INDUCED MORPHOLOGICAL DEFENSES IN THE WILD: PREDATOR EFFECTS ON A MAYFLY, *DRUNELLA COLORADENSIS*

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Abstract. Phenotypic plasticity may enable organisms to optimize their phenotypes in environments that are heterogeneous over time or space. For example, inducible defenses are favored for prey populations faced with variable predation risk. We studied the impact of brook trout (*Salvelinus fontinalis*) on the development of defensive morphological characters in larvae of the mayfly, *Drunella coloradensis*, both in natural streams and in a series of experiments carried out in streamside, circular, flow-through chambers. *Drunella* larvae in natural streams with trout had longer caudal filaments per unit body size and relatively heavier exoskeletons than *Drunella* in fishless streams. Female larvae that developed in fish streams were also significantly smaller and less fecund at metamorphosis than those living in fishless streams, suggesting a possible fitness cost associated with development of morphological defenses. However, timing of metamorphosis of *Drunella* larvae to the adult stage did not differ between fish and fishless streams.

Larvae originating from fish and fishless streams were reared and exposed to water with fish cues or fishless control water for three weeks (1998) or until they developed black wing pads, i.e., just before emergence (1999). In both experiments waterborne fish cues induced the development of relatively longer caudal filaments, but only in *Drunella* originating from sites without fish. Waterborne fish cues had no effect on *Drunella* growth rates, behavior, or size at emergence. *Drunella* originating from fish streams had significantly lower mortality when exposed to predation by trout than *Drunella* originating from fishless streams. Caudal filament length appears to enhance survival, as *Drunella* with intact caudal filaments had lower predation rates than *Drunella* with their tails artificially shortened. This study provides evidence of chemically induced morphological plasticity that could reduce predation rates of these mayflies in natural stream environments.

Key words: adaptive phenotypic plasticity; development; Drunella coloradensis; *fecundity costs of induced morphological defenses; inducible defenses; mayflies; morphological defenses; predation; streams; trout.*

INTRODUCTION

The environment not only establishes the relationship between the phenotype of an individual and its fitness, but also plays a role in determining the optimal phenotype (Scheiner 1993, Via 1994, Scheiner and Berrigan 1998, Schlichting and Piggliuchi 1998). However, there may not be one optimal phenotype for organisms living in environments that vary over time and space. In such heterogeneous environments organisms may evolve phenotypic plasticity to optimize morphology, behavior, or life-history traits under variable conditions (Bradshaw 1965, Stearns 1992, DeWitt et al. 1998, VanBuskirk and Relyea 1998).

Variable predation risk is one important example of environmental heterogeneity for prey individuals faced with the problem of optimizing their phenotypes. Pred-

ators not only affect prey abundance, species, or size composition (Brooks and Dodson 1965, Sih et al. 1985, Kerfoot and Sih 1987, Carpenter 1988), but also influence prey behavior, life histories, and morphology (Lima and Dill 1990, Wellborn et al. 1996, Brönmark et al. 1997, Lima 1998, McPeek and Peckarsky 1998). Examples of prey traits that have been shown to evolve under selection pressure from predators are prey crypsis (Endler 1986), distasteful chemistry (Schultz 1988), and morphological defenses (Harvell 1990, Brönmark and Miner 1992).

While many defensive traits are constitutive (fixed), environmentally induced morphological defenses have been described in a variety of organisms including plants (Karban 1993), bryozoans (Harvell 1984, 1990), cladocerans (Dodson 1984, Havel 1987, Tollrian and Harvell 1999), barnacles (Reimer and Tedengren 1996), gastropods (Lively 1986, Appleton and Palmer 1988), fish (Brönmark and Miner 1992, Brönmark and Pettersson 1994, Pettersson and Brönmark 1997), and amphibians (McCollum and Leimberger 1997, Van Buskirk and Relyea 1998). While induction of behav-
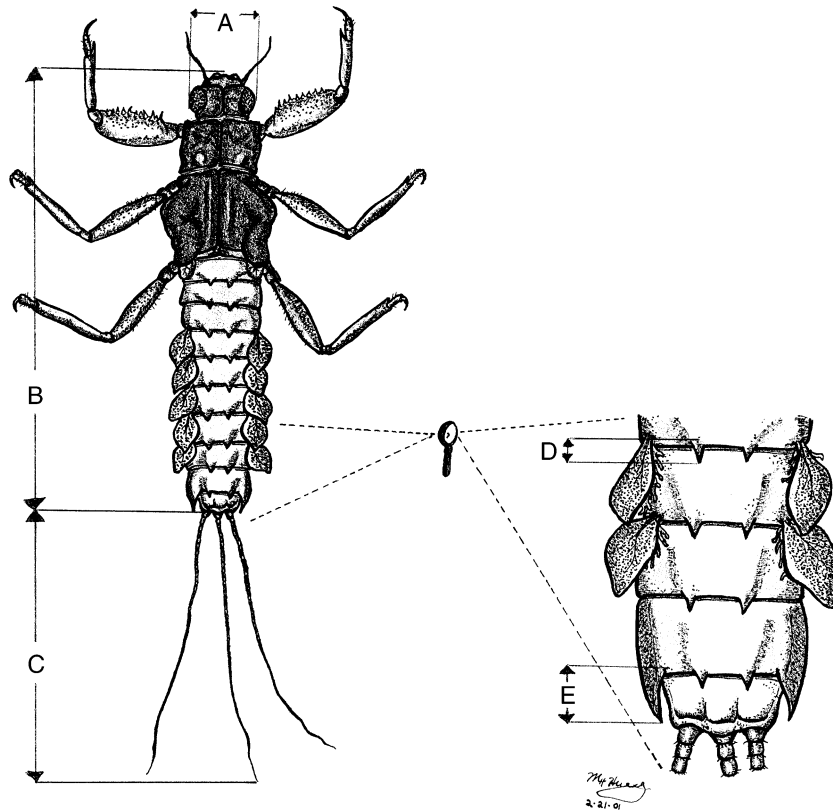
FIG. 1.　*Drunella coloradensis* larvae in a dorsal view showing structures measured for the morphometric analysis. Arrows indicate (A) head capsule width, (B) body length, (C) caudal-filament length, (D) dorsal spine VI length, and (E) lateral spine XI length. Illustration by Ming Huang.

ioral defenses of insect prey is common (Kohler and McPeek 1989, Douglas et al. 1994, Forrester 1994, McIntosh and Peckarsky 1999), examples of predator-induced morphological defenses in insects are rare. In one example, the presence of yellow perch (*Perca fluviatilis*) induced the growth of longer abdominal spines in a dragonfly larva (*Leucorhinia dubia*) compared to spines of individuals living in fishless ponds (Johansson and Samuelson 1994, Arnqvist and Johansson 1998).

We know very little about how phenotypes are expressed or how natural selection affects phenotypic plasticity in unmanipulated natural populations living in heterogeneous environments (Arnqvist and Johansson 1998, Pettersson 1999). Most studies of phenotypic plasticity and inducible morphological defenses have been conducted in the laboratory where single environmental factors have been manipulated (see Schlichting and Piggliuchi [1998] and Tollrian and Harvell [1999] for reviews). Our knowledge about the ontogeny of phenotypic plasticity in animals is even more limited, because the relationship between organismal development, plasticity, and environmental heterogeneity can be very complex. Several different environmental variables can affect morphological characters, physiology, behaviors, and life-history strategies (Schlicht-

ing and Pigliucci 1998). Therefore, studies are needed that observe phenotypic plasticity in natural systems, where all environmental variables are integrated, and test the phenotypic responses of organisms to single variables in controlled experiments.

