Nonconsumptive effects of Brook Trout predators reduce secondary production of mayfly prey

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Abstract: The nonconsumptive effects of predators on prey include behavioral, physiological, and life-history changes that reduce the risk of predation but have associated energetic or fitness costs to prey individuals and populations. Biologists have documented such changes for a wide array of predator–prey interactions in a variety of ecosystems; however, the energetic cost of nonconsumptive effects to prey populations has rarely been measured directly. Using a reach-scale manipulation of a naturally-fishless stream, we added chemical cues produced by live Brook Trout (*Salvelinus fontinalis* Mitchill, 1814) to simulate the presence of trout predation risk, and we quantified the impact of nonconsumptive effects on the secondary production of mayfly prey in the stream. The addition of trout chemical cues reduced the secondary production of larval *Baetis* mayflies in the treatment reach by 17% compared to an upstream, unmanipulated reference reach. This reduction was driven by smaller body size and earlier emergence of mayflies from the reach with added predator cues. The nonconsumptive effects of a predator can consequently reduce the flux of energy through a dominant stream invertebrate by altering individual life-history and development patterns. Furthermore, quantifying the population-level impact of nonconsumptive effects enables understanding the extent to which these widespread predator effects shape food-web dynamics and ecosystem processes.

Key words: *Baetis*, field experiment, mayfly, nonconsumptive effects, predator–prey interactions, risk effects, *Salvelinus fontinalis*, secondary production, trout

Predators, in addition to killing prey, often elicit behavioral, physiological, and life-history changes that reduce prey individuals' risk of being eaten. Such nonconsumptive effects impose a cost to prey individuals, which, despite those changes, must continue to acquire energy, grow, and reproduce (Sih 1980, Dill 1987, Lima and Dill 1990). The phenotypic manifestations of nonconsumptive effects of predators on prey have been widely reported in terrestrial, marine, and freshwater ecosystems for a wide variety of taxa (Lima 1998, Preisser et al. 2005, Heithaus et al. 2008, Peckarsky et al. 2008a, Zanette et al. 2011, Hermann and Landis 2017, Creel et al. 2019). For example, the presence of wolves alters the spatial foraging patterns of elk (Creel et al. 2005), the invertebrate predator *Chaoborus* induces the development of defensive spines in pelagic *Daphnia* (Krueger and Dodson 1981), and predatory crabs suppress spawning in marine intertidal snails (Rawlings 1994). However, in those cases, and indeed in most others, the emphasis has been on describing the altered behavioral, physiological, or life-history traits of prey individuals and not on measuring the costs of those changes to entire populations (but see Preisser and Bolnick 2008).

Quantifying how predator-induced changes in prey traits affect prey populations is a necessary step toward understanding the consequences of nonconsumptive effects within different ecosystems. However, few studies have examined such population-level effects, despite the diversity and apparent ubiquity of the changes in prey traits induced

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by predators. For example, sea turtles may alter their foraging behaviors when sharks are abundant (Heithaus et al. 2007), but do those changes make a difference to sea turtle population dynamics? If so, how does the magnitude of this effect compare to the loss of turtle biomass due to shark consumption? When are nonconsumptive effects of predators on prey strong enough to propagate throughout the food web? Answering those types of questions requires using population-level currencies, such as prey secondary production or prey population growth rate, to measure the nonconsumptive effects of predators (McPeek and Peckarsky 1998, Nelson et al. 2004, Pangle et al. 2007, Koch 2011, Reynolds and Bruno 2013, Marino et al. 2019).

Here, we assess the degree to which nonconsumptive effects of trout predators alter the secondary production of a population of larval mayflies. In montane and subalpine streams of western Colorado, USA, naturalized Brook Trout (*Salvelinus fontinalis* Mitchill, 1814) feed on drifting mayfly larvae and other aquatic invertebrates (Allan 1981). Certain mayflies in the genus *Baetis* can detect the presence of trout predators through chemical cues released by fish into the water column (McIntosh and Peckarsky 1996, Peckarsky and McIntosh 1998). *Baetis* larvae respond to the threat of predation by altering when and where they forage (Cowan and Peckarsky 1994), by drifting less during the day and becoming more nocturnal (McIntosh et al. 1999, 2002), and by accelerating their development to reach maturity faster and at a smaller body size (Peckarsky et al. 2001, 2002). A model examining the consequences of those previously-observed nonconsumptive effects predicted that accelerated larval development and earlier emergence in the presence of trout would decrease *Baetis* secondary production by leaving fewer and smaller individuals in the stream (Koch 2011). In this study, we tested that prediction by adding trout chemical cues to a naturally-fishless stream and measuring *Baetis* secondary production in the treatment reach and in an upstream, unmanipulated reference reach.

