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Large-scale manipulation of mayfly recruitment affects population size

Andrea C. Encalada · Barbara L. Peckarsky

Abstract Recruitment establishes the initial size of populations and may influence subsequent population dynamics. Although strong inference can be made from empirical relationships between recruitment and population sizes, a definitive test of recruitment limitation requires manipulating recruitment at relevant spatial and temporal scales. We manipulated oviposition of the mayfly *Baetis bicaudatus* in multiple streams and measured the abundance of late-stage larvae at the end of the cohort. Based on fundamental knowledge of mayfly behavior, we increased, eliminated, or left unmodified preferred mayfly oviposition sites in 45-m reaches of streams ($N = 4$) of one high-altitude drainage basin in western Colorado, USA. We compared egg densities before (2001) and after the manipulation (2002) using paired t tests and compared larval densities before and after the manipulation among treatments using repeated measures analysis of variance. This manipulation altered not only egg densities, but also larval abundances 1 year later. Compared to the previous year, we experimentally increased egg densities at the addition sites by approximately fourfold, reduced egg densities to zero in the subtraction sites, and maintained egg densities in the control sites. After the manipulation, larval densities increased significantly by a factor of approximately 2.0 in the addition sites and decreased by a factor of approximately 2.5 in the subtraction sites. This outcome demonstrates that dramatic changes in recruitment can limit larval population size at the scale of a stream reach, potentially masking previously observed post-recruitment processes explaining the patterns of variation in abundance of a stream insect. Furthermore, our results emphasize the importance of preferred oviposition habitats to population sizes of organisms.

Keywords Ephemeroptera · Large-scale experiment · Life histories · Oviposition behavior · Recruitment limitation · Streams

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Introduction

The contribution of recruitment to the population dynamics of organisms is often overlooked, but in systems where it has been studied, recruitment may have a substantial influence on the size of animal populations (Doherty 1983; Caley et al. 1996). Furthermore, inputs of new individuals are especially important to populations in open systems (Palmer et al. 1996) where immigration may swamp the effects of biological interactions, such as predation (Cooper et al. 1990) or competition (Kohler 1992). Several studies have reported patterns of variability in abundance consistent

with recruitment limitation in populations living in marine intertidal habitats (Connell 1985; Underwood and Fairweather 1989; Hughes 1990). Despite similarities between the complex life histories of stream benthos and marine benthic organisms (Palmer et al. 1996; Downes and Keough 1998), less is known about the influence of recruitment by oviposition on population sizes of stream insects (but see Bunn and Hughes 1997; Peckarsky et al. 2000; Hildrew et al. 2004; Reich and Downes 2004; Lancaster et al. 2010). Here, we define "recruitment" as the initial establishment of new individuals to populations (by oviposition or birth), and treat the subsequent addition of dispersing larvae (drift) as a post-recruitment process.

Although hypotheses can be generated from empirical relationships between recruitment and population sizes of organisms (Underwood and Fairweather 1989; Pfister 1996), a definitive test of recruitment limitation requires manipulating recruitment at relevant spatial and temporal scales (Lancaster et al. 2010) and measuring the subsequent responses of populations (Wilson and Osenberg 2002). While large-scale field experiments are challenging and vulnerable to uncontrolled variation (Resetarits and Bernardo 1998), the successful implementation of such experiments can result in substantial progress being made in our understanding of the underlying processes influencing population dynamics of organisms (e.g., Jones 1990). Well-designed experiments carried out at realistic scales also provide definitive tests of hypotheses suggested by field surveys (Encalada and Peckarsky 2011) and models (Lancaster et al. 2010).

Previous studies of recruitment limitation in stream invertebrates have inferred process from the pattern by relating the abundance of eggs to the abundance of larvae (Reich and Downes 2004; Lancaster et al. 2010; Encalada and Peckarsky 2011). While many small-scale experiments have been published testing mechanisms affecting the population dynamics of stream invertebrates (e.g., Kohler and McPeek 1989; Fonseca and Hart 2001), innovative large-scale experiments are rare, generally restricted to less than one generation (but see Hildrew et al. 2004), and carried out in a single stream (Allan 1982; Nakano 1999) or in an unreplicated treatment and reference streams (Wallace et al. 1997; Taylor et al. 2002; Hildrew et al. 2004). Replicated large-scale experimental studies in streams are difficult (Peckarsky et al. 2002), but this approach is well worth the effort considering the advances that can be made by such experiments by providing robust approaches to testing ecological questions. The goal of this study was to manipulate the initial recruitment of individuals (oviposition) of one species of insect in multiple streams during 1 year, and then to measure the abundance of late-stage larvae at the end of the same cohort (second year), which would provide an estimate of the population size that will contribute offspring to the next generation.

