Incorporating invertebrate predators into theory regarding the timing of invertebrate drift

**Bruce G. Hammock · Nickilou Y. Krigbaum ·
Michael L. Johnson**

Abstract Theory concerning the timing of lotic invertebrate drift suggests that daytime-feeding fish cause invertebrates to restrict their drift behavior to the nighttime. However, there is growing evidence that the nighttime foraging of invertebrate predators also contributes to the nocturnal timing of drift, though it is unclear whether the nocturnal behavior of invertebrate predators is innate or proximately caused by fish. In two experiments, one conducted in a fish-bearing stream and a second in a fishless stream, we compared the drift patterns of Baetidae (Ephemeroptera) from channels with and without benthic invertebrate predators. We tested whether invertebrate predators affect the timing of drift, either as a proximate cause of nocturnal drift in the fishless stream (diel periodicity) or as a proximate cause of a pre-dawn peak in drift in the fish-bearing stream (nocturnal periodicity). In the fish-bearing stream experiment, a pre-dawn increase of baetid drift occurred independently of invertebrate predators, indicating that invertebrate predators were not the proximate cause of nocturnal periodicity in the stream. In the fishless stream experiment, invertebrate predators caused more baetid drift at night than during

B. G. Hammock (✉) · N. Y. Krigbaum · M. L. Johnson
Center for Watershed Sciences, John Muir Institute
of the Environment, University of California, Davis,
One Shields Avenue, Davis, CA 95616, USA
e-mail: brucehammock@yahoo.com

the day, indicating that invertebrate predators caused the nocturnal drift pattern we observed in the stream, and that invertebrate predators can influence drift timing independently of fish. Therefore, we suggest that both visually feeding fish and nocturnally foraging benthic predators, when present, affect the timing of invertebrate drift; visually feeding fish by reducing daytime drift, and benthic predators by increasing nighttime drift.

Introduction

Invertebrate drift, defined as the downstream movement of benthic macro-invertebrates via the water column, is an ecosystem function of lotic systems. Much of the research concerning invertebrate drift can be divided into two (related) categories: studies on drift timing and studies on drift causes. Factors that cause drift include predation by a variety of benthic predators (Peckarsky 1980; Culp et al. 1991), competition for space (Wiley and Kohler 1981), food limitation (Kohler 1985; Siler et al. 2001), increases and decreases in flow (Poff and Ward 1991; Gibbins et al. 2007), sedimentation (Culp et al. 1986), and pollution (e.g., Schulz and Liess 1999). In addition, Elliott (1967) suggested that, even in the absence of disturbance, invertebrates may accidentally drift if they lose their purchase on stream substrate.

Questions concerning the timing of drift engendered strong interest following the discovery that drift rates often are highest at night (e.g., Tanaka 1960; Waters 1962; Müller 1963). Allan's (1978) fish avoidance hypothesis (hereafter FAH) is a well supported explanation for the diel periodicity of invertebrate drift (Merritt et al. 2008). The FAH, paraphrased from Allan (1978), states that invertebrates drift nocturnally to avoid heightened risk of mortality from visual predators (fish) during the day. The FAH is supported primarily by three pieces of evidence. First, Allan (1978) established that the drift behavior of several aquatic insect taxa becomes increasingly periodic at larger instars. Because invertebrate vulnerability to visually feeding fish also increases with size, Allan (1978) hypothesized that the drift behavior of fish prey becomes nocturnal ontogenetically. Second, mayfly (Ephemeroptera) drift in fishless streams is relatively aperiodic, while mayfly drift in fish-bearing streams is distinctly nocturnal (Flecker 1992; McIntosh et al. 2002), although drift can be aperiodic in the presence of drift-feeding fish at high latitudes when the sun does not set (e.g., Hinterleitner-Anderson et al. 1992; Johansen et al. 2000). Flecker (1992) and McIntosh et al. (2002) concluded, like Allan (1978), that mayflies wait until dusk to drift, thereby avoiding daytime fish predation. Third, mayflies drift more nocturnally in the presence of trout-conditioned water (e.g., Douglas et al. 1994; Tikkanen et al. 1994), suggesting that mayflies detect chemical cues from fish and adjust their drift behavior to reduce predation risk.