METHODS

Experimental design

We conducted the experiment during June to September 2007 in a 350-m fishless reach of Rock Creek (lat 38.98°N, long 107.02°W), a tributary of the East River in the Elk Mountains of western Colorado. Like many headwater streams in this watershed, the upper reaches of Rock Creek lack fish because of natural barriers to fish dispersal (Peckarsky et al. 2001, 2008b). To minimize the variability between reaches with different treatments, we used a single stream for both the reference and treatment conditions (within-subject design: same stream, different reaches) rather than establishing separate reference and treatment streams. The inaccessibility of suitable fishless streams within the study area made replication in multiple streams infeasible. Our study design required a relatively large stream supporting high *Baetis* densities to enable accurate measurements of cohort production, and establishing and maintaining the fish-cue manipulation required nearly daily visits for 17 wk to the study site, which was located 1.6 km and 270 m in elevation above the nearest trailhead.

To simulate the presence of Brook Trout predators without allowing predation, we added trout chemical cues to the downstream 200 m of the study reach by placing 6 live Brook Trout (total length: 118–164 mm) in each of four 110-L flow-through streamside containers (plastic garbage cans) spaced every 50 m (Fig. 1). This method has been demonstrated to elicit nonconsumptive effects of trout predators on *Baetis* mayflies in smaller streams (Peckarsky et al. 2002). Containers were gravity fed with 1.9-cm diameter hoses delivering fishless stream water into each container and back into the stream at a rate of 2 to 9 L/min. We collected Brook Trout from the East River using a backpack electrofishing unit (model LR-24; Smith-Root Incorporated, Vancouver, Washington). Every 2 d, fish in each container were fed a mixed diet of live aquatic invertebrates collected >1 km downstream of the study reach.

The upstream 150 m of the study reach received no trout chemical cues and served as a reference reach. This reach was separated from the 150-m treatment reach by a 50-m buffer reach that received trout chemical cues, but which was not sampled for mayfly larvae (Fig. 1). The buffer reach was intended to minimize the number of mayfly larvae drifting from the reference reach into the downstream treatment reach where we sampled. Drift distances of *Baetis* larvae foraging in similar streams of the East River watershed have been estimated at <0.5 m (Ode 2002) and, in general, tend to be <5 m for this genus (Allan and Feifarek 1989).

Baetis secondary production

Two common species of *Baetis* occur in the streams at this site. *Baetis bicaudatus* (Dodds, 1923) individuals overwinter as early-instar larvae and emerge in July. Individuals of an undescribed species, the univoltine *Baetis* B, overwinter as eggs and complete their entire larval development during the summer, emerging in late August and early September. Because the timing of its life cycle enabled us to calculate secondary production for 1 complete cohort during the period of the fish-cue manipulation (5 June–29 September 2007), we targeted *Baetis* B for our secondary production measurements.

We quantitatively sampled *Baetis* B larvae in the reference and treatment reaches every 8 to 14 d for a total of 11 sampling dates. We used area-restricted electrobug sampling, where an electric current is used to induce invertebrates to drift into a collection net (Taylor et al. 2001). We placed a custom-built Hess-style sampler with a mesh size of 250 μm and a sample area of 0.104 m^2 on the stream bottom and used the backpack electrofisher fitted with a