Larvae of the mayfly *Baetis bicaudatus* are abundant in high-altitude streams of western Colorado, USA (Peckarsky et al. 2001). Females exhibit a specialized oviposition behavior that is unusual for mayflies (Brittain 1982) and makes manipulation of recruitment feasible for this species. As in other species of the genus *Baetis*, each female attaches one egg mass onto the wetted underside of a rock protruding from the water surface (Eaton 1888; Peckarsky et al. 2000). Female *B. bicaudatus* selectively oviposit under large rocks located in fast current velocity locations (Encalada and Peckarsky 2006). A low availability of optimal oviposition sites can force winged adult females to disperse (fly) to find suitable oviposition habitats (Peckarsky et al. 2000), which could result in high rates of female mortality before oviposition (Encalada and Peckarsky 2007) and affect recruitment limitation at regional scales. Previous studies of the empirical relationship between densities of eggs (initial recruits) and subsequent densities of larvae suggest the potential for recruitment limitation in *B. bicaudatus* population sizes in some streams and in some life stages (Encalada and Peckarsky 2011).

This objectives of this study were twofold: (1) to alter experimentally the preferred oviposition habitat of females of *B. bicaudatus* in large sections of multiple streams and then to quantify oviposition in order to determine whether egg densities were modified; (2) to estimate the abundance of late-instar larvae before and after the manipulation to test whether the effect of recruitment limitation altered larval population size at the end of the cohort.

Materials and methods

Study sites

The 12 experimental streams were located at approximately 2,900 m a.s.l. in the upper East River drainage basin near the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, CO, USA (38°57′30″N, 106°59′15″W). Some streams in this drainage basin originate from snowmelt, and others are lake outlets or of groundwater origin. All study streams were highly oxygenated, had relatively low levels of dissolved ions (Peckarsky et al. 2001), and mineral substrates were dominated by boulders, cobbles, and pebbles with mixed geology (Wilcox et al. 2008). Streams included in this study varied in a number of physical attributes, including size (drainage basin area, stream width, stream discharge) and water temperature (Table 1). Water temperature fluctuated daily and seasonally in all streams, but the average temperature during the time period when oviposition occurs varied among streams (5.5–9.5°C). Brook trout (*Salvelinus fontinalis* Mitchell) were the only fish present in some streams, while others were

Table 1 Environmental variables of 12 experimental stream reaches measured during the summer of 2003 in the East River drainage basin near Gothic, Colorado, USA

Streams[a]	Treatment[a]	Drainage basin area[b] (km²)	Mean discharge (m³ s⁻¹)	Mean stream width (m)	Mean stream temperature (°C)	Mean fish density[c] (ind. m⁻²)
Avery	A	1.8	0.09	1.7	10.70	0.658
B10	A	0.11	0.007	0.5	7.22	0
Benthette	A	0.34	0.09	1.5	8.43	0
Copper	A	24.3	1.6	6.2	7.33	0.018
	Average	6.64	0.45	2.48	8.42	0.169
	SE	5.90	0.38	1.27	0.81	0.16
B9	C	0.11	0.004	0.6	6.27	0
Bradley	C	3.8	0.28	1.1	5.95	0
Lower rock	C	3.6	0.22	2.3	9.21	0.619
Rustlers	C	15.1	1.3	6.8	7.03	0.145
	Average	5.65	0.45	2.70	7.12	0.191
	SE	3.26	0.29	1.41	0.73	0.15
B2	S	0.05	0.016	0.4	5.47	0
East	S	45.9	2.6	10.1	9.69	0.176
Marmot	S	0.93	0.05	1.0	6.13	0
Quigley	S	2.1	0.22	1.4	6.83	0.007
	Average	12.25	0.72	3.23	7.03	0.04575
	SE	11.23	0.63	2.30	0.93	0.04

Differences among treatments of interdependent physical variables were analyzed with a multivariate analysis of variance (MANOVA)

[a] Different streams were allocated to three alternative oviposition site manipulation treatments: addition (A), control (C), and subtraction (S) sites

[b] Data on drainage basin areas are from Wilcox et al. (2008)

[c] Brook trout (*Salvelinus fontinalis*) densities [mean ± 1 standard error (SE)] of 3 years: 2001–2003 data are provided by B.L. Peckarsky (unpublished)

completely fishless due to natural dispersal barriers (waterfalls). Brook trout densities also varied among the study streams during the 3 years of this study (Table 1).