The major goals of this study were to determine whether defensive morphological characters of mayfly larvae living in stream environments could be induced by the presence of brook trout, and the costs (if any) associated with developing these defensive structures. Harvell (1990) proposed four factors as prerequisites for the evolution of inducible defenses: (1) prey should have access to reliable cues for detecting predators; (2) the defense should be effective; (3) predation pressure should be variable but sometimes strong; and (4) the defense should be costly (see also Pettersson 1999). In this study we addressed all four of these factors.

METHODS

Study organisms

Larvae of mayflies in the family Ephemerellidae possess morphological characters that have been suggested to decrease predation risk (Peckarsky 1980, Soluk 1990). For example, larvae of *Drunella coloradensis* (L.) (Ephemeroptera: Ephemerellidae) (Fig. 1) have

well-developed lateral and dorsal abdominal spines, thick exoskeletons, and long caudal filaments. Larvae of this species are common in high-altitude streams of western Colorado. The main predators in these streams are brook trout (*Salvelinus fontinalis*), and predatory stoneflies (Peckarsky 1985, McPeek and Peckarsky 1998). The ability of trout to disperse is limited in some streams by waterfalls or other physical obstacles, resulting in environmental heterogeneity with fish present or absent in different stream reaches (Peckarsky et al. 2000, 2001). *Drunella* larvae emerge to the adult stage and reproduce in late August and September in East River valley, Colorado (J. Dahl, *personal observation*). Most larval growth occurs during the summer when stream water temperatures warm and trout activity is highest (B. L. Peckarsky, *unpublished data*).

Field study

This study was performed in the East River and it tributaries at ~2900 m elevation near the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, Colorado, USA. To determine whether natural variation in defensive morphology of mayfly larvae was associated with presence or absence of brook trout, we conducted a two-year field survey in sites where trout distributions were known. We collected *Drunella coloradensis* larvae at weekly intervals at 8 stream reaches during July 1998 and 10 stream reaches from late June through mid-September 1999 to establish the ontogeny of development of defensive characters.

Stream reaches (sites) were selected that contained abundant populations of *Drunella* larvae. Reaches ranged from 0.5–10 m wide, and lengths of reaches sampled (10–30 m) were chosen so that the areas sampled were ~100 m² at all sites. Some sites contained reproductive populations of brook trout (*Salvelinus fontinalis*), and others were completely fishless as a result of barriers to fish dispersal (waterfalls >1 m high). In 1998 we sampled four sites in streams where trout occurred over the entire stream (East River, Copper Creek, Rustlers Gulch, and Quigley Creek), and four sites in two streams where trout only occurred in the lower reaches but not in the upper reaches (Lower and Upper Rock Creek and Lower and Upper Gothic Creek). In the 1999 field survey we added two sites from one more stream that was fishless in its upper reaches but had trout in the lower reaches (Upper and Lower Bradley Creek). (See Peckarsky et al. [2000] for a map of sites.)

Presence or absence of fish was established by electrofishing. All sites had been fish or fishless 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). However 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.

Morphometric analyses.—We preserved *Drunella* larvae in 70% ethanol and brought them to the laboratory where we determined developmental stages (as in Delucchi and Peckarsky 1986) and measured morphological characters, size at emergence, and female fecundity. We dry-weighed final-instar (stage IV—with black wing pads) females and males from the three streams that had both fish and fishless sites (Rock Creek, Gothic Creek, and Bradley Creek). When mayflies develop black wing pads (stage IV) they have ceased feeding and growing and will metamorphose within 24 h (Peckarsky et al. 2001). We also collected black-wing-pad females to determine the relationship of body size to fecundity. We measured body length, dissected each female and counted the number of eggs, and then weighed each female together with her eggs. Dry mass was obtained by drying each individual at 60°C for 3 d, and weighing them to the nearest 0.01 mg on a Cahn microbalance (Cahn Instruments, Cerritos, California, USA).

We measured five structures on all collected mayflies using a binocular dissecting microscope equipped with an ocular micrometer. Measurements included head width, body length, length of the middle caudal filament, length of the lateral IX spine (hereafter called "lateral spine"), and length of the dorsal VI spine (hereafter called "dorsal spine") (Fig. 1).

Error estimates for each variable were obtained by making 20 measurements of each parameter for 20 individuals for each year and calculating their deviation from the first measurement. These deviations were then divided by the first measurement and averaged to determine a percentage error.

We used analysis of covariance with body length as a covariate to characterize the variation in allometry of abdominal spine and caudal-filament morphology of *Drunella* in natural populations and in experiments. We first tested the assumption of homogeneity of slopes between treatments (fish or fishless). If there was no significant interaction between the treatment and relationship between body length and a morphological character (i.e., slopes of allometric relationships were the same for fish and fishless *Drunella*), further analyses of main effects were conducted using nested MANCOVA (with site as a random factor nested within stream type) unless we only had one character to analyze, in which case we used univariate nested ANCOVA. Site means for each date were used as the unit of replication in these analyses, and we used site (type) for the error degrees of freedom. Also, differences between fish and fishless streams for *Drunella* size at emergence and timing of emergence (emergence date) were analyzed using one-way nested MANOVA. In all cases, significant multivariate tests were followed by univariate analyses on individual response variables to determine the significant source(s) of variation.

Investment in exoskeleton.—We also determined whether variation of *Drunella* investment in protective exoskeleton per unit body mass was associated with the presence or absence of trout. In August 1999 we collected *Drunella* with black wing pads from five sites with fish (East River, Copper Creek, Lower Rock Creek, Lower Gothic Creek, and Rustlers Gulch) and from two fishless sites (Upper Rock Creek and Upper Gothic Creek). Individuals were placed in separate circular stream chambers (described in *Induction experiments*, below) until they emerged, at which time we measured the length of the exoskeleton and then dry-weighed the exoskeleton ($N > 15$ individuals from each stream). Investments in exoskeletons were compared between individuals collected from fish and fishless streams by one-way nested ANOVA of mass of exoskeleton per unit body length of exoskeleton.

Experiments

As a mechanistic test of whether predator cues (trout) could induce morphological differentiation in *Drunella* larvae we conducted two rearing experiments. The first experiment (1998) determined whether fish chemical cues could induce the development of defensive morphological characters. In the second experiment we tested whether defensive morphology could be induced in later larval development, and whether defensive morphology affected *Drunella* size at metamorphosis and female fecundity. To test the effectiveness of morphological defenses, we compared mortality during predation trials in artificial streamside channels between *Drunella* originating from fish and fishless streams, and between *Drunella* with intact caudal filaments and artificially shortened filaments. Finally, we compared behavior of *Drunella* from fish and fishless streams to determine whether presence of trout induced defensive behaviors.

Induction experiments.—Experiments were carried out in a stream-side system of circular flow-through chambers (15-cm diameter) (described and illustrated in Peckarsky and Cowan [1991]) using natural stream water from a fishless first-order tributary of the East River near RMBL. Chambers were shaded by a white portable greenhouse (Weatherport). These experiments were conducted from July to August 1998 and from August to September 1999. Waterborne fish cues originated from a plastic 100-L holding container where two brook trout were held (size range: 100–130 mm total length). Trout were fed a mixed mayfly diet including *Drunella* larvae for the duration of the experiments. Water was gravity-fed from the holding tank, divided, and distributed (dripped) into the appropriate stream chambers for the entire experiment. Fishless water was similarly dripped into control chambers.