Although a distinction can be drawn between factors that cause drift and factors that affect the timing of drift, the causes of drift may influence its timing if the causes exhibit periodicity. In a laboratory experiment, Culp et al. (1991) found that the diel periodicity of drift of *Paraleptophlebia heteronea* McDunnough (Ephemeroptera) increased in the presence of a nocturnally foraging benthivorous fish. Huhta et al. (2000) suggested a modification of the FAH, hypothesizing that while visually feeding fish decrease daytime drift, benthivorous fish increase nighttime drift. Two experiments supported their hypothesis. In two fishless streams, the drift behavior of *Baetis* sp. (Ephemeroptera) was roughly aperiodic, while in a stream with only benthivorous fish, drift of *Baetis* sp. was nocturnal (Huhta et al. 2000). The

benthivorous fish also caused more nighttime drift than daytime drift in a laboratory experiment (Huhta et al. 2000).

Like benthivorous fish, invertebrate predators cause prey to drift following non-consumptive encounters (Peckarsky 1980; Tikkanen et al. 1997) and are generally nocturnal foragers (e.g., Elliott 1973; Peckarsky and Cowan 1995; Huhta et al. 1999; Elliott 2000, 2005), suggesting that benthic invertebrate predators may contribute to the diel periodicity of invertebrate drift by causing more nocturnal drift than diurnal. Several studies provide evidence for this argument. Corkum and Pointing (1979) showed that a stonefly predator increased the night/day drift ratio of a mayfly in the laboratory, and Malmqvist and Sjöström (1987) demonstrated the same in a fish-bearing stream. Huhta et al. (1999) showed that a stonefly can increase the night/day drift ratio of Baetidae in the absence of fish, although, in a similar experiment, McIntosh and Peckarsky (1999) found that while a stonefly predator increased baetid drift, it did not affect the diel periodicity of baetid drift.

In addition to causing diel periodicity, invertebrate predators may affect the timing of drift by causing peaks in drift during the night (i.e., nocturnal periodicity) (Kohler 1985). Some invertebrate predators show increased foraging activity at dusk and dawn (Elliott 2000, 2005), the same time periods that invertebrate drift sometimes peaks (e.g., Waters 1962; Reisen and Prins 1972). Therefore, periods of heightened invertebrate predator foraging may be responsible for causing nocturnal periodicity of drift (Kohler 1985).

Despite the evidence that invertebrate predators affect drift timing, recent reviews do not include benthic invertebrate predators in their explanations for the diel periodicity of invertebrate drift (Allan and Castillo 2007; Merritt et al. 2008; Elliott and Humpesch 2010). We suggest three reasons for why benthic predators were not included in these explanations. First, although Kohler (1985) suggested that invertebrate predators may be the proximate cause of nocturnal drift periodicity, to our knowledge the hypothesis has never been tested. Second, although there is extensive literature on the FAH, relatively few studies have demonstrated an interaction between night and day and benthic invertebrate predators on drift (Corkum and Pointing 1979; Malmqvist and Sjöström 1987; Huhta et al. 1999). Finally,

invertebrate predators may feed at night because of the threat of diurnal fish predation (Huhta et al. 1999), so although invertebrate predators may be a proximate cause of the diel periodicity of drift, the ultimate cause may remain fish predation.