Study organism

Larvae of *Baetis bicaudatus* Dodds (Ephemeroptera: Baetidae) are highly efficient grazers of attached algae in high-elevation Rocky Mountain streams (Álvarez and Peckarsky 2005). They are ubiquitous in streams of the drainage basin, are generally among the most abundant species, and their biology and ecology have been studied extensively near RMBL (e.g., Peckarsky et al. 2001). Larvae of these mayflies are highly mobile and prefer to graze medium-sized cobble substrates in moderate- to high-velocity locations. Larvae often drift in the water column (McIntosh et al. 2002) and, consequently, their populations constitute an important food source for salmonid fishes (Allan 1981). Populations at this location are univoltine, emergence is somewhat synchronous, occurring during the summer months (June–August) (Peckarsky et al. 2000), and short-lived non-feeding females generally disperse upstream before ovipositing (Flecker and Allan 1988; Vance and Peckarsky 1997). Each female lays one egg mass during her lifetime, with fecundity ranging over an order of magnitude (approx. 200–3,000 eggs per female), depending on the size of the female (Peckarsky et al. 1993). Eggs hatch in 14 days, larvae overwinter as first instars and advance through later developmental stages starting in April and completing larval development the next summer (Peckarsky et al. 2000; Encalada and Peckarsky 2011).

Experimental design

To test the effect of variation in recruitment by oviposition on subsequent larval densities of one cohort of *B. bicaudatus*, in the summer of 2002 we experimentally increased the availability of oviposition substrates ("addition sites"), eliminated oviposition substrates ("subtraction sites"), or did not modify oviposition sites ("control sites") in 45-m reaches of four different streams for each treatment. This stream length was the largest spatial scale we could manage and all that was available in the smaller streams. Previous sampling of these reaches also suggested that within-reach dynamics of *Baetis* populations were relatively homogeneous, and that relevant population measurements could be

performed (as suggested by personal communication with G. Englund).

To test the effect of the manipulation on recruitment, we censused oviposition (egg densities) in all 12 reaches during the summer of 2002 (during the manipulation) and compared those levels to egg densities measured in the same stream reaches during the summer of 2001, i.e., 1 year before the manipulation. We then compared densities of late-instar larvae of the manipulated cohort in June 2003 to those measured in the same stream reaches in June 2002 before the manipulation. If initial egg densities limited subsequent larval densities, we expected larvae to be more abundant in 2003 than in 2002 in the addition streams, less abundant in the subtraction streams, and to vary unsystematically between years in control streams. The experiment took 3 years to accomplish and followed a replicated modified BACI (before–after, control-impact) design (Underwood 1991). Therefore, in this experiment, we analyzed the change in mayfly densities from before to after the manipulation across all treatments (see below for more details on the statistical analysis).

Manipulation of oviposition sites

A stratified random method was applied to allocate treatments to the 12 streams, taking into account the natural variation among streams in several physical and biological characteristics (Table 1). To avoid systematic variation in *Baetis* biology that could be associated with physical attributes in streams, we replicated each treatment in a representative mixture of stream types, assigning streams to treatments based on stream size, presence/absence of brook trout and *B. bicaudatus* egg, and larval densities measured in previous years (Encalada and Peckarsky 2011). The densities of brook trout are variable among the experimental stream reaches and among years within streams (B.L. Peckarsky, unpublished data); during the years of the experiment the fish densities ranged from zero to 0.658 m^{-2} in the study reaches (Table 1). Each treatment included similar numbers of small and large streams, fishless and fish streams, streams with few or many preferred oviposition substrates, and streams with previously low and high *Baetis* densities (Peckarsky et al. 2001). A multiple analysis of variance (MANOVA) indicated that streams allocated to different treatments did not differ significantly in other attributes (Table 1) that could have confounded effects of the manipulation on *Baetis* populations (Wilks' lambda = 0.32, $F_{(8,12)} = 1.16$, $P = 0.396$). However, unexpectedly, streams allocated to the addition treatment had lower egg densities the year before the manipulation than did the subtraction and control streams, an effect that we did not predict from previous estimates of egg densities (Encalada 2005).