Drunella larvae used in these experiments were obtained from fish and fishless streams and reared in either fish or fishless water in a completely cross-classified design. Twenty individuals from each stream were pre-served for measurement of initial length, body mass, and defensive morphological structures. Then five larvae originating from either a fish or fishless site were placed into each chamber and exposed to waterborne fish cues or fishless control water (four treatments: fish or fishless *Drunella* × fish or fishless water). The measurement of morphological characters followed the same procedure as in the analyses of morphological differences in the field survey. Chamber means were used as the unit of replication for statistical analysis. Effects of fish cues on growth rates (1998 and 1999), size at emergence (1999), and emergence date (1999) were analyzed using two-way ANOVA (1998) or MANOVA (1999) (fish cue treatment × and origin of individuals).

1. *1998 experiment.*—*Drunella* larvae used in this experiment were obtained from the upper (fishless) or lower (fish) sections of Rock Creek and were in late stage II (wing pads wider than long) or early stage III (wing pads longer than wide) (Delucchi and Peckarsky 1986). The purpose of the experiment was to determine whether fish cues induced the development of defensive structures in either population. We replicated each treatment five times. We started the experiment on 17 July and ended it on 10 August.

2. *1999 experiment.*—*Drunella* larvae used in the 1999 experiment were collected from either Upper Rock Creek (fishless) or from Copper Creek (fish) and were in middle to late stage III. The goals of this study were to determine whether defensive structures could be induced late during mayfly development, and whether predator cues would affect size at emergence, growth rates, or development of *Drunella*. We used 10 replicates of each treatment, starting the experiment on 17 August and ending it on 15 September after the last *Drunella* developed black wing pads.

Predation trials.—We conducted predation trials to determine whether *Drunella* from fish and fishless streams differed in vulnerability to trout predation. Experiments were carried out in 16 flow-through cattle tanks with length × width dimensions of 1.21 × 0.68 m, providing a water surface area of 0.823 m². Tanks were located beside the East River, a trout stream, and received gravity-fed water from a fishless first-order stream on the east side of Gothic Mountain at RMBL. We created circular flow by partially dividing tanks with a central baffle, and using two water jets facing in opposite directions on each side of the tanks. Water drained from mesh windows in a center standpipe in each tank. Current velocity was similar among tanks (31.9 ± 2.2 cm/s), but heterogeneous within tanks (range: 12–58 cm/s), depending on the closeness to the water jets. Ten large rocks were evenly distributed within each tank. (See Peckarsky and McIntosh [1998] for an illustration and more information about the system.)

Predation trials were conducted three times during *Drunella* larval development (early, 6–7 July; middle,

23–24 July; and late, 23–24 August 1999). In each experiment, *Drunella* from a fishless site (Upper Rock Creek) and from a fish site (Copper Creek) were exposed to trout predation: (1) 20 *Drunella* from a fish stream plus one brook trout ($n = 5$ replicates), (2) 20 *Drunella* from a fishless stream plus one brook trout ($n = 5$), (3) 20 *Drunella* from a fish stream and no trout ($n = 3$), and (4) 20 fishless *Drunella* plus no trout ($n = 3$). Due to the limited number of cattle tanks (16), we chose an unbalanced design with more replication of the predation treatments than the control treatments. Each experiment ran from 1600 to 0900 next morning (17 h). Sizes of trout were 107.3 ± 3.9 mm, 125.9 ± 5.9 mm, and 124.5 ± 4.4 mm total length (means ± 1 SD), respectively, for these three trials. When the experiments were completed, trout were removed and the remaining *Drunella* were counted. The losses of *Drunella* from controls were subtracted from losses in predation treatments to correct for non-predator mortality. The numbers of individuals consumed by trout were compared between *Drunella* originating from fish and fishless streams using a two-way ANOVA. Sizes of *Drunella* used in the predation trials did not differ between treatments at any date ($N = 20$ replicates, means ± 1 SD: 7–8 July, 5.20 ± 0.93 mm [fish] vs. 4.60 ± 0.46 [fishless], $t = 1.96$, $P = 0.09$; 22–23 July, 7.09 ± 0.71 mm [fish] vs. 7.00 ± 0.86 mm [fishless]; 24–25 August, 10.68 ± 1.02 [fish] vs. 10.24 ± 1.18 [fishless]). However, sizes of *Drunella* increased from the first to the last predation trial.

During the third predation trial, we conducted behavioral observations, both during the evening (1900 hours) and at night (0200 hours). We made observations at night using dim red light from flashlights covered with red acetate, and during the day by staying motionless, neither of which appeared to affect *Drunella* or trout behavior. The objective of these observations was to compare drift rates and numbers of individual mayflies visible on rocks among treatments. We measured *Drunella* per capita drift in the tanks by counting the number moving in the water column through a cross-section of each tank over 5 min. We measured number of visible *Drunella* on rocks by visually searching every rock in each tank. We used one-way MANOVA to compare drift rates and visible numbers of *Drunella* originating from fish and fishless streams using only control treatments without trout, where densities of *Drunella* remained constant.

On 5 September one additional experiment was conducted to test directly whether longer caudal filaments protected *Drunella* from trout predation. We used *Drunella* from a fish stream (Copper Creek) and clipped 50% off all three caudal filaments from half of the individuals using a dissecting scissors. We handled the other half in a similar manner as a sham control, but left their caudal filaments intact. Clipped and unclipped larvae were then exposed to one brook trout in cattle tanks (size of trout was 126.0 ± 5.0 mm total length),

and numbers of *Drunella* consumed were compared to controls with no trout using two-way ANOVA as above. Four treatments were used in this experiment; (1) 20 *Drunella* with intact filaments plus one trout ($n = 4$ replicates), (2) 20 *Drunella* with reduced filaments plus one trout ($n = 4$), (3) 20 *Drunella* with intact filaments and no trout ($n = 4$), and (4) 20 *Drunella* with reduced filaments and no trout ($n = 4$). The body length of *Drunella* used in this experiment was 10.12 ± 1.24 mm (mean ± 1 SD).

RESULTS

Field survey

Secondary eyes develop in male *Drunella* during the larval stage III, which enabled us to distinguish males from females in 1999, but not in 1998. Thus, males and females were analyzed separately only in 1999. Error estimates for measurements ranged from 0.50% to 1.00%, demonstrating that measurement error was not a significant source of variability in this study.

In the ANCOVA of 1998 morphometric data, the slopes of the relationships between body length and dorsal spine length differed between *Drunella* from fish and fishless streams ($F_{1,20} = 4.76$, $P = 0.04$). Since the length of the dorsal spines of *Drunella* increased with body length faster in fish than fishless streams, we were unable to analyze main treatment effects on spine length. However, the slopes did not differ between fish and fishless streams for the relationships between body length and caudal filament length ($F_{1,20} = 0.004$, $P = 0.10$) or lateral spine length ($F_{1,20} = 0.10$, $P = 0.75$). Subsequent tests of main effects in the nested MANCOVA showed that both caudal filaments (Fig. 2A) and lateral spines (Table 1) were relatively longer in fish streams than in fishless streams.