Our study focused on the drift behavior of Baetidae (Ephemeroptera) because baetids are ubiquitous and abundant in the study region and the taxon is used as a model organism in drift research (e.g., Douglas et al. 1994; Tikkanen et al. 1994; McIntosh and Peckarsky 1996; Huhta et al. 2000). The study was further restricted to baetids >2 mm in length because larger baetids exhibit the strongest diel periodicity (Allan 1978), and to invertebrate predators considered large enough to consume them. We tested two hypotheses to determine whether invertebrate predators should be included in explanations of the timing of invertebrate drift. First, we hypothesized that the locally dominant invertebrate predator *Doroneuria baumanni* Stark and Gaufin (Plecoptera) was the proximate cause of a pre-dawn peak in baetid drift we observed in a fish-bearing stream (see "Results"). Second, we hypothesized that an invertebrate predator community was the proximate cause of the diel periodicity with nocturnal maxima in baetid drift we (unexpectedly) observed in a fishless stream (see "Results"). By testing the second hypothesis in a fishless stream, we isolated the effect of benthic invertebrate predators on baetid drift from fish (although not from possible evolutionary effects of fish, see "Discussion").

We tested these hypotheses by comparing 24-h drift patterns of baetids from channels with and without benthic invertebrate predators, one experiment in a fish-bearing stream, and a second in a fishless stream. In addition, the experiment in the fish-bearing stream allowed us to corroborate the findings of Malmqvist and Sjöström (1987) that benthic invertebrate predators are a proximate cause of nocturnal drift in a fish-bearing stream. The experiment in the fishless stream was coupled with observations of *Doroneuria* sp., the most common large invertebrate predator in the stream, to determine whether the taxon was nocturnal, diurnal, or aperiodic in its activity. Finally, because of the FAH, we were surprised to observe a nocturnal drift pattern in a fishless stream, so we measured 24-h drift in two additional fishless streams to determine whether the nocturnal drift we observed was isolated or typical of streams in the region.

Materials and methods

Study sites

Our study was conducted at four locations: one perennial fish-bearing stream and three perennial fishless streams. The fish-bearing stream, Convict Creek, is a second-order stream located at the Sierra Nevada Aquatic Research Laboratory (SNARL), ca. 13 km east of Mammoth Lakes, California, USA (37°37′ N, 118°50′ W; elevation 2,200 m). The channel of Convict Creek in which we worked was at (or near) base flow and was ∼4 m wide and 0.2 m deep. Mean water column velocity was 0.51 m s^{-1} ($n = 12$). The fish assemblage includes brown trout (*Salmo trutta* Linnaeus) and rainbow trout (*Oncorhynchus mykiss* Walbaum) (Jenkins et al. 1999). Benthivorous fish are absent, and *B. tricaudatus* is the dominant baetid in Convict Creek (Kratz 1996).

The fishless streams are located in Sequoia-Kings Canyon National Park, California, USA and include a second-order stream draining Observation Basin (37°01′ N, 118°34′ W, elevation 2,540 m), a first-order stream draining Goethe Basin (37°11′ N, 118°42′ W, elevation 3,460 m), and a second-order stream on Darwin Bench (37°12′ N, 118°47′ W, elevation 3,080 m). Like Convict Creek, the streams support stoneflies, mayflies, and caddisflies (*personal observation*). The three fishless stream sites were located within 1 km of a confluence with a fish-bearing stream. Cascades between the confluence with fish-bearing streams and each site prevented colonization by fish.

Two of the fishless streams (Goethe Basin and Darwin Bench) were similar in size, with approximate widths of 1 m and depths of 0.15 m. These sites were likely near base flow. However, the fishless stream draining Observation Basin was larger and visited earlier, so flow was probably above base flow both years we worked there. The study reach was ∼5 m wide, mid-channel depth ranged from 0.1 to 0.4 m, and mid-water column velocity ranged from 0.3 to 0.7 m s^{-1}. Among the baetids, both *Diphetor hageni* (Eaton) and *Baetis* sp. were observed. The ratio of *Baetis* sp. to *D. hageni* density was 26:1.

