activity is highest (B.L. Peckarsky, unpublished data).

Previously we have shown that in streams with brook trout, *D. coloradensis* larvae develop longer caudal filaments and heavier exoskeletons and emerge at smaller sizes compared with *D. coloradensis* in streams without brook trout (Dahl and Peckarsky 2002). Experimental studies have shown that longer caudal filaments are induced by chemical cues from brook trout and reduce trout predation on *D. coloradensis* (Dahl and Peckarsky 2002).

Smaller size at maturity in mayflies (including ephemerellids) is generally associated with lower fecundity (Sweeney and Vannote 1981; Peckarsky et al. 1993). Thus, we suspected that induction of smaller female *D. coloradensis* by trout could be a cost of developing morphological defences. Specifically, in this study we determined whether there was variation among *D. coloradensis* populations with regard to the relative investment in eggs versus defensive characters, and whether this variation was associated with the presence or absence of brook trout in nature. Thus, we investigated whether there was an association between allocation to eggs and defensive morphology, which could potentially be ascribed to a cost of induced defences.

Methods

We performed a survey of tributaries of the East River that contained abundant populations of *D. coloradensis* larvae at ~2900 m elevation near the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, Colorado. To determine the relative investment by *D. coloradensis* females in eggs compared with the rest of their body mass, we collected and measured 20 mature *D. coloradensis* larvae with black wing pads (BWPs) at seven stream reaches (sites) during August 2000. When mayflies develop BWPs (stage IV), they have ceased feeding and growing, their eggs are fully developed, and they will metamorphose within 24 h (Peckarsky et al. 2001).

Stream reaches ranged from 0.5 to 5 m wide, and reaches with lengths between 10 and 30 m were chosen to standardize the area sampled. Some sites contained reproductive populations of brook trout, whereas others were completely without fish as a result of barriers to fish dispersal (waterfalls > 2 m high). We sampled two sites in streams where trout occurred over the entire stream (Copper Creek and Rustlers Gulch), two streams where trout occurred in the lower reaches but not in the upper reaches (Lower and Upper Rock Creek and Lower and Upper Bradley Creek), and one stream that was completely without fish (Marmot Creek). Presence or absence of fish was established by electrofishing. All sites had been with or without fish since at least 1994 (Peckarsky et al. 2001). Sites differed in other physical and biological factors that could influence growth and development of mayflies (Peckarsky et al. 2001), but Peckarsky et al. (2001) found no linear combination of environmental variables (elevation, stream width, discharge, conductivity, degree-days, and nutrient chemistry) that separated streams with and without fish.

We preserved *D. coloradensis* females in 70% EtOH and in the laboratory we determined developmental stages (as in Delucchi and Peckarsky 1989), measured the relative length of the caudal filament (as in Dahl and Peckarsky 2002), and dissected eggs from the bodies. Females (minus eggs) and egg masses were dried at 60 °C for 3 days and weighed to the nearest 0.01 mg on a Cahn microbalance. To calculate the relative investment in eggs of each female, we divided the mass of the female's eggs by the mass of the female's body. We used this variable as a surrogate of fecundity because previous work suggests that egg size does not vary in this species. Thus, as in other species of mayflies (Peckarsky et al. 1993), heavier egg masses have higher numbers of eggs rather than larger eggs (Dahl and Peckarsky 2002).

Using a binocular dissecting microscope equipped with an ocular micrometer, we measured the length of the middle caudal filament and the body length of all mayflies collected. To calculate the relative investment in defensive structures, we divided the caudal filament length by the body length. This character was shown previously to be induced by chemical cues from brook trout and to affect the vulnerability of *D. coloradensis* larvae to predation by brook trout (Dahl and Peckarsky 2002). Error estimates for length and mass variables were obtained by making 20 measurements of each parameter for each of 20 mayflies and calculating their deviation from the first measurement. These deviations were then divided by the first measurement and averaged to determine the percent measurement error.