Separate analyses of females and males in 1999 removed much of the variability observed in the 1998 morphometric data (Fig. 2). The slopes did not differ between fish and fishless streams for the relationships between body length and length of caudal filaments, lateral spines, or dorsal spines (females, $F_{1,32} < 0.03$, $P > 0.86$, for all three characters; males, $F_{1,32} < 3.06$, $P > 0.09$, for all three characters). Thus, we were able to test for main treatment effects on all three structures. Nested MANCOVAs showed that both males and females developed relatively longer caudal filaments in the presence of trout (Fig. 2B, Table 2) but there was no effect of fish on abdominal spines (Figs. 3 and 4, Table 2).

Thus, data from both surveys showed that the caudal filaments of *Drunella* were relatively longer in streams with fish compared to fishless streams. Furthermore, the 1999 data suggest that divergence in defensive morphology of *Drunella* between fish and fishless streams had already occurred by the time we began sampling in late June (i.e., pattern already evident in smaller individuals—see Figs. 2B and 3). The 1999 data also
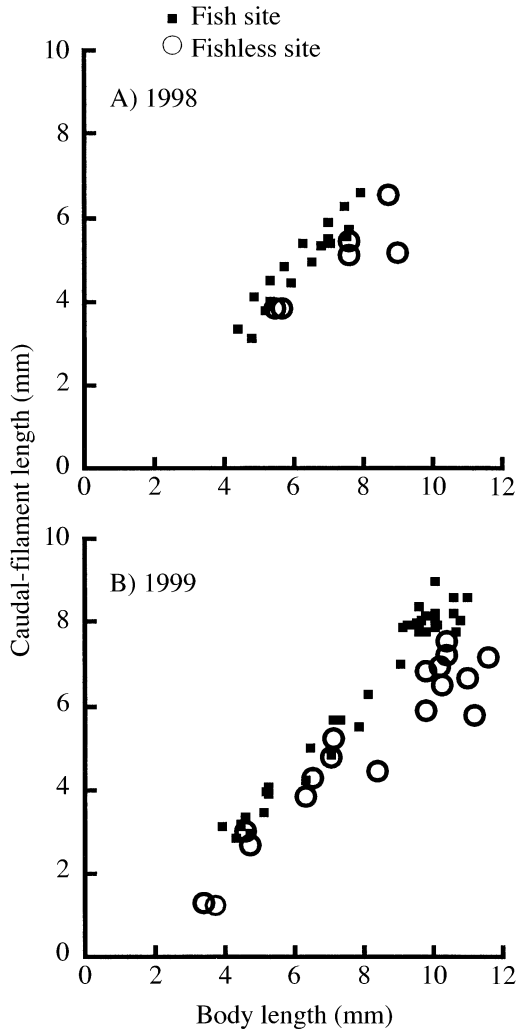
FIG. 2. The allometric relationship between body length and caudal-filament length in natural *Drunella* populations sampled in natural streams, the East River and its tributaries, Gunnison County, Colorado, USA. Each point (replicate) represents the mean of individuals collected on each date at each site. (A) July 1998: fish streams—East River, Cooper Creek, Rustlers Gulch, Quigley Creek, Lower Rock Creek, and Lower Gothic Creek; fishless streams—Upper Rock Creek and Upper Gothic Creek. (B) June, July, August, and September 1999: same streams as in 1998 plus fish stream Lower Bradley Creek and fishless stream Upper Bradley Creek. For 1999 data, the x-axis (body length) roughly approximates time.

suggest that defensive morphological responses were similar in both males and females.

There was no significant difference in the timing of emergence of *Drunella* between fish and fishless streams during the 1999 survey (nested ANOVA: $F_{1,2} = 1.00$, $P = 0.42$). All individuals were at stage II when we started sampling in June. The first black-wing-pad *Drunella* individuals were found in Upper Rock Creek (fishless) and East River (fish) (18 August) followed by Lower Rock Creek (fish), Quigley Creek (fish), Upper Gothic Creek (fishless), Rustlers Gulch (fish), and Lower Gothic Creek (fish) (24 August). The last black-wing-pad *Drunella* were found in Copper Creek (fish) (26 August), Upper Bradley (fishless), and Lower Bradley (fish) (28 August).

The size at emergence (black-wing-pad individuals) of females was significantly smaller in fish sites than in fishless sites (nested ANOVA; $F_{1,2} = 15.76$, $P = 0.05$) (Fig. 5), and there was a tendency for males to emerge at smaller sizes from fish streams (nested ANOVA; $F_{1,2} = 4.82$, $P = 0.16$) (Fig. 5).

As has been reported for other mayflies (Sweeney and Vannote 1981), smaller females produced fewer eggs (Fig. 6). The number of eggs produced per female can be predicted from body mass of black-wing-pad-stage females using the equation: egg number = 239.40 + 61.39x ($r^2 = 0.72$, $n = 20$ replicates).

Investment in exoskeleton.—Individuals originating from fish streams had significantly heavier exoskeletons per unit body length than individuals from the fishless sites (nested ANOVA: $F_{1,5} = 9.09$, $P = 0.003$) (Fig. 7). These data suggest that *Drunella* from fish streams allocate more resources to exoskeletons than do those from fishless streams.

Experiments

Induction of morphological defenses, 1998.—If defensive caudal filament and spine morphology is induced by chemical cues from trout, we expected to see a pattern in our experiments similar to the one observed in nature. Similar to the field data from 1999, the slopes of the relationships between *Drunella* body length and lengths of caudal filaments, lateral spines and dorsal spines did not differ among treatments (fishless, $F_{1,6} < 1.68$, $P > 0.24$; fish, $F_{1,6} < 0.70$, $P > 0.39$ for all characters). However, further analyses of main treatment effects of the MANCOVA showed that relatively

TABLE 1. Results of nested MANCOVA and univariate nested ANCOVA tests for effects of presence/absence of trout on caudal-filament length and length of abdominal spines of *Drunella* in the 1998 field survey.

Test	Response variables	df	Wilks' lambda	F	P
MANCOVA		3, 14	0.518	4.34	0.02
ANCOVA	caudal filament	1, 16		13.19	0.002
	lateral spine IX	1, 16		6.16	0.025

Note: Body length was used as covariate in the analyses.

TABLE 2. Results of nested MANCOVA and univariate nested ANCOVA tests for effects of presence/absence of trout on caudal-filament length, ventral spine IX length, and dorsal spine VI length (see illustration, Fig. 1).

Test	Response variables	df	Wilks' lambda	F	P
Females					
MANCOVA		3, 26	0.24	26.42	<0.001
ANCOVA	caudal filaments	1, 28		70.91	<0.001
	lateral spine IX	1, 28		0.42	0.52
	dorsal spine VI	1, 28		1.48	0.23
Males					
MANCOVA		3, 26	0.47	9.66	<0.001
ANCOVA	caudal filaments	1, 28		25.79	<0.001
	lateral spine IX	1, 28		0.78	0.38
	dorsal spine VI	1, 28		2.17	0.15

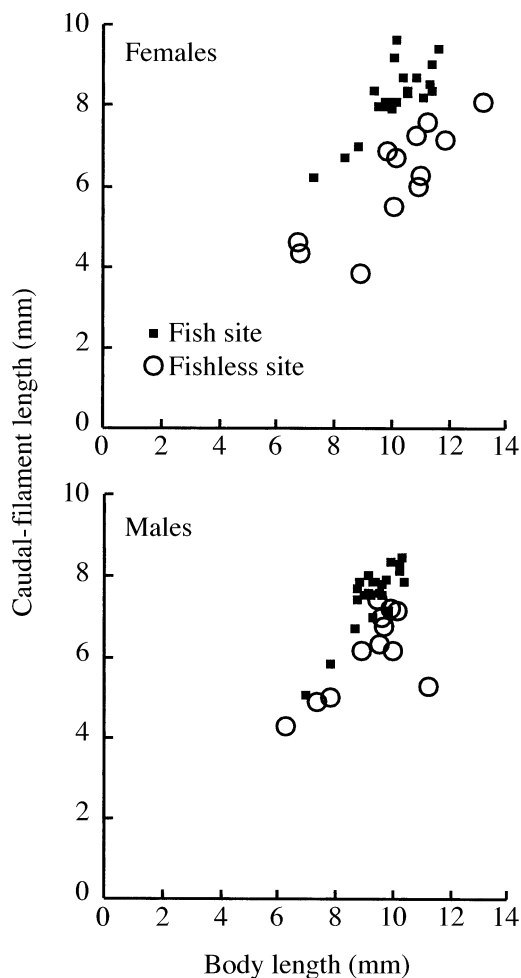*Note:* Body length was used as covariate in the analyses.