Channels

The experimental channels used at Convict Creek and the stream draining Observation Basin differed

slightly, though at both sites channels were placed in the stream and lined with substrate collected from the creek. At Convict Creek, channels were constructed from three plastic flower boxes cut and glued end-to-end to form 1.97-m long × 0.18-m wide channels (total area = 0.36 m^2), while at Observation Basin, channels were constructed from two flower boxes and were 1.38 m × 0.18 m (total area = 0.25 m^2). A metal screen (mesh size: 3×3 mm) was inserted between the channels and the rear driftnets in the Observation Basin channels to prevent predators, which were far more mobile in Observation Basin than in Convict Creek, from emigrating. The mesh screen allowed baetids to drift from the channels, but predators could not. Aside from length and the presence of the metal screen, the channels used in Convict Creek and Observation Basin were identical.

Channels were isolated from stream fauna with upstream and downstream driftnets (aperture 300 μm) and by the sides of the channels, which rose above the stream surface. Upstream nets filtered water flowing into the channel and were 70-cm long. Nets were cone-shaped and contained within a length of PVC pipe (inside diameter of 10.2 cm). Downstream driftnets were 34-cm long cones. They had an upstream opening of 10.2 cm (inside diameter) and were equipped with a screened collection vial to filter drifting invertebrates from the channel outflow. The sides and bottoms of each channel were roughened with a file to provide purchase for experimental animals. Driftnets that were used to collect 24-h drift data were identical to the rear driftnets on channels, although they were anchored to the stream bottom rather than to channels.

In addition to the experimental channels described above, a third channel type was used to make observations of *Doroneuria* sp. in Observation Basin. The behavior of *Doroneuria* sp. was observed in a raised, flow-through, flower-box channel with clear Plexiglas bottom and walls (channel length: 0.72 m, width: 0.18 m). Black plastic was draped from the channel to the ground to darken the underside of the channel and provide the stoneflies with a more realistic light environment.

Drift sampling

Twenty-four-hour drift data were collected in Observation Basin in July 2008 and in August 2008 at Goethe Basin, Darwin Bench, and Convict Creek. At three of the four sites, driftnets were deployed for two 24-h periods (Darwin Bench, Observation Basin, and Convict Creek). At the fourth site, Goethe Basin, baetids were relatively rare, so the site was sampled for three 24-h periods. During each 24-h sampling period, 3 or 4 driftnets were deployed in the stream. At the three fishless sites (Darwin Bench, Observation Basin, and Goethe Basin), nets were removed from the stream every 2 h, contents were rinsed into sorting trays, and nets were replaced in the stream within 1 min of their removal. At Convict Creek, drift was sampled hourly because nets clogged more quickly, but otherwise the process was the same. During the period between net removal, baetids >2 mm in length were counted.

Behavior

In the absence of fish (at Observation Basin), observations were made of the foraging behavior of *Doroneuria* sp., the most common invertebrate predator in the stream (head capsule width >3 mm). To begin each replicate, channels were lined with substrate collected from the stream (sand, gravel, and cobble). The channel received stream water diverted through a PVC pipe (water velocity inside the channel ranged from 0 to 0.29 m s^{-1}, mean = 0.14 m s^{-1}). One *Doroneuria* sp. individual was collected from the stream and introduced to the channel. Following Elliott (2000), each stonefly was given 3 days to acclimate to the channels. A variety of non-predacious invertebrates were provided for food. After the acclimation period, each stonefly was observed for the first 20 min out of every 30 for 24 h (16 h of observation for each stonefly). Nighttime observations were made with a dim red light. To quantify stonefly activity, we set a metronome to beat at a rate of one beat s^{-1} and observed the stonefly. If the observer saw the stonefly change location or orientation between beats of the metronome (a period of one second), one "movement" was recorded. Thus, a stonefly that was active for the entire 16 h of observation would have received a movement score of 57,600 movements (hours × min × sec). The process was replicated five times ($n = 5$).