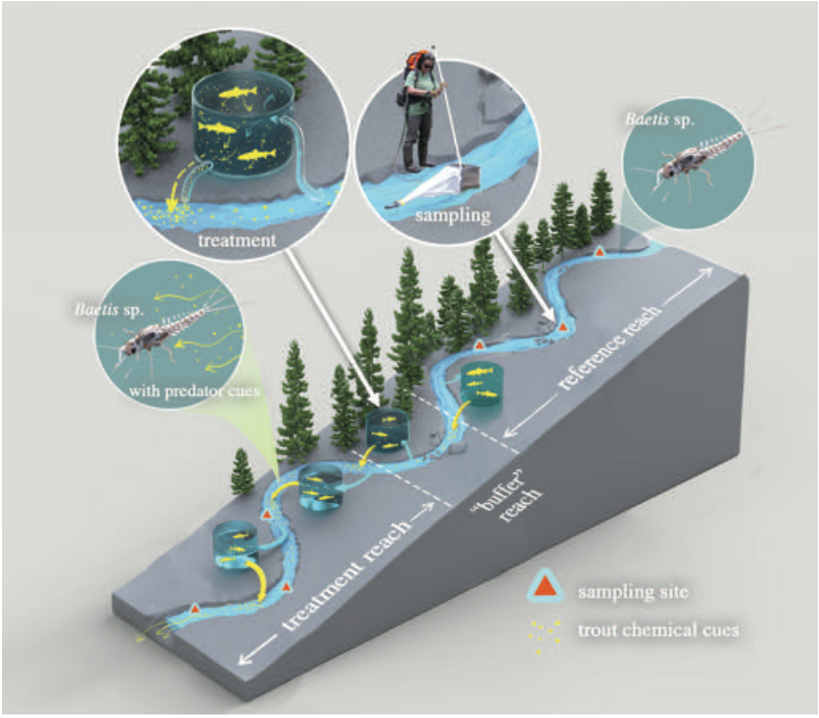

Figure 1. The field experiment assessed the population-level costs incurred by *Baetis* mayfly prey in avoiding trout predation. We simulated the presence of trout predators by adding chemical cues produced by live Brook Trout held in flow-through streamside containers to a naturally-fishless stream (Rock Creek) in Colorado, USA. We sampled mayfly larvae at 3 locations in each reach every 8 to 14 d to estimate cohort secondary production of the univoltine *Baetis* B in the unmanipulated upstream reference reach (150 m) and in the downstream treatment reach (150 m). A 50-m-buffer reach also received trout chemical cues but was not sampled. The buffer reach reduced the likelihood of mayflies drifting into the treatment sampling reach from the fishless reference reach upstream.

15-cm anode to shock continuously within the sampler for 90 s with a pulsed DC waveform (60 pulses/s, pulse width = 6 ms) following the procedure described by Taylor et al. (2001). Voltage ranged from 300 to 600 V depending on stream water conductivity. This electrobugging method efficiently and quantitatively samples *Baetis* mayfly larvae in these streams (Taylor et al. 2001), although it may undersample certain other benthic invertebrate taxa.

In each 150-m stream reach, we collected 3 samples on each sampling date. The location for each sample was determined by randomly choosing a meter mark within each of the upper, middle, and lower sections of the 150-m study reach (Fig. 1). After electrobugging, we rinsed invertebrates from the collection net, preserved each sample individually in 70% ethanol in the field, and subsequently processed samples in the laboratory under 10 to 30× magnification with an Olympus SZ60 stereo microscope (Olympus Corporation, Shinjuku, Tokyo, Japan). In each sample we counted all *Baetis* B larvae and measured the length of each individual to the nearest 0.1 mm. We scored the sex of each individual ≥3.0 mm in length based on the presence or absence of developing secondary eyes. We developed and used sex-specific length–mass regressions for *Baetis* B mayflies (Table S1) to calculate the biomass (as ash-free dry mass; AFDM) of each individual. The sex of individuals <3.0 mm could not be reliably scored, and we used a separate *unknown sex* length–mass regression to calculate biomass for individuals in that size class (Table S1). To estimate cohort secondary production (P), we used the increment summation method in which interval production values are calculated for all sampling intervals and summed with the initial biomass (B_{initial}) present on the 1st sampling date (Benke 1984, Benke and Huryn 2006):

$$P = B_{\text{initial}} + \sum_i^I \bar{N}_i \Delta W_i \qquad \text{(Eq. 1)},$$

where $\bar{N}_i$ is the mean abundance and ΔW_i is the change in mean body size over each sampling interval i. I represents the total number of sampling intervals. Our sampling began before *Baetis* B larvae hatched from eggs in the stream, thus, B_{initial} was 0 for both reaches.

We used bootstrapping to estimate uncertainty for all abundance, biomass, and secondary production estimates (Manly 2007). We randomly sampled abundance and biomass data sets with replacement and computed the mean 1000× for each reach and date. Those bootstrapped vectors were used to produce vectors of cohort secondary production. We calculated the median (median_b) and 90% confidence interval (CI_b) for all bootstrapped vectors using the

percentile method (Manly 2007). Bootstrapping analyses were conducted in R (version 3.3.3; R Project for Statistical Computing, Vienna, Austria).

Timing of *Baetis* emergence

We estimated the timing of emergence for the reference and treatment populations of *Baetis* B larvae. *Baetis* larvae progress through an indeterminate number of instars and, for convenience, are classified into 4 developmental stages based on characteristics of developing wing pads (Delucchi and Peckarsky 1989). Larvae spend 24 h or less in the final stage (IV) before emerging, and those mature individuals are easily recognized by their blackened wing pads (Peckarsky et al. 2001). We assessed the timing of *Baetis* B emergence in the reference and treatment reaches by collecting stage-IV larvae every 3 to 4 d from 1 August to 25 September 2007. We used a kick net with equal sampling effort in each reach, placing the net in the water for 90 s/reach. Kick net sampling was conducted ≥3 m from all secondary production sampling locations. We preserved stage-IV larvae in 70% ethanol and identified the sex of all individuals based on the presence or absence of developing secondary eyes. For each reach and sex on each sampling date, we calculated the cumulative proportion of the total number of stage-IV *Baetis* B larvae collected over the entire emergence period and used a permutation Kolmogorov–Smirnov test in R to detect differences in the temporal distribution of stage-IV mayflies among sexes and reaches (Praestgaard 1995).