Differences in investment in eggs (proportion of female mass allocated to eggs) and proportion of body length allocated to defensive structures (caudal filaments) between *D. coloradensis* in streams with fish and those in streams without fish were analysed using a nested ANOVA, with stream sites nested within stream type (with or without fish). In this analysis, streams were replicates. Correlation analysis was used to test whether there was a significant association between investment in defensive structures (relative length of the middle caudal filament) and investment in eggs (proportion of body mass allocated to eggs). Individual *D. coloradensis* females were used as replicates because the trade-off between allocation to defence and allocation to reproduction is expected to occur at the level of individuals. Proportional variables were arcsine square-root transformed to conform to the assumptions of normality and homogeneity of variance.

Results

Error estimates for measurements ranged from 0.45% to 1.2%, suggesting that measurement error was not a significant source of variability in this study. The proportion of body mass allocated to eggs was significantly greater in females in streams without fish than in streams with fish (nested ANOVA: $F_{[1,4]} = 10.1$, $P = 0.034$; Fig. 1), and, as in Dahl and Peckarsky (2002), *D. coloradensis* larvae in streams with fish had significantly longer caudal filaments per unit body length than larvae in streams without fish (nested ANOVA: $F_{[1,4]} = 14.2$, $P = 0.019$). There was a significant negative correlation between investment in defensive

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Fig. 1. The proportion (mean ± 1 SE) of female body mass allocated to eggs of mature (black wing pad) *Drunella coloradensis* larvae collected from streams with and without fish (20 larvae from each stream). Streams with fish (solid bars): CO, Copper Creek; LB, Lower Bradley; LR, Lower Rock Creek; RU, Rustlers Gulch. Streams without fish (open bars): UB, Upper Bradley; UM, Marmot Creek; UR, Upper Rock Creek.

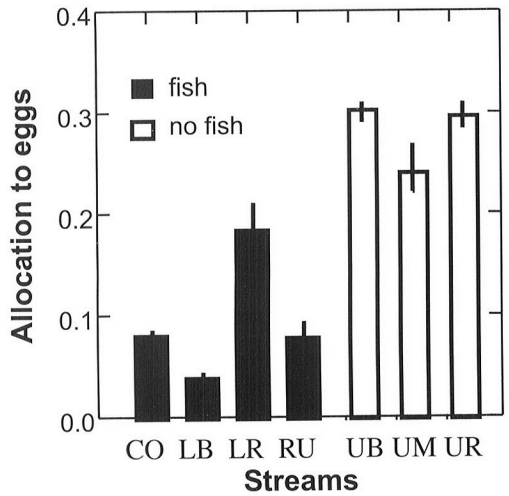
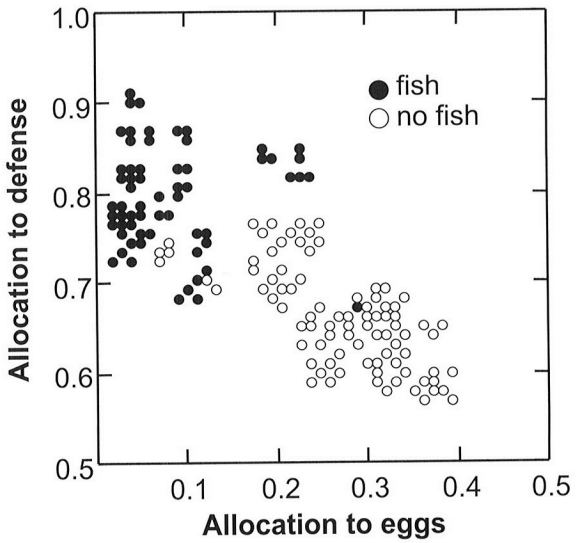


Fig. 2. Relative investment in defensive structures (length of the caudal filament as a proportion of total body length) versus relative investment in eggs (proportion of total body mass allocated to eggs) for mature (black wing pad) *D. coloradensis* larvae collected from streams with (●) and without fish (○).



structures (relative length of caudal filaments) and investment in eggs (relative mass of eggs); i.e., individuals with higher allocation to defensive structures had lower allocation to eggs ($R^2 = 0.54$, $n = 139$, $P < 0.001$; Fig 2). Furthermore, females with higher investment in eggs and lower investment in defensive structures were from streams without fish, and those with greater investment in caudal filaments and fewer eggs were from streams with fish (Fig. 2).