Convict Creek experiment

The goal of this experiment was to test the hypothesis that *D. baumanni*, the most common invertebrate

predator in Convict Creek (length > 1.3 cm), contributed to the nocturnal drift periodicity of *B. tricaudatus*, though we were also able to determine whether *D. baumanni* contributed to the diel periodicity of *B. tricaudatus* drift. The experiment had 3 temporal blocks (channels were cleaned and removed from the stream between blocks), 2 treatments (channels with and without *D. baumanni*), and 2 replicates per block-treatment combination. The experiment was conducted in September 2008.

To begin the experiment, four channels were placed on a shaded concrete slab in Convict Creek (block 1). Each channel received ambient flow (inside channel velocity ranged from 0.04 to 0.19 m s^{-1}, mean = 0.11 m s^{-1}) and cobble, gravel, and sand collected from the stream. Before introducing substrate to channels, invertebrate predators and *B. tricaudatus* were removed, but other invertebrates were not purposefully excluded. Two randomly selected channels received nine *D. baumanni* to produce a density of 25.4 *D. baumanni* m^{-2}, as close as possible to its natural density of 25 m^{-2} in Convict Creek (Kratz 1996). Again following the methodology of Elliott (2000), we waited 3 days (two nights) following the introduction of stoneflies before *B. tricaudatus* were introduced. At dusk on the first two days of acclimation, 50 larval Simuliidae (Diptera) were introduced to each of the four channels to provide stoneflies with food.

On the morning of the third day of stonefly acclimation, 1000 *B. tricaudatus* were collected into four flow-through buckets (250 *B. tricaudatus* per bucket), and 250 *B. tricaudatus* were introduced to each channel at 16:00 h. During the 1-min long mayfly introductions, flow was reduced to minimize disoriented mayflies from being washed from the channels. Initial density was 705 *B. tricaudatus* m^{-2}, a density within the range of *B. tricaudatus* density (>2 mm) observed in Convict Creek. Live *B. tricaudatus* were removed from the downstream driftnets and counted every 2 h for 24 h, and the four upstream driftnets were cleaned to maintain constant flow-through the channels.

Observation Basin experiment

Observation Basin is remote and extremely difficult to access, which necessitated several differences between the Observation Basin and Convict Creek experiments, though both compared drift patterns of baetids from channels with and without benthic predators. Differences were that in Observation Basin (1) two channels were used per block rather than four, although six blocks were run rather than three, so six replicates were run in both cases; (2) invertebrate predators were provided one night to acclimate to the channels rather than two; (3) invertebrate predators with head capsule widths >3 mm were randomly chosen from the stream for use in the experiment; (4) baetid and predator density were both lower (100 baetids per channel, or 403 baetids m^{-2}, and 5 predator individuals per channel, or 20 predators m^{-2}); (5) baetids were introduced to each channel at 13:30 h rather than 16:00 h; and (6) the experiment was conducted from mid-June to mid-July 2009 rather than in September 2008, when the Convict Creek experiment was run.

The reason for differences 1 and 2 was that the site was remote. For the same reason, we could not measure water velocity in the experimental channels, but we estimate it to be somewhat faster than in the Convict Creek experiment ($\sim$0.2 m s^{-1}). Predators were randomly chosen from the stream because we were interested in how the invertebrate predator community affected baetid drift patterns rather than the effect of any particular taxon. The three predator taxa used in the experiment were *Doroneuria* sp. (making up 63.3% of the predators used), *Hesperoperla* sp. (20.0%), and *Orohermes crepusculus* (16.7%). Percentages approximate relative abundances of large invertebrate predators in the stream. Experimental densities were based on feasibility of collecting individuals and did not necessarily reflect natural densities. The three-week Observation Basin experiment was begun in June to ensure adequate stream flow throughout.