Baetis cohort duration

We estimated cohort duration of *Baetis* B larvae in the reference and treatment reaches by analyzing temporal distributions of the 1st (I) and final (IV) developmental stages. We estimated the date on which the cohort began as the date at which 50% of all stage-I *Baetis* B larvae were observed. From the set of regularly-collected electrobugging benthic samples, we calculated the cumulative proportion of total stage-I abundance over time using rectangular integration under the curve delineated by successive dates. We then fit a lognormal cumulative distribution function and calculated the cohort starting date as the median of that distribution. We used bootstrapping to estimate uncertainty by repeating this procedure 1000×, sampling the stage-I abundance data with replacement at each iteration. Similarly, we estimated the ending date of the cohort as the date at which 50% of all stage-IV *Baetis* B larvae were observed. We used the *Baetis* B emergence-timing dataset (described above) for this calculation because of its finer temporal resolution relative to the regularly-collected electrobugging benthic samples. We calculated the cumulative proportion of all stage-IV larvae observed over time using rectangular integration under the curve delineated by successive dates. We fit a locally estimated scatterplot smoothing (LOESS) regression model ($\alpha = 0.6$, degree = 2) and solved for the date at which 50% of all stage-IV *Baetis* B larvae were detected. We calculated cohort duration as the number of elapsed days from the estimated starting date to ending date of the cohort. Males and females were grouped together for cohort-duration calculations. All cohort-duration analyses were conducted in R.

Abiotic and biotic stream characteristics

Because our experiment used a within-subject design, we quantified abiotic and biotic characteristics of the 2 reaches that could confound treatment effects. We measured discharge at the upstream and downstream extents of each reach at the end of the experiment and also measured wetted stream width and specific conductance every 10 m during the *Baetis* B emergence period. We used pulse additions of a conservative tracer (NaCl) to estimate average water velocity and average depth for each reach at the end of the experiment. We estimated canopy cover by viewing the canopy through a 25-cm long, 5-cm diameter tube vertically and at 45° angles in the 4 cardinal directions every 15 m and calculating the mean. Mean stream temperature was computed from measurements every 30 min in each reach using HOBO™ Pendant® data loggers (±0.5°C accuracy, model UA-001-064; Onset Computer Corporation, Bourne, Massachusetts). We measured chlorophyll *a* (Chl *a*) by randomly selecting a rock from the stream bottom every 7.5 m in both the reference ($n = 20$) and treatment ($n = 20$) reaches, scrubbing the biofilm with a hard-bristled toothbrush, and filtering 1 to 10 mL of the resulting slurry onto Gelman A/E filters (Gelman Sciences Incorporated, Ann Arbor, Michigan), which were frozen for later analysis. We extracted Chl *a* in 90% buffered ethanol and measured it with a fluorometer (model TD-700; Turner Designs, San Jose, California).

To assess differences in the mayfly assemblages of the reference and treatment reaches that could confound any observed effects on *Baetis*, we estimated larval biomass of all mayfly taxa in each reach on 3 dates: prior to the fish-cue addition (4 June 2007), during the period of peak *Baetis* larval abundance (13 August 2007), and near the end of the experiment after the *Baetis* emergence period (28 September 2007). We used the electrobugging benthic sampling methods described above and published length–mass regressions (Benke et al. 1999, Johnston and Cunjak 1999) to calculate biomass for each taxon. All mayfly larvae were identified to genus and most were identified to species. We tested for reach-specific differences in total mayfly assemblage biomass with a permutation test for a linear model that included reach, date, and a reach × date interaction term as predictor variables. To test for differences in mayfly assemblage composition between the 2 reaches, we conducted a permutational multivariate analysis of variance (PERMANOVA) and used non-metric multidimensional scaling (NMDS) to visualize the results. We then tested for

homogeneity of multivariate group dispersions (PERMDISP). Statistical analyses were conducted in R, and we used the *vegan* package (version 2.4-5; Oksanen et al. 2017) for the PERMANOVA, PERMDISP, and NMDS analyses.

RESULTS

Cohort secondary production of *Baetis* B was 17% lower in the stream reach with added fish cues (median$_b$: 150 mg AFDM m^{-2} cohort^{-1}; 90% CI$_b$: 120–180 mg AFDM m^{-2} cohort^{-1}) compared to the reference reach (median$_b$: 180 mg AFDM m^{-2} cohort^{-1}, 90% CI$_b$: 140–220 mg AFDM m^{-2} cohort^{-1}). Although peak abundance of *Baetis* B larvae was higher in the reference reach than the treatment reach, abundances were similar for all other sampling dates of the larval cohort period (Fig. 2A). The mean body size of *Baetis* B larvae, measured as AFDM, increased over the summer and remained similar in the reference and treatment reaches until late in the cohort period, when the larvae remaining in the reference reach attained final body sizes that were, on average, 11% greater than that of larvae in the treatment reach (Fig. 2B). These differences were reflected in the Allen curves for the 2 reaches, which show abundance vs mean body size (Fig. 2C). Cohort production for the 2 reaches can be estimated as the areas under the 2 curves defined by the data points (Fig. 2C). The largest difference in interval production occurred during the emergence period of *Baetis* B within the final sampling intervals, where secondary production from 26 August to 14 September was 28% lower in the reach with added fish cues relative to the reference reach (Fig. 2D). Average standing stock biomass of *Baetis* B over the duration of the cohort was 25% lower in the treatment reach (median$_b$: 39 mg AFDM/m^2; 90% CI$_b$: 32–45 mg AFDM/m^2) compared to the reference reach (median$_b$: 52 mg AFDM/m^2; 90% CI$_b$: 44–61 mg AFDM/m^2).