In the addition sites, we attempted to increase recruitment by experimentally increasing the availability of preferred oviposition substrates for females of *B. bicaudatus*, based on previous experimental evidence (Encalada and Peckarsky 2006). We increased oviposition substrates by approximately 50% by reorienting large rocks (>500 cm^2) already present in each addition reach to protrude from the water surface in fast current velocity locations (>50 cm^{-1}). In the subtraction sites, we submerged all protruding rocks to prevent females from ovipositing in those stream reaches. Since stream water levels recede throughout the oviposition period, during weekly visits to the experimental sites we submerged all newly protruding rocks in the subtraction sites. We used those methods rather than adding or removing rocks from stream reaches to avoid altering the abundance of substrates used by larvae for the habitat. In the control sites, we left all potential oviposition sites unmodified to allow natural levels of oviposition by females. However, all sites received a similar "disturbance" since we moved all protruding rocks to count the oviposited egg masses during weekly visits to each stream. We began the manipulation on 6 June 2002 and finished the last week of July, which was the end of the oviposition period of *B. bicaudatus* during the summer of 2002.

Estimates of recruitment

During weekly visits to each stream, we also conducted censuses of egg masses oviposited on each rock in the addition and control streams. We used a soft-bristled toothbrush to remove all egg masses found on newly protruding rocks in the subtraction streams and released eggs downstream of the experimental reach, thereby maintaining recruitment in those streams at zero. We estimated the cumulative number of egg masses in each addition and control stream for the entire cohort by adjusting weekly counts of egg masses by egg development time (Hairston et al. 1987) (14 days), egg desiccation (measured directly), and hatching success, which is 97% under field conditions (Peckarsky et al. 2000). We then divided numbers of egg masses (=females ovipositing) by the area of each reach to estimate egg masses per square meter.

Because it was not possible to count individual *Baetis* eggs (ranging from approx. 200 to 3,000 eggs per egg mass), we estimated the total number of eggs from the number of egg masses censused in each of the 12 experimental reaches by incorporating information from female size–fecundity regressions (Peckarsky et al. 1993). First, since *Baetis* fecundity depends on female size, we measured the head capsule width (HCW) of 36 final-stage females with black wing-pads (BWP: a 24-h non-feeding larval stage immediately preceding metamorphosis into adults) collected at random over the summer of 2002 at

locations throughout the drainage basin and predicted their dry weight (DW) based on the equation: female DW = $1.5304 \times HCW^{3.9816}$ ($r^2 = 0.90$) (Peckarsky et al. 2001). We then predicted the number of eggs (f) per BWP female based on the relationship between female DW and fecundity using the equation: $f = 413.0 \times (BWP larval DW)^{1.89}$, ($r^2 = 0.89$) (Peckarsky et al. 1993). From separate regressions for females collected each week of the experiment, we drew a random residual value from the observed distribution of residuals, which were normally distributed with a mean of zero. Using a randomization procedure, with 1,000 iterations (SAS Institute 2003), we assigned to each egg mass a mean number of eggs (and corresponding variance) that would be produced by females collected during the week of that recruitment survey and used these estimates of eggs per egg mass to calculate the total density of eggs per square meter of stream area for each site. We mapped the distributions of egg masses on the undersides of all rocks protruding from streams weekly to distinguish new egg masses and compiled a cumulative estimate of the total egg densities oviposited over the entire flight period in each stream to establish the initial population sizes of *Baetis*.

Estimates of larval densities

To test whether manipulated egg densities (summer 2002) affected subsequent densities of larvae of this cohort, we collected benthic samples from all 12 stream reaches in June 2003. An electroshocker was used to collect six benthic invertebrate samples (Taylor et al. 2001) at random locations of each 45-m length of stream reach by applying 90 s of electric current inside a modified Hess sampler placed on the stream bottom. This method sampled a 0.09-m² area of stream bottom, and organisms were collected in a Wilco 30×25 cm drift net with a 202-μm mesh attached to the downstream end of the sampler. This collection method is highly effective for collecting mobile organisms like *Baetis* (Taylor et al. 2001). Invertebrate samples were preserved in 90% ethanol with rose bengal dye added to help distinguish mayflies from detritus. We estimated larval density per square meter by dividing the number of larvae per sample by the area of the sampler. We used the same methods to estimate larval densities in June 2002 before the manipulation.

Data analyses

We compared estimates of egg densities in the test reaches after the manipulation (2002) to estimates of natural egg densities obtained in 2001 within the same stream reaches. To test for significant changes in recruitment in experimental reaches, we used a paired t test to compare egg densities before (2001) and after the manipulation (2002) for the addition and controls sites only. Subtraction sites were not analyzed statistically because initial recruitment by ovipositing females was effectively zero in 2002.