FIG. 3. The allometric relationship between body length and caudal-filament length for females and males in the natural *Drunella* populations sampled during June–September in 1999. Streams sampled are indicated in the Fig. 2B legend. Each point (replicate) represents the mean of individuals collected on each date at each site. The x-axis (body length) roughly approximates time.

longer caudal filaments were induced by waterborne cues only in *Drunella* originating from fishless streams (Fig. 8A, Table 3). In *Drunella* originating from the fish site, morphological defenses did not differ between individuals reared with and without fish cues (Table 3). Growth rate was not affected by fish cues or origin of larvae (two-way ANOVA; $F_{1,16} < 0.06$, $P > 0.52$), nor was there any interaction between treatment and origin of larvae ($F_{1,16} = 3.31$, $P = 0.09$).

Induction of morphological defenses and body size at emergence, 1999.—Similarly, the analyses of the 1999 experiment showed no significant effect of treatments on the allometric relationship between body length and caudal-filament length or abdominal-spine lengths (no significant interaction terms). Analysis of the main effects showed, as in the 1998 experiment, that fish cues induced longer caudal filaments per unit body length in both males and females originating from fishless streams (Fig. 8B and C, Table 4), but treatments had no effects on *Drunella* originating from the fish stream.

In contrast to the field data, there were no significant treatment effects on the size (either length or mass) at emergence for either males or females originating from either fish or fishless sites (two-way ANOVA: $F_{1,16} < 0.36$, $P > 0.55$ for all comparisons). Similarly, there were no significant differences in growth rate between treatments for males or females originating from fish or fishless sites (two-way ANOVA: $F_{1,16} < 0.10$, $P > 0.75$ for all comparisons). Finally, there were no treatment effects on *Drunella* emergence date (one-way ANOVA: $F_{1,16} < 1.17$, $P > 0.29$ for all comparisons).

Predation trials: fish vs. fishless Drunella.—Trout consumed fewer *Drunella* originating from the fish site than from the fishless site in all three experiments (Fig. 9) (two-way ANOVA: fish vs. no fish, $F_{1,22} = 38.78$, $P < 0.001$). There was also a significant time effect in the analysis ($F_{2,22} = 12.21$, $P = 0.003$), but no interaction between predator treatment and time ($F_{2,22} = 1.96$, $P = 0.16$). The time effect indicates that trout consumed fewer *Drunella* later in the summer, possibly
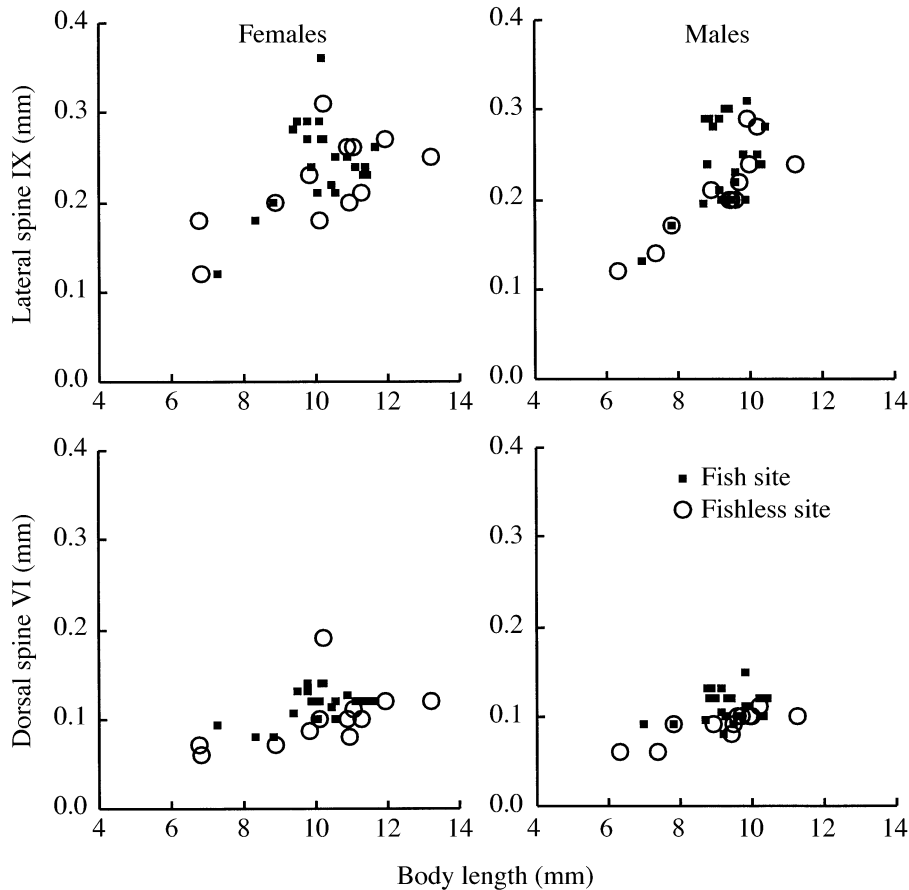
FIG. 4. The allometric relationship between body length and lengths of dorsal VI and lateral IX spines for females and males in the natural *Drunella* populations sampled in June–September 1999. Each point (replicate) represents the mean of individuals collected on each date at each site. The *x*-axis (body length) roughly approximates time.

because *Drunella* size and defensive morphology increased over time.

Univariate ANCOVAs of the *Drunella* used in these predation trials showed that *Drunella* from fish streams had relatively longer caudal filaments in all three experiments (7–8 July, $F_{1,34} = 5.54$, $P = 0.03$; 22–23 July, $F_{1,34} = 6.84$, $P = 0.018$; 24–25 August, $F_{1,34} = 20.93$, $P = 0.003$). Furthermore, neither lateral spine length nor dorsal spine length differed between fish and fishless *Drunella* on any date ($F_{1,34} < 1.52$, $P > 0.23$ in all cases).

Behavioral observations showed that *Drunella* had very low drift rates, and were rarely observed on rocks during the day or night. Both of these behaviors (drifting and residing on the exposed surfaces of rocks) would make them more vulnerable to visually feeding trout (number of individuals drifting in 5 min per tank, fish *Drunella* 0.42 ± 0.22, no fish *Drunella* 0.40 ± 0.20; individuals seen on rocks, fish *Drunella* 0.26 ± 0.20, no fish *Drunella* 0.28 ± 0.12). There were no effects of origin of *Drunella* on these behaviors of individuals. Per capita drift and numbers of *Drunella* exposed did not differ between individuals originating

from fish and fishless streams. We could not test the effects of trout on these behaviors because *Drunella* individuals were consumed during the trout treatments, resulting in unknown population sizes in the tanks during the observations.