Larvae of female *Baetis* B achieved their final stage (IV) earlier in the treatment reach than in the reference reach and earlier than males in both reaches. These results indicate that females emerged earlier in the treatment reach than did females in the reference reach and earlier than males in both reaches (permutation Kolmogorov–Smirnov: D = 0.170, n = 15, p < 0.001; Fig. 3). We found no difference in emergence timing among the males in the treatment reach and males and females in the reference reach (D = 0.150, n = 15, p = 0.575; Fig. 3). Larval cohort duration was ~4 d

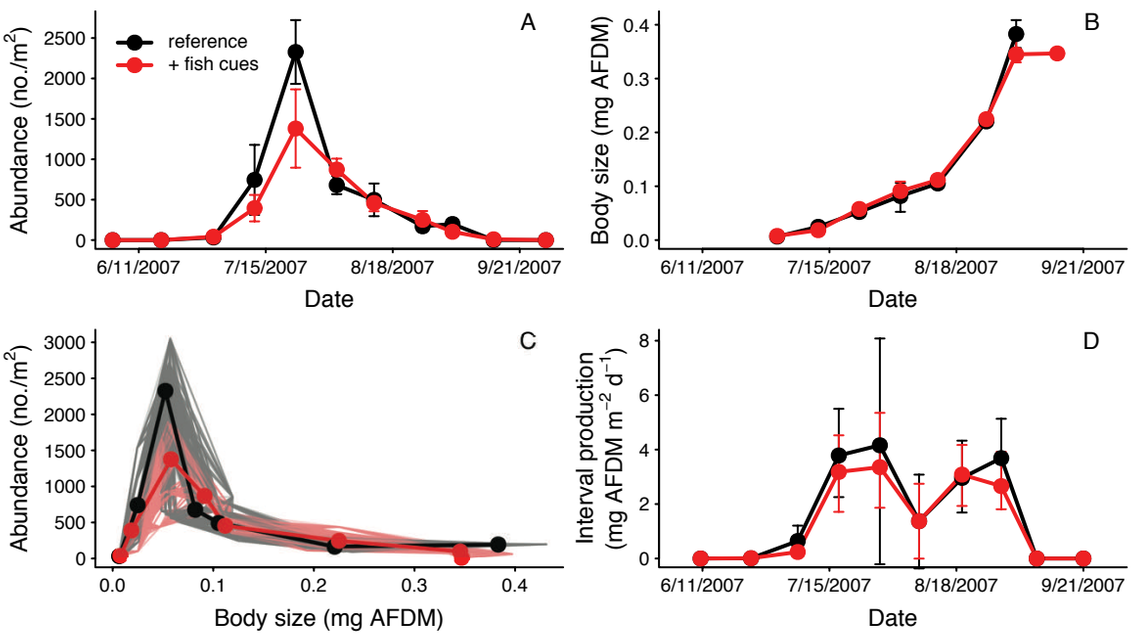

Figure 2. A.—Abundance of *Baetis* B mayfly larvae (males and females combined) was similar throughout the summer in the reference reach and in the treatment reach with added fish cues but peaked at a higher abundance in the reference reach. B.—*Baetis* B larvae (males and females combined) in the reference reach attained greater mean body size (mg ash-free dry mass, AFDM) at the end of the cohort period than larvae in the treatment reach. C.—Allen curves for the reference and treatment reaches reflect the differences in peak abundance and mean body size. The area under each curve equals the total secondary production across all sampling intervals. Gray and light red lines indicate 1000 bootstrapped Allen curves for the reference reach and treatment reach, respectively. D.—Larval interval production was lower in the treatment reach than in the reference reach. This difference was greatest during the period of emergence in the last sampling interval. Bootstrapped medians are plotted in all panels. All error bars are bootstrapped 90% confidence intervals.