Similarly, we compared larval densities estimated in June 2002 before the manipulation to larval densities after the manipulation, estimated in June 2003. In this case, larvae densities were log transformed to meet the normality assumptions. To test the effects of the recruitment manipulation on larval abundance, we performed repeated measures ANOVA (Proc GLM; SAS Institute 2003) where the response variable was density of larvae and the repeated measures were the years (before and after the manipulation in each stream). This analysis takes into account the interdependence between the repeated larval-density estimates taken in each stream reach over time (year) and was performed on the means of the six invertebrate samples taken in each stream/year ($n = 4$ stream reaches for each treatment). Hence, our analysis follows a modified BACI design in which we analyzed the change in larvae densities from before to after the manipulation across all treatments—addition, control, and subtraction—with a particular focus on the interaction terms between treatments and years.

Specifically, we tested whether larval density differed between years depending on treatment, which would be indicated by a significant year × treatment interaction. We performed post-hoc univariate tests of significance for planned comparisons on the contrasts between years within treatments, after meeting the assumptions for the Mauchley sphericity test.

Results

Effects of the manipulation on recruitment by oviposition

By manipulating the preferred oviposition substrates of *B. bicaudatus* females in experimental stream reaches during 2002, we increased average egg densities per stream at the addition sites by fourfold compared to the same streams during the previous year (2001) (paired t test: $t_3 = 3.6$, $P = 0.037$) and reduced egg densities to zero in the subtraction sites (Fig. 1). Egg densities were not significantly different in the control reaches between 2001 and 2002 (paired t test: $t_2 = 0.88$, $P = 0.472$). Therefore, by modifying oviposition substrates within experimental stream reaches we were able to successfully alter mayfly recruitment and thus could test the effects of the treatments on the change in the sizes of *Baetis* populations from before to after the manipulation the next year at the end of the larval cohort.

Effects of the manipulation on larval densities

The repeated measures ANOVA indicated that differences in the densities of larvae per stream between the year before
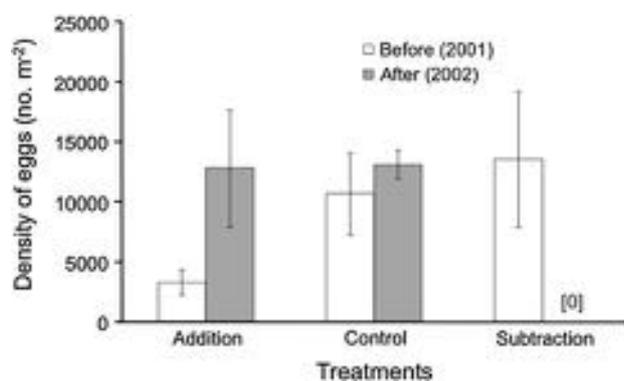
Fig. 1 Density of *Baetis bicaudatus* eggs [mean no. eggs m^{-2} ± standard error (SE)] before (2001) and after (2002) oviposition site treatments in the upper East River drainage basin near the Rocky Mountain Biological Laboratory (RMBL) at Gothic, Colorado, USA ($n = 4$, 45-m stream reaches per treatment). Preferred oviposition substrates were added to streams (*Addition*), not modified (*Control*), or removed (*Subtraction*). In the subtraction treatment, egg densities were reduced to zero by the removal of all oviposition substrates. Egg densities were analyzed by paired *t* tests, with the treatments as factors and observations paired within streams between years (see methods for SE calculation)

the recruitment manipulation and the year after the recruitment manipulation reached significance depending on the treatment (significant year × treatment interaction: $F_{2,9} = 6.39$, $P = 0.019$). Contrast estimates for planned comparisons revealed that from 2002 to 2003 larval densities doubled in the addition sites and decreased by a factor of approx. 2.5 in the subtraction sites after the manipulation (contrast M1: $F_{1,9} = 35.06$, $P = 0.00023$) (Fig. 2). No significant differences in larvae densities between years were found in the control sites (Fig. 2).

It is remarkable that despite the considerable variation we intentionally included when allocating streams to treatments (Table 1), the overall effects of the manipulation were significant, as illustrated by consistent effects within treatments for 11 of the 12 study streams (Fig. 2), including both fish and fishless streams (Fig. 2). However, adding oviposition substrates in 2002 to one fish stream (Copper Creek) neither increased the egg nor the larval densities from 2002 to 2003. Copper Creek was the largest addition stream, and our results suggest that the manipulation of oviposition substrates was not effective at that site.