Predation trial: fish Drunella *with intact vs. reduced caudal filaments.*—*Drunella* originating from the trout stream with intact caudal filaments had significantly lower mortality from trout predation compared to *Drunella* from the same site with reduced caudal-filament length. On average 0.25 ± 0.19 individuals with intact caudal filaments were consumed, while 1.50 ± 0.25 individuals with reduced tails were consumed in 17 h (means $\pm$ 1 SD; $F_{1,6} = 9.58$, $P = 0.02$). The reduction of caudal-filament length had no effect on *Drunella* survival during the experiment as all individuals in the controls were recaptured alive.

DISCUSSION

Two years of field data showed a clear pattern of variation in defensive structures of *Drunella coloradensis* larvae. In sites that contained brook trout, *Drunella* had longer caudal filaments and allocated more
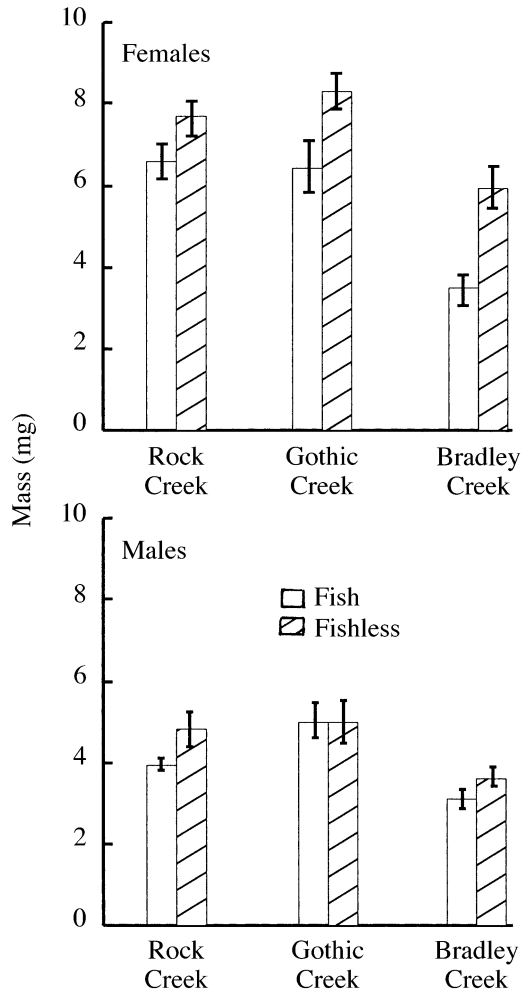
FIG. 5. Dry mass of mature larvae (black-wing-pad stage) of *Drunella coloradensis* females and males from fish and fishless stream sites in the East River watershed (Colorado, USA) in 1999. Data are means ± 1 SE.
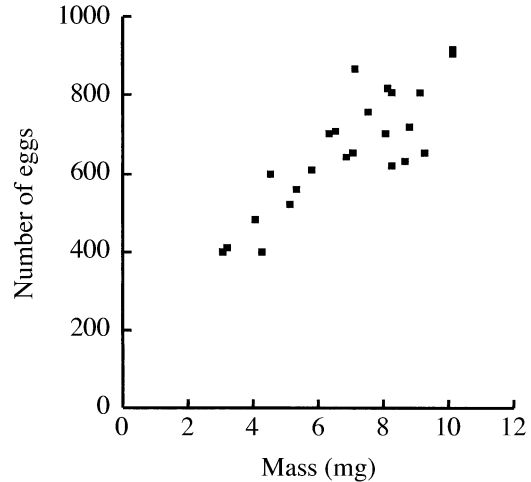

FIG. 6. Fecundity vs. dry body mass for mature (black-wing-pad) female larvae of *Drunella coloradensis*. Individuals were taken from all streams sampled in this study.

1990, Arnqvist and Johansson 1998, Schlichting and Pigliucci 1998).

The phenotypic plasticity observed in this study meets most of the prerequisites proposed by Harvell (1990) for the evolution of inducible defenses. First, to express the optimal antipredator phenotype, prey must have reliable cues by which to obtain information on the local predator regime. Induced defenses in aquatic systems are often triggered by waterborne

body mass to exoskeletons compared to *Drunella* in fishless sites. Results of two different rearing experiments suggest that chemical cues from trout induced developmental differentiation in defensive characters of *Drunella* larvae. Larvae originating from a fishless stream and reared in water carrying environmental cues from brook trout developed longer caudal filaments relative to their body length than larvae from the same stream reared without predator cues.

The induction of longer caudal filaments in *Drunella* was graded, in the sense that caudal filaments were present in all environments, but became longer in environments containing trout. Such graded plasticity is much less apparent than discrete polymorphisms (e.g., spines or no spines) and, therefore, may be potentially overlooked. We suspect that these more subtle phenotypic responses to variable environments could be more widespread than previously thought (Harvell
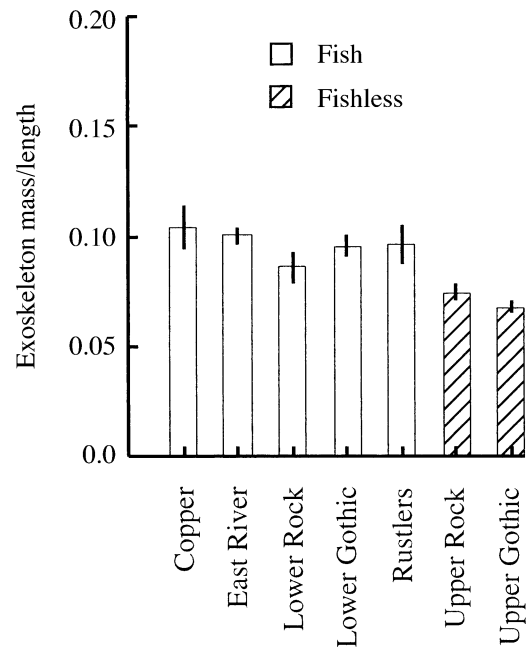