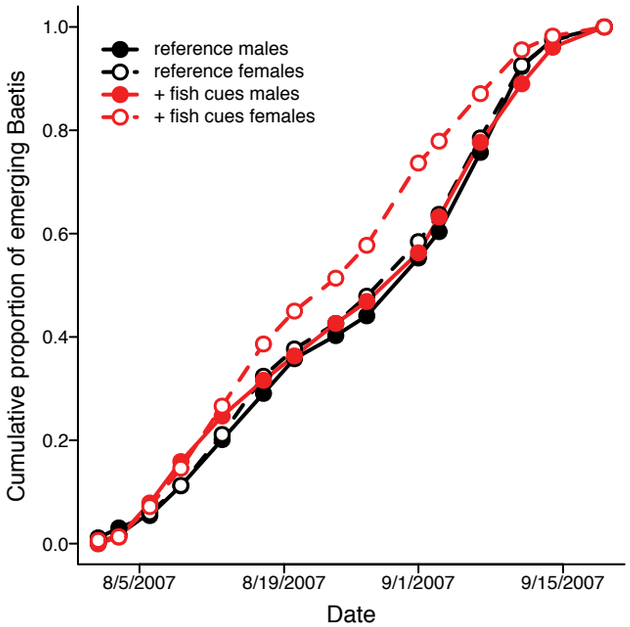

Figure 3. The cumulative proportion of *Baetis* B larvae with blackened wing pads (i.e., those that will emerge within 24 h) sampled over the emergence period. Female *Baetis* B mayfly larvae from the reach with added fish cues emerged earlier than males as well as males and females from the reference reach.

shorter for *Baetis* B in the treatment reach (median_b: 46 d; 90% CI_b: 43–50 d) compared to the reference reach (median_b: 50 d; 90% CI_b: 47–52 d).

Geomorphic, hydrologic, and other biophysical attributes of the reference and treatment reaches were similar (Table 1) and within the ranges reported for multiple streams within the upper East River catchment (Peckarsky et al. 2001, McIntosh et al. 2004). These factors were, therefore, unlikely to have contributed to systematic variation in mayfly behavior, physiology, and life history. Water temperatures followed a congruent seasonal pattern in the reference and treatment reaches, with the reference reach remaining slightly warmer (by ~0.9°C) in late July and August (Fig. S1).

Total mayfly assemblage biomass was similar in the reference and treatment reaches before, during, and at the end of the experiment (Table 2) and did not vary by reach ($p = 0.347$) or date ($p = 0.340$). A linear model permutation test revealed no substantial reach × date interaction; therefore, this term was dropped from the model. *Baetis* B biomass composed a similar proportion of total mayfly biomass before, during, and at the end of the experiment (Table 2). PERMANOVA showed seasonal shifts (*pseudo* $F = 8.426$, $p = 0.001$; Fig. 4) but no substantial differences in the taxonomic composition of mayfly biomass between the reaches (*pseudo* $F = 0.445$, $p = 0.872$; Table S2). The reach × date term had a negligible effect and was dropped from the PERMANOVA model. PERMDISP indicated that group dispersions did not differ by date (*pseudo* $F = 2.122$, $p = 0.158$; Table S3).

DISCUSSION

Nonconsumptive effects of predators are widespread and alter prey behavior, physiology, and life history in diverse animal taxa. Yet, the ecological consequences of those trait changes are poorly understood, in large part because their population-level costs have rarely been measured (Zanette et al. 2011, Peacor et al. 2012). Our field experiment revealed that the nonconsumptive effects of a predator can lower the secondary production of a prey population. The strengths of food-web interactions are often assessed using fluxes of energy consumed by predators (Novak and Wootton 2008). Similarly, the relative reduction in energy flux through the *Baetis* population represents the nonconsumptive species impact of Brook Trout. Measuring the costs of nonconsumptive effects to prey populations, therefore, enables estimating nonconsumptive interaction strengths that can be incorporated into food-web models of multi-species interactions.

Results of our experiment support predictions that nonconsumptive effects of Brook Trout alter *Baetis* population dynamics and reduce biomass fluxes (McPeek and Peckarsky 1998, Peckarsky et al. 2002, Koch 2011). A model of *Baetis* secondary production predicted that earlier emergence of mayflies in the presence of trout would decrease cohort production by leaving fewer and smaller larvae in the stream during the emergence period (Koch 2011).

Table 1. Geomorphic, hydrologic, and riparian variables in the reference and treatment reaches. The treatment reach received Brook Trout chemical cues continuously for 116 d and was located downstream of the fishless reference reach in Rock Creek, Colorado, USA. SC = specific conductance.

Reach	Width (m)	Depth (cm)	Water velocity (m/s)	Discharge (L/s)	SC at 25°C (μS/cm)	Water temperature (°C)	Canopy cover (%)	Chlorophyll *a* (mg/m^2)
Reference	2.38 (0.74)[a]	4.6[b]	0.11[b]	12.5	186 (21.5)[a]	8.75 (3.84)[a]	61.6 (35.5)[a]	5.38 (1.66)[a]
+ fish cues	2.98 (1.30)[a]	4.3[b]	0.10[b]	13.4	226 (0.87)[a]	8.28 (3.54)[a]	61.5 (37.5)[a]	6.46 (3.15)[a]

[a] Mean (standard deviation).
[b] Reach-scale average calculated based on hydraulic continuity.