Discussion

Based on fundamental knowledge of *B. bicaudatus* oviposition behavior, we were able to successfully modify the density of recruits (eggs) at multiple experimental stream reaches by altering the availability of preferred oviposition substrates. Notably, despite the previously reported existence of post-recruitment density-dependent losses of
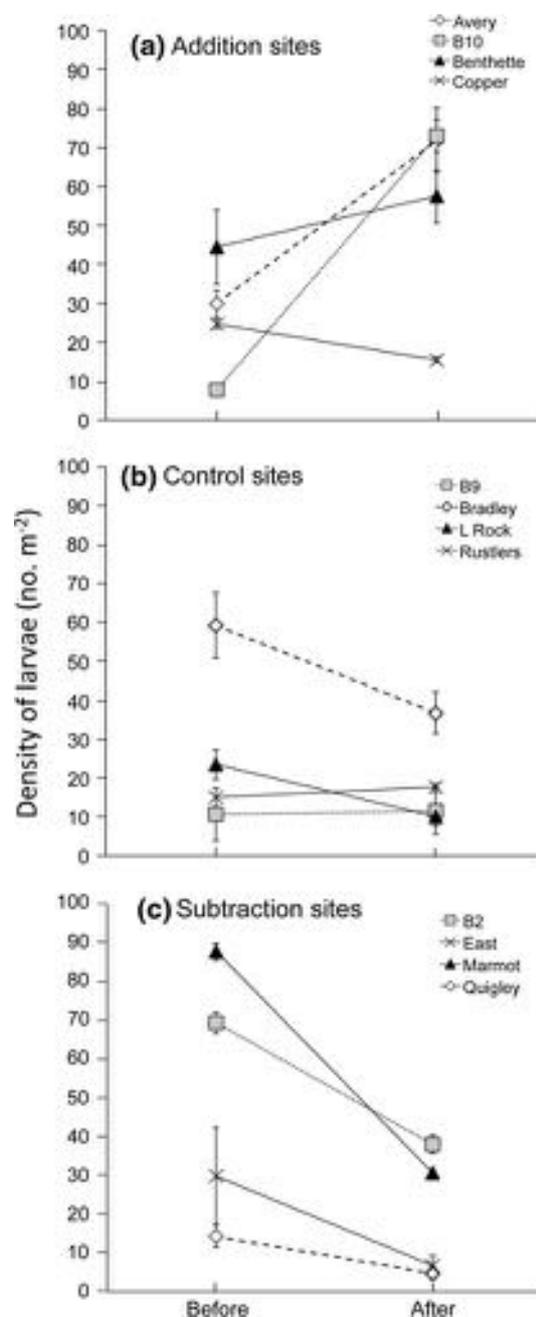
Fig. 2 Density of *B. bicaudatus* late-instar larvae per stream before (June 2002) and after (June 2003) the recruitment manipulation in the addition, control, and subtraction treatments (mean no. m^{-2} ± SE; $n = 6$ benthic samples per stream site). Sampled streams are listed in each panel and are identified as either fishless (*open symbols*, *dashed lines*) or with fish (*solid symbols and lines*). *Lines* are included to emphasize the direction of the change in densities between years within each stream. Larval densities were compared among treatments between years using repeated measures analysis of variance (ANOVA)

larvae (Encalada and Peckarsky 2011), their densities remained altered 1 year after the manipulation. This replicated experiment carried out at a large spatial and temporal scale provides evidence that recruitment limitation can affect population size.

Remarkably, modifying the availability of oviposition sites not only affected the recruitment of *B. bicaudatus* at the scale of 45-m-long stream reaches, but also altered the population sizes of the late-stage larvae 1 year after the manipulation. Compared to the 2001–2002 cohort (before the manipulation), the 2002–2003 cohort achieved higher population sizes of late instars in stream reaches with added oviposition substrates, and lower population sizes in reaches where oviposition sites were removed. Average population sizes of *Baetis* in control reaches varied unsystematically between years. This outcome is consistent with the hypothesis that recruitment can limit larval abundance at the scale of a stream reach. Higher population sizes of the manipulated cohort were achieved by increasing initial egg densities in the addition treatment. However, in subtraction sites with zero oviposition, low larval densities in 2003 must have been attained by immigration of larvae from adjacent stream reaches.