FIG. 7. The ratio between the mass of the exoskeleton and the length of the exoskeleton of *Drunella* from streams with and without fish. Black-wing-pad individuals were collected from these streams and reared in circular stream tanks until they emerged. Data are means ± 1 SE.
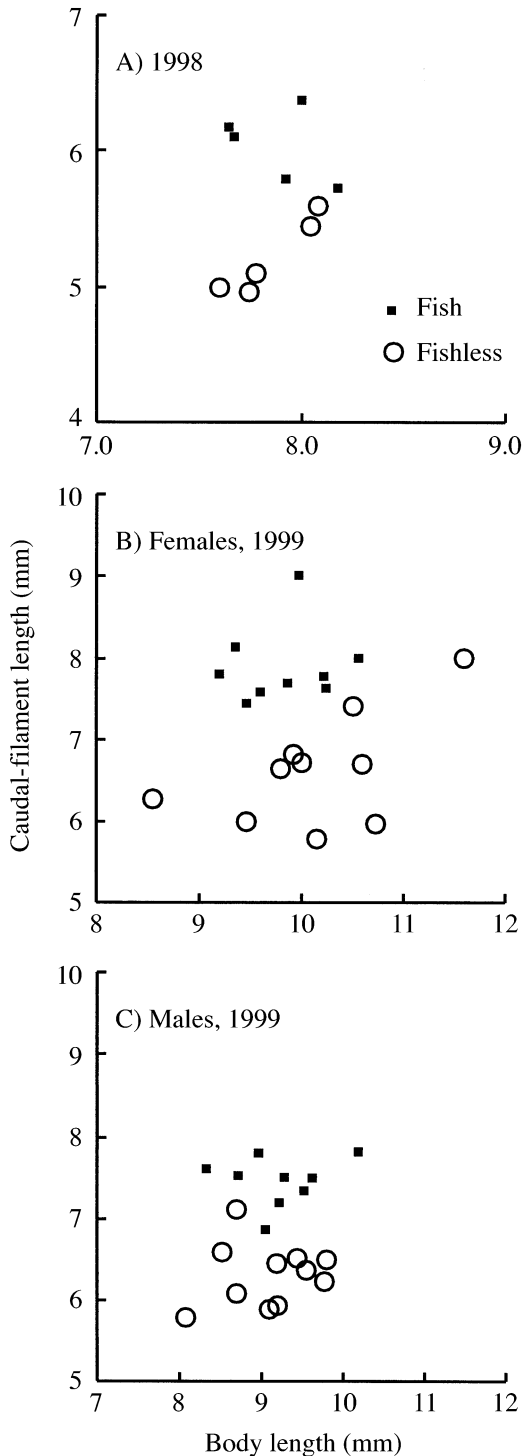
FIG. 8. The allometric relationship between body length and caudal-filament length for *Drunella* originating from a fishless stream reared with either control water or waterborne trout cues. (A) Rearing experiment conducted in 1998 from 17 July to 10 August; (B) and (C) rearing experiment conducted in 1999 from 17 August to 15 September. Each point (replicate) represents the mean of all individuals per tank.

chemical cues released by the predator or by injured conspecifics (Havel 1987, Dodson 1989, Adler and Harvell 1990). The recognition of predators through chemical stimuli has been reported for a large number of freshwater invertebrates (Dodson et al. 1994), including mayflies (Peckarsky 1980, Peckarsky and McIntosh 1998). Recognition of predators through chemical cues may be particularly important for prey with poorly developed visual acuity (Dodson et al. 1994), or when the predator is cryptic or adopts a "sit and wait" strategy (Malyushina et al. 1991, Mathis and Smith 1992). Mayfly larvae lack well-developed compound eyes (Needham et al. 1935), and brook trout are ambush predators that spend most of their time motionless behind cobbles in complex habitats and attack prey at short distances (Fausch and White 1981). Therefore, it may be advantageous for *Drunella* and other stream invertebrates to use chemical cues to detect the presence of trout (see Dahl et al. 1998).

Second, an induced defense must be effective (Harvell 1990). Induced defensive morphology may explain why *Drunella* from fish streams had a lower mortality during the predation trials compared to *Drunella* originating from fishless streams. The presence of spines is a widely adopted antipredator morphology (Edmunds 1974), and spines and caudal filaments have been shown to be effective defenses for insects against predators (Otto and Sjöström 1983, Johansson and Samuelsson 1994, Arnqvist and Johansson 1998). Predatory fish have difficulty handling and swallowing dragonflies with longer spines (Johansson and Samuelsson 1994). Defensive spines also provide protection for *Daphnia* from predation by sunfish (Tollrian 1994, Swaffar and O'Brian 1996), sticklebacks (Hoogland et al. 1957, Reist 1980), and crucian carp (Brönmark and Miner 1992, Brönmark and Pettersson 1994, Nilsson et al. 1995, Brönmark et al. 1999) (see also Tollrian and Harvell [1999] for more examples). Furthermore, Soluk (1990) showed that another species of ephemerellid was more susceptible to stonefly predation immediately after molting, supporting the notion that higher investment in exoskeleton by *Drunella* in trout streams may be an effective defense.

An alternative explanation of the results from predation trials may be that behavioral differences between mayflies in fish and fishless streams caused naive mayflies to be more susceptible to predation by trout. However, behavioral observations during the predation trials did not support this explanation, since we observed no differences in drift rates or proportions of individuals residing on exposed substrates between *Drunella* from fish and fishless streams. *Drunella* from both streams had very low drift rates, which should generally reduce their exposure to trout predation. Since per capita drift rates were similar between populations, we could not attribute higher predation rates on inexperienced *Drunella* to behavioral differences.

The effectiveness of the induced morphology is fur-

TABLE 3. Results of MANCOVA and univariate ANCOVA tests for effects of presence/absence of trout cues on caudal-filament length, and length of abdominal spines of *Drunella* in the 1998 induction experiment.

Test	Response variables	df	Wilks' lambda	*F*	*P*
Drunella from fishless stream					
MANCOVA		3, 5	0.26	8.46	0.017
ANCOVA	caudal filament	1, 7		19.75	0.003
	lateral spine IX	1, 7		1.64	0.24
	dorsal spine VI	1, 7		0.87	0.87
Drunella from fish stream					
MANCOVA		3, 5	0.48	1.78	0.26

Note: Body length was used as covariate in the analysis.

ther substantiated by the observation that experienced *Drunella* individuals with artificially reduced caudal filaments had significantly higher rates of trout predation than those with intact caudal filaments. This result provides the strongest evidence that longer caudal filaments function as a protection against trout predation. The exact mechanism for the increased capture success of mayflies with reduced caudal filaments is unknown. One hypothesis is that longer caudal filaments increase the perceived size of the prey, especially as *Drunella* postures like a scorpion when a predator approaches, similar to other ephemerellids (Peckarsky 1987, Peckarsky and Penton 1988). This posture should also make it more difficult for a fish to consume *Drunella*. We suspect that longer caudal filaments and heavier exoskeletons may increase handling time and escape probability during the predation event similar to the crucian carp–northern pike system (Nilsson et al. 1995).

Third, inducible defenses are thought to be favored when selection pressure exerted by predators is variable (Harvell 1990, Tollrian and Harvell 1999). Higher rates of attack and more uniform predator impacts favor the evolution of constitutive defenses (Tollrian and Harvell 1999). We suspect that temporally and spatially variable risk of predation from trout in the East River (Colorado, USA) and its tributaries maintains morphological plasticity in these *Drunella* populations. Although little is known about *Drunella* dispersal, they have winged adults that oviposit in both fish and fishless streams, subjecting them to spatial variation in risk of predation. Although the presence or absence of trout among streams has been constant for at least seven years in this system (Peckarsky et al. 2001), trout density is temporally variable (B. L. Peckarsky and A. R. McIntosh, *unpublished data*). Furthermore, we suspect that the presence or absence of fish in these streams may be more variable over longer time periods (decades, centuries). The risk of mortality in high-altitude streams from severe physical conditions such as floods, avalanches, droughts, and freezing has been shown to severely affect other fish species (Seegrist and Gard 1972, Harvey 1987).

Fourth, there is usually assumed to be some fitness

TABLE 4. Results of MANCOVA and univariate ANCOVA tests for effects of presence/absence of trout cues on caudal-filament length and length of abdominal spines of *Drunella* in the 1999 induction experiment.