Data analysis

For observational drift data from each of the four sites, the measurements of daytime baetid drift were summed, as were the nighttime measurements. Nighttime measurements included all 2-h periods that included any time after sunset or before sunrise. For each site, the total nighttime drift was divided by the total daytime drift to produce a ratio of night/day drift. However, although each fishless stream had six daytime and six nighttime drift measurements per

24-h sampling period, the Convict Creek drift pattern had 11 nighttime and 13 daytime measurements, making its night/day ratio incomparable to the fishless streams. To allow for comparisons, we provide both the night/day drift ratio and the night/day drift rate ratio for Convict Creek (see "Results").

The *Doroneuria* sp. behavioral data were analyzed with a paired *t*-test. The daytime movements were summed for each individual, as were the nighttime movements, and a paired *t*-test was used to determine whether there was a difference in activity level between day and night. The paired *t*-test and all other analyses were performed in the statistical software package SAS (SAS Institute Inc., version 9.1). For all hypothesis tests, significance was determined using $\alpha = 0.05$.

The channel experiments were analyzed with repeated measures ANOVAs (rmANOVA) because multiple measurements were taken from the same experimental unit. In these experiments, baetid individuals that drifted from the channels were not replaced, causing populations in each channel to decline during the 24-h drift sampling period. Our rationale for not replacing mayflies was twofold. We preferred to not disturb the experiment following the initial introductions because disturbance could have elevated rates of drift, and it was infeasible to collect baetids throughout the night. In consequence, population loss from channels early in the 24-h period reduced the number of baetids available to drift from channels later in the period. Therefore, a per capita drift (PCD) rate for each time period was calculated and used as the response variable. The following equation was used:

$$\mathrm{PCD}_{t=n} = \frac{\mathrm{Drift}_{t=n}}{T - \sum_{t=1}^{n-1} \mathrm{Drift}_t} \times 100$$

where PCD is expressed as a percent at time *t*, *T* is the total number of baetids introduced, *t* is the time period during which drift was sampled, and Drift is the number of baetids that drifted during time *t*.

To some extent, the use of PCD corrected for population loss during the 24-h period, but for two reasons the correction was not complete. First, we could not account for the consumption of *B. tricaudatus* in predator treatments. However, rates of consumption were low compared with rates of drift. For example, in the Observation Basin experiment, 3.5%

of baetids introduced to predator channels were unaccounted for (presumably consumed) after 24 h, while 51.7% of the baetids in the predator channels drifted. Second, because fewer mayflies were present in channels during later time periods, negative density-dependent effects may have been reduced during the 24-h drift sampling period, particularly in the predator treatment channels. Because of the asymmetric population loss between treatments (due to consumption and drift), only analyses that would be biased against detecting a time × invertebrate predator interaction are reported (see below). Three rmANOVAs were run, two for the Convict Creek experiment and one for the Observation Basin experiment.

The first rmANOVA addressed whether *D. baumanni* presence contributed to *B. tricaudatus* diel periodicity in drift (i.e., caused more drift at night than during the day). To account for the asymmetry in negative density dependence problem, data from day 2 (06:00–16:00 h) were not included in the analysis, leaving one daytime drift measurement for each treatment (day 1), and six nighttime measurements. The nighttime measurements were summed for each channel to make a single "nighttime" measurement ("nighttime" lasted from 18:00 to 06:00 h and "daytime" from 16:00 to 18:00 h), and the PCD equation was used to calculate per capita drift. Thus, the analysis had two levels of time (day and night) and two levels of predation (*D. baumanni* present and absent), and we were interested in the time × predation interaction. By only including two levels of time (day and night) rather than three (day 1, night, and day 2), we biased our analysis against detecting the time × predation interaction (the bias occurred because drift rates were higher from *D. baumanni* channels than control during day 1) and made the need to satisfy the assumption of sphericity unnecessary (it can only be failed if a repeated measures analysis has more than two levels of time) (Gurevitch and Chester 1986). Because the variances increased with the mean, the data were natural log $(x + 1)$-transformed prior to the analysis.

The second rmANOVA addressed whether *D. baumanni