Recruitment limitation has also been demonstrated by other experiments carried out in marine (Doherty 1983; Hughes 1990) and terrestrial systems (Connell and Green 2000; Cole and Wiernasz 2002). Our findings are consistent with "habitat determination" of recruitment, instead of "supply determination" (sensu Schmitt and Holbrook 2000), since the supply of adult females is not limited, but rather there is a habitat constraint (availability of preferred oviposition habitat), which we successfully modified. In contrast to the outcome of our manipulation, the effects of recruitment limitation observed in early stages of marine fish populations are often overridden by density-dependent post-recruitment processes (Jones 1990; Forrester 1995; Pfister 1996). Likewise, in a detailed study of the 2001–2002 cohort of *B. bicaudatus* in unmanipulated streams, we showed natural variation in recruitment-limited population sizes of only small-instar larvae and in only fishless streams, which had lower egg densities than fish streams (Encalada and Peckarsky 2011). In that study, the effects of recruitment on population sizes were eventually overridden by post-recruitment negative density-dependent processes operating on late instars in fishless streams, and there was no evidence of recruitment limitation in the population sizes of any larval stages in natural fish streams (Encalada and Peckarsky 2011), suggesting that density dependence is an especially strong regulatory process in those streams.

In contrast, the manipulation of egg density in this study was strong enough to sustain an effect on *Baetis* population sizes all the way to the end of the 2002–2003 cohort (Fig. 2), even in fish streams that exhibited stronger negative density dependence when unmanipulated (Encalada and Peckarsky 2011). We considered several potential explanations for this apparent contradiction between the results of the survey and this experiment. First, the survey was not a definitive test of the role of density dependence;

second, the strength of different processes could vary among years (between 2001–2003). Third, although egg densities increased fourfold and larval densities doubled after the manipulation at the addition sites, larval abundance at the addition sites was within the range of variation observed at the control sites; therefore, it is plausible that larval densities never reached a level at which density-dependent processes occur. However, we believe the most parsimonious explanation for the differences between the survey and the experiment is that our manipulation of oviposition was powerful enough to mask density-dependent post-recruitment processes that were nonetheless occurring in these streams, thereby inducing more persistent effects of recruitment limitation than we observed in unmanipulated streams. We suggest that our survey data provide good evidence that both recruitment and post-recruitment processes operate in this system and are not mutually exclusive (Encalada and Peckarsky 2011), as has been demonstrated in other systems (Caley et al. 1996; Hixon et al. 2002). The precise mechanisms involved in post-recruitment processes are complex, but previous studies suggest that both consumptive and, more importantly, non-consumptive effects of fish on behavior and life histories influence the population dynamics of *B. bicaudatus* (Peckarsky et al. 2001; Peckarsky et al. 2008). Nonetheless, exaggerating recruitment variation experimentally changed the population sizes of late instars within fish and fishless streams from those of the previous cohorts, despite the existence of these density-dependent processes.

In another experimental manipulation of recruitment of megalopteran eggs in one stream in the UK (Hildrew et al. 2004), initial responses of larval densities to treatments (addition, removal, and control) were transient, and strong density-dependent survival stabilized populations in all treatments at similar levels within 4–5 months. The difference between our study and that of Hildrew et al. (2004) may reflect the increased power of testing effects of recruitment limitation in multiple streams rather than in sections of one stream, or may be due to inherent differences in the development and population dynamics of Megaloptera, which are predatory holometabolous insects, and Ephemeroptera, which are herbivorous hemimetabolous insects.

As a consequence of the removal of oviposition sites, densities of *B.* larvae were reduced in all of the subtraction reaches between 2002 and 2003. Interestingly, the only mechanism available for the colonization of new individuals in these reaches was immigration of larvae from adjacent reaches, most likely by downstream dispersal in the water column (drift), which did not compensate completely for the loss of oviposition as a colonization pathway. In mayflies and other stream invertebrates, downstream drift is a ubiquitous process and has been widely studied (Allan and Russek 1985). Mayflies drift to find food or avoid

competition and predation (Kohler and McPeek 1989; McIntosh et al. 2002); however, density-dependent drift has rarely being shown (but see Dimond 1967). In fact, some have argued that drift is a density-independent process (Humphries 2002).

Drift density into and out of the stream reaches manipulated for this study have been previously reported in detail in Wilcox et al. (2008, see Table II). Here, we elaborate on these data as related to the potential that recruitment by drift could have contributed to the observed differences among treatments. First, while the presence of any mayfly individuals can be attributed to drift into the subtraction reaches, emigration from upstream of the test reaches was not density-dependent; i.e., the probability that a *Baetis* individual would drift (estimated as drift propensity = drift into the study reach as a ratio of the benthic density of the stream reach immediately upstream) did not depend on the density of the upstream source population (Wilcox et al. 2008). Furthermore, drift propensity from upstream reaches into the study reaches did not differ among treatments (addition = 0.028 ± 0.024, control = 0.027 ± 0.018, and subtraction sites = 0.038 ± 0.032; data from Wilcox et al. 2008). Given this observation, it is perhaps less surprising that emigration did not overwhelm the effects of the addition treatment, which has further implications for the potential of the site fidelity of these relatively mobile mayflies.