Table 2. Total mayfly assemblage biomass and *Baetis* B biomass in the reference and treatment reaches. The treatment reach received Brook Trout chemical cues continuously for 116 d and was located downstream of the fishless reference reach in Rock Creek, Colorado, USA. Values are bootstrapped median ash-free dry mass (mg/m^2) with 90% confidence intervals indicated in parentheses.

	Reference reach		+ fish-cues reach	
Date	Total biomass	*Baetis* B biomass	Total biomass	*Baetis* B biomass
6/4/2007	161 (75–269)	0	143 (76–207)	0
8/13/2007	276 (213–331)	52 (35–70)	189 (143–241)	51 (42–60)
9/28/2007	310 (188–455)	0	237 (137–327)	0

Moreover, this model predicted that interval production would decline sharply during the period of emergence. Consistent with the model's predictions, we observed smaller mayflies in the treatment reach vs the reference reach during the emergence period. In addition, the greatest difference in interval production occurred during the final sampling intervals of our experiment, which was the period of peak *Baetis* B emergence. During that time, interval production was 28% lower in the reach with added fish cues. Furthermore, the semiweekly collections of mature *Baetis* B larvae provide evidence that the early emergence of female mayflies drove the observed decline in secondary production in the fish-cue treatment reach. Similar total mayfly assemblage biomass and taxonomic composition between the reference and treatment reaches suggest that reach-specific differences in biotic interactions among mayfly taxa were minimal and were, therefore, unlikely to have caused the observed variation in *Baetis* B production between the reference and treatment reaches.

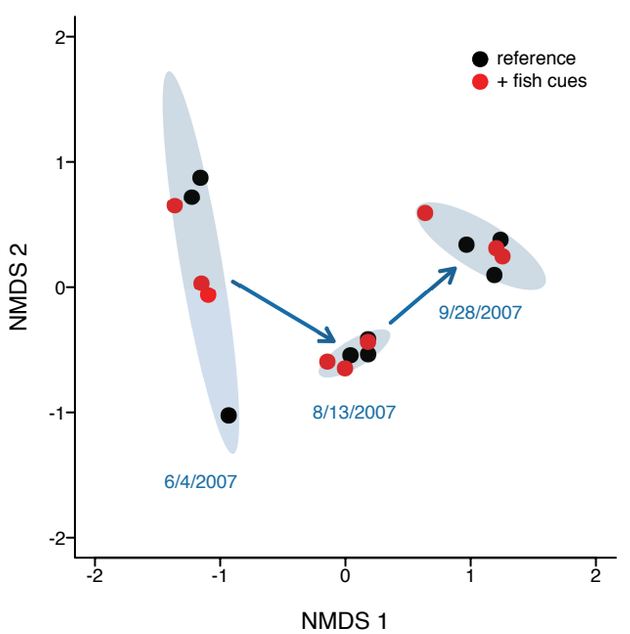

Figure 4. A non-metric multidimensional scaling (NMDS) plot showing how the taxonomic composition of the mayfly assemblage (measured in units of biomass) changed similarly in the reference (black) and treatment (red) reaches during the field experiment (stress = 0.05). Shaded ovals denote the 90% confidence ellipses for all samples from the indicated sampling dates, irrespective of stream reach. There were 3 samples from each reach on each date.

The result that females tended to emerge earlier than males in the fish-cue reach suggests that females were more responsive to trout chemical cues than males. Females may experience high selection pressure to minimize exposure to perceived predators by accelerating emergence, potentially at a cost of reduced body size and fecundity (McPeek and Peckarsky 1998). The observed shift in the relative timing of female and male emergence may have also disrupted the mating pattern of protandry, common among mayflies, where males tend to emerge earlier than females (Takemon 1993). Such a disruption could reduce female mating success, thereby compounding the negative impacts of size–fecundity effects (McPeek and Peckarsky 1998) on population growth.

Because the inaccessibility of suitable fishless streams in the East River drainage basin precluded replicating our experimental treatments in multiple streams, we used a reference–treatment reach approach similar to that of other large-scale field manipulations of streams (Wallace et al. 1997, Slavik et al. 2004, Taylor et al. 2006, Davis et al. 2010), lakes (Schindler 1974, Carpenter et al. 1987), and watersheds (Likens et al. 1970, Webster et al. 2016). This design limits the ability to ascribe the observed secondary production patterns to the treatment and limits the spatial scale of inference. However, the phenomenological mechanisms underlying our experimental findings (e.g., reduced body size, accelerated development) have been demonstrated using replicated designs observationally and experimentally for *Baetis* larvae from many streams throughout the East River drainage basin (Peckarsky et al. 2001, 2002). For instance, Peckarsky et al. (2002) performed a similar replicated field experiment in which trout chemical cues were added to small fishless streams and measured a resultant 20% reduction in *Baetis* B body size at emergence, an effect size nearly identical to the 17% reduction in secondary production we measured in our field experiment. The congruence of our results with those previous studies lends

strong support to the hypothesis that trout chemical cues reduced *Baetis* secondary production via the same mechanisms demonstrated in other nearby streams.