Discussion

The field data in this study showed that variation in defensive investment (caudal filament length) and reproductive investment (proportion of body mass allocated to eggs) by *D. coloradensis* females was associated with the presence or absence of fish. At sites that contained brook trout, *D. coloradensis* females had relatively longer caudal filaments and allocated a smaller proportion of their body mass to eggs compared with females from streams without brook trout, providing evidence of a fitness cost for *D. coloradensis* females inhabiting streams with fish. Females maturing in streams with fish were significantly less fecund than females that matured in streams without fish. Furthermore, females with longer caudal filaments were less fecund. These data, in conjunction with those of previous studies (Dahl and Peckarsky 2002), are consistent with the hypothesis that *D. coloradensis* females developing in streams with fish incur a fitness (fecundity) cost of developing defensive morphology to reduce mortality.

It is difficult, however, to attribute lower fecundity in fish streams directly to a cost of the induced morphological defences. Lower fecundity could also be caused by abiotic or behavioural differences or by life-history shifts between mayflies in streams with fish and those in streams without fish. Data from previous studies enable us to rule out some of these alternative hypotheses. First, behavioural observations in previous studies (Dahl and Peckarsky 2002) showed no differences in the movement rates of mayflies (drift in the water column) or proportions of mayflies residing on exposed substrata between streams with fish and streams without fish. Mayflies from both stream types had very low drift rates, which should generally reduce their exposure to trout predation. Second, previous studies showed no clear separation between streams with fish and streams without fish based on linear combinations of environmental variables (elevation, stream width, conductivity, degree-days, and nutrient chemistry) (Peckarsky et al. 2001). Third, previous studies have not shown differences in timing of emergence of *D. coloradensis* between streams with fish and streams without fish, suggesting that mayflies in both stream types have similar development times (Dahl and Peckarsky 2002). It is conceivable, however, that differences in other variables affecting growth rates, size, and fecundity (e.g., resource abundance) could cause the patterns we observed. Nonetheless, our data support costs of inducible defences as one reasonable mechanism explaining variation in fitness of female mayflies.

Costs of induced defences are defined as fitness deficits associated with the induced character (DeWitt et al. 1998; Scheiner and Berrigan 1998). In our study, the negative relationship between investment in defensive characters and investment in eggs may represent such a cost; i.e., more investment in longer caudal filaments could result in fewer eggs per female. Thus, there appears to be a trade-off between reproductive and defensive efforts by *D. coloradensis* females living in environments with variable risk of predation.

Conceivably, the patterns of relative fecundity of *D. coloradensis* in streams with or without fish could be differences that are fixed between these populations. Previous studies, however, have demonstrated that reduction in fecundity can be induced experimentally (Dahl and Peckarsky 2002). Fur-

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thermore, many streams with and without fish are connected and not separated by barriers to dispersal of adult mayflies. Thus, dispersal of larvae and winged adults should facilitate genetic mixing of populations derived from different stream sites. Therefore, the defensive and life-history traits observed in streams with and without fish appear to be phenotypically plastic rather than fixed in these mayfly populations.

Costs of induced defences, such as those demonstrated in this study, have rarely been demonstrated in natural environments (Pettersson 1999). Ultimately, to quantify costs of plasticity, one would compare individual organisms that exhibit the same phenotype in a given environment but differ in their plasticity (Relyea 2002). Because such organisms are difficult to find, recent studies have used genetically distinct groups and applied multiple regression to analyse the effects of phenotypic variation and variation in plasticity (DeWitt et al. 1998). Those studies suggest that costs of plasticity are rare under laboratory conditions. On the other hand, Relyea (2002) found that fitness effects of plasticity were widespread in wood frogs (*Rana sylvatica*) studied in outdoor mesocosms. Future work should examine the potential fitness costs of induced morphological defences under natural field conditions.

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