Data on immigration and emigration out of the experimental reaches have also been previously published in Wilcox et al. 2008 (see Fig. 3), demonstrating a positive correlation between benthic density and the "immigration ratio", which is the ratio of drift into and the drift out of each reach. Reaches with high drift ratios (>1) gain individuals by immigration, and reaches with low drift ratios (<1) lose individuals to emigration. If drift were density dependent, benthic density and immigration ratio would be negatively correlated, indicating more emigration, and thus lower ratios from stream reaches with higher benthic density. Further analysis of the drift ratios of the study reaches shows that addition streams had high drift ratios and were thus gaining individuals by immigration, and that subtraction reaches had low drift ratios (<1), thereby losing individuals. Standard error bars of the drift ratios of control streams suggest that they were not different from one another, and thereby neither gained nor lost individuals on average (Fig. 3). Although drift ratios were not significantly different among treatments ($F_{(2,9)} = 1.75$, $P = 0.227$), the observed patterns are consistent with the hypothesis that recruitment of drifting larvae could have contributed to the observed effects of the manipulation. However, more firm conclusions on the influence of drift on observed changes in larval densities would have required comparison to pre-manipulation immigration ratios, which were not measured.
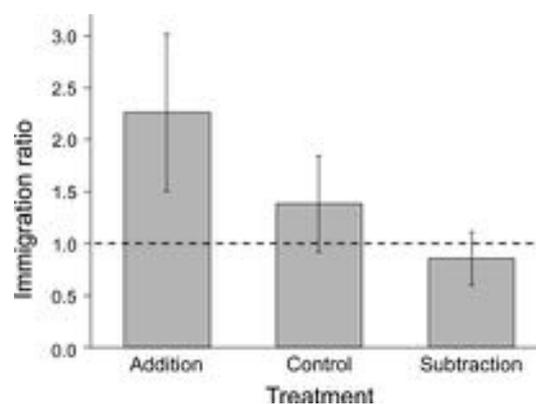
Fig. 3 *Baetis bicaudatus* immigration ratio (drift density in: drift density out; mean $\pm$ SE; $n = 4$) in stream reaches for the addition, control, and subtraction treatments. Stream reaches with immigration ratios >1 gain individuals, whereas those with ratios <1 (*dotted line*) lose individuals. Immigration ratios were not significantly different among treatments ($F_{2,9} = 1.75$, $P = 0.227$) when compared using one-way ANOVA. This analysis is implemented on data reported by Wilcox et al. (2008)

In summary, the result of this study demonstrate experimentally that manipulating the availability of preferred oviposition substrates of females caused a local limitation in the population sizes of *B. bicaudatus* at the scale of stream reaches. Similar cases of recruitment limitation may be linked to environmental or habitat constraints, as has been observed in other aquatic insects (Fonseca and Hart 2001; Spencer et al. 2002), marine fishes (Forrester 1995; Holbrook et al. 2000; Schmitt and Holbrook 2000), and terrestrial plant and animal systems (Connell and Green 2000; Cole and Wiernasz 2002). Uniquely, it is noteworthy that significant changes in *Baetis* population sizes resulted without directly manipulating egg densities (as done by Hildrew et al. 2004), but by exploiting the knowledge of a particular behavior to test the influence of recruitment. Oviposition behavior has been considered to be an adaptive trait that generates fundamental patterns of population dynamics in other insects (Price and Ohgushi 1995). Thus, the specialized oviposition behavior of *Baetis* resulted in females responding to the manipulation of available oviposition sites, which contributed to the subsequent variation in population size.

Finally, this observed response to manipulation of the physical habitat has important implications for the sustainability of *Baetis* populations in these and other streams throughout the world. Given the sensitivity of population size to the availability of preferred oviposition substrates, recruitment limitation in this mayfly could be exacerbated by disruption of the natural hydrologic regimes of streams (Poff et al. 1996). Human influences on natural seasonal water level fluctuations, such as those caused by water abstractions and hydro-peaking dams, have the potential to disrupt the recruitment process of this important species.

Furthermore, increases in extreme hydrologic events, such as droughts and floods, associated with climate change (McKnight 2001) can affect the timing of the availability of rocks protruding out of streams that are used by these mayflies for oviposition, which could critically alter the ability of *Baetis* populations to recruit new individuals.

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