We suspect that the observed difference in $median_b$ cohort production values in our experiment may be biologically significant, although the 90% CI_b of cohort production in the treatment and reference reaches overlapped. Secondary production of aquatic invertebrates is difficult to measure with high precision. Our estimates of cohort production had coefficients of variation (CV) of 12 to 14% and are consistent with most estimates of secondary production, which have CVs no smaller than 10% (Huryn 1996). The difference in $median_b$ cohort production of 30 mg AFDM m^{-2} $cohort^{-1}$ between the treatment and reference reaches represented 17% of *Baetis* B production in the reference reach, about half the 30% decline predicted by an independently-calibrated model of *Baetis* B secondary production (Koch 2011).

Baetis mayflies are dominant grazers in Rock Creek (Peckarsky et al. 2001) and in many high-elevation streams throughout western North America, and the loss of $^1/_5$ of the production of this taxon could have energetic consequences for stream food webs. For example, relative to fishless streams, a decrease in *Baetis* production due to the presence of trout may facilitate a greater flux of primary production into competing grazer populations (e.g., Heptageniidae), which show no evidence of exhibiting strong nonconsumptive effects from trout (McIntosh et al. 2002; BLP, unpublished data). Indeed, *Baetis* larvae are particularly-strong interactors in East River drainage-basin stream food webs (McIntosh et al. 2004, Álvarez and Peckarsky 2005) and can be considered foundation species because their abundance and functional attributes modulate food-web structure and ecosystem processes (Ellison et al. 2005). Nonconsumptive effects of trout on *Baetis* may, therefore, have large rippling effects through the rest of the stream food web.

The difference in *Baetis* secondary production between the reference and treatment reaches can be used to estimate the strength of the nonconsumptive interaction between trout and mayflies. Wootton (1997) defined the top-down consumptive species impact of a predator on prey as the number (or biomass) of prey consumed by the predator population per unit time divided by the total number (or biomass) of prey. Similarly, the biomass lost per day due to the nonconsumptive effect of a predator divided by the standing stock of prey biomass represents an analogous estimate of the nonconsumptive species impact of a predator on a prey population. $Median_b$ standing stock biomass of *Baetis* B in the reference reach of our experiment was 52 mg AFDM/m^2 (90% CI_b: 44–61 mg AFDM/m^2), and the $median_b$ cohort duration was 50 d (90% CI_b: 47–52 d); thus, the estimated nonconsumptive species impact of Brook Trout on *Baetis* B mayfly larvae was roughly (30 mg AFDM m^{-2} 50 d^{-1} / 52 mg AFDM m^{-2}) = 0.01 d^{-1}. Consequently, our results suggest that *Baetis* lose 1% of their biomass each day to nonconsumptive effects of Brook Trout. We can also consider alternative methods of estimating species impact. For example, the magnitude of the reduction of *Baetis* production in the reach with added fish cues was 17% of *fishless* production in the reference reach, indicating that over the period of the *Baetis* cohort, the threat of predation alone can reduce mayfly production appreciably. Estimating the strength of nonconsumptive interactions in these ways offers a common currency for incorporating nonconsumptive interactions into food-web models based only on consumptive interactions.

The nonconsumptive species impact of trout on *Baetis* B biomass of 1% per day is similar in magnitude to the estimated consumptive effect derived from previously-published data from streams in the upper East River catchment. Across 11 streams, Peckarsky et al. (2008b) found that the mean predation rate of trout on *Baetis* B larvae was 1.1 individuals m^{-2} d^{-1}. Working in nearby Cement Creek, Allan (1978) measured median head-capsule widths of *Baetis* larvae consumed by trout during daytime (median: 0.83 mm) and at night (median: 0.75 mm). Using the allometric equation in Table S1 to convert head-capsule width to AFDM yields an estimate of overall average body mass of 0.32 mg AFDM/individual for *Baetis* mayfly larvae consumed by trout. When scaled by the standing stock biomass of *Baetis* B in the reference reach of our experiment, the consumptive species impact of Brook Trout on *Baetis* B mayfly larvae is, therefore, ~0.007/d (i.e., 1.1 individuals m^{-2} d^{-1} × 0.32 mg AFDM $individual^{-1}$ / 52 mg AFDM m^{-2}). The concordance of estimated consumptive and nonconsumptive species impacts suggests that the nonconsumptive pathways by which Brook Trout can alter prey secondary production merit consideration in models of interspecific interactions.

In conclusion, our experiment showed that the nonconsumptive effects of trout predators can reduce the organic matter flux through a population of a foundation species of mayfly prey by altering the development patterns of individuals. Measuring the cost of nonconsumptive effects at the population level and placing them in a currency common to that of consumptive effects enables assessing their strength relative to other food-web links and provides a basis for evaluating the ecological consequences of these widespread predator–prey interactions.

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