Test	Response variables	df	Wilks' lambda	*F*	*P*
Drunella from fishless stream					
MANCOVA					
Females		3, 14	0.35	8.53	0.002
Males		3, 15	0.25	14.88	<0.001
ANCOVA					
Females	caudal filament	1, 16		29.21	<0.001
	lateral spine IX	1, 16		0.12	0.73
	dorsal spine VI	1, 16		0.26	0.62
Males	caudal filament	1, 17		49.58	<0.001
	lateral spine IX	1, 17		0.35	0.56
	dorsal spine VI	1, 17		3.61	0.07
Drunella from fish stream					
MANCOVA					
Females		3, 10	0.56	2.64	0.11
Males		3, 14	0.78	1.30	0.31

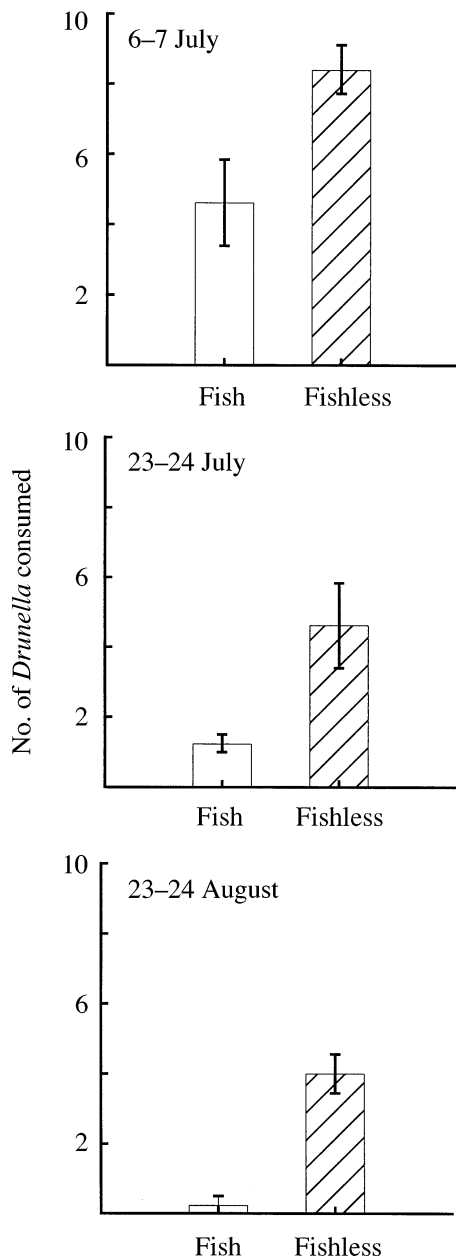*Note:* Body length was used as covariate in this analysis.

FIG. 9. Results of 1999 predation trials conducted during early, middle, and late *Drunella* larval development, showing number of prey consumed by one brook trout in 17 h, corrected for prey missing from the controls, with *Drunella* originating from fish or fishless streams. Original prey density was 20 individuals per tank. Error bars are ±1 SD.

vell [1999] for reviews). However, several previous studies have been unable to detect such costs; and recent theoretical work suggests that under certain conditions, inducible defenses can evolve even in the absence of costs (Spitze 1992, Karban 1993, Adler and Karban 1994, Tollrian 1995, Padilla and Adolph 1996). Nonetheless most studies have reported costs associated with morphological defenses in a wide variety of species (Bradshaw 1965, Lively 1986, Harvell 1992, Pettersson and Brönmark 1997, Schlichting and Pigliucci 1998, Tollrian and Harvell 1999).

Our field data show a clear cost for *Drunella* females inhabiting fish streams. Females emerging from fish streams were significantly smaller, and thereby less fecund, than females emerging from fishless streams, which could be attributed to the cost of producing morphological defenses. In contrast to the pattern in the field, we did not observe effects of trout chemicals on *Drunella* growth rates or size at emergence in experiments, even though these cues induced defensive morphology. Therefore, no costs of induction of defensive morphology were observed in these experiments. We suspect that either costs are initiated earlier in ontogeny, or that other environmental cues present in natural streams, but not rearing chambers, combined with predator cues to cause *Drunella* females to emerge at smaller sizes from trout streams than from fishless streams. It is also possible that developing the entire set of defensive characters in the field contributed to fitness costs in natural populations, while the development of only the longer caudal filaments in experiments was less costly. Further studies are needed to separate these mechanisms and to understand the causes of these fitness costs to *Drunella* of living in fish streams.

If there are costs of expressing defensive morphological traits, we would expect such defenses to be most pronounced during stages where benefits are largest (see Tollrian and Harvell [1999] for a review). For example, defensive traits in *Daphnia* vary during ontogeny and are most pronounced during stages when individuals are more exposed to predation (Vourinen et al. 1989, Harvell 1990, Parjeko and Dodson 1990, Tollrian and Harvell 1999). *Drunella* larvae are probably most vulnerable to predators late in their ontogeny, since salmonids become more active throughout the summer and are generally large-size-selective predators (Newman and Waters 1984, Bechara et al. 1993, Dahl 1998a, Peckarsky et al. 2001). The induction experiments showed that fish cues elicited the change in caudal-filament morphology in stage III and stage IV *Drunella*; and our field data suggest that this differentiation was already present by late June when larvae were in stage II. *Drunella* reproduce in late August and early September in the East River system and eggs develop for two weeks before hatching (Needham et al. 1935). They overwinter as stage I (no wing pads) larvae, and begin to grow and develop after snow melt (B. L. Peckarsky, *unpublished data*). We suspect that

cost when induced morphological defenses are expressed (Harvell 1990, Clark and Harvell 1992), because allocation of resources to defensive structures may result in fewer resources available for growth, reproduction, or longevity (Stearns 1992). A number of mechanisms by which inducible morphological defenses may result in direct fitness costs have been suggested (see DeWitt et al. [1998] and Tollrian and Har-

differentiation of defensive morphology begins after the spring runoff recedes, and the risk of trout predation increases (Peckarsky et al. 2001), thereby protecting the later, more vulnerable developmental stages.

Other insects have been shown to speed up their development rates to avoid predation (Bradshaw and Johnsson 1995, Arnqvist and Johansson 1998). These life-history shifts may also be accompanied by fitness costs. Peckarsky et al. (2001) found that while densities of *Baetis bicaudatus* were not reduced in streams with brook trout, the presence of trout induced life-history shifts in these mayflies. With trout present, *Baetis* increased their development rates, but emerged at smaller sizes compared to *Baetis* inhabiting fishless streams. However, our study provided no evidence that the smaller size of *Drunella* emerging from fish streams was caused by an induced acceleration of development, since time of emergence was not different between fish and fishless streams. Thus, in *Drunella* we conclude that trout induced morphological defenses rather than altering behavioral or life-history traits.

Stream ecologists have debated the issue of whether predatory fish can explain patterns of invertebrate distribution and abundance. Although extensive empirical and theoretical work has been conducted, the results are equivocal (see Cooper et al. [1990] or Wooster et al. [1997] for reviews). Most studies have focused on effects of fish on prey densities or biomass. However, to better understand processes producing patterns in streams, we must look beyond measures of abundance (Dahl 1998b). This and other studies show clearly that the effects of predators are not only mediated through prey mortality, but they are also mediated through prey traits, such as behavior, physiology, morphology, and life history (Werner 1991, Peacor and Werner 1997, McPeek and Peckarsky 1998). By affecting different traits, predators can have important consequences to prey populations as well as other members of communities (Werner and Anholt 1996, Peacor and Werner 1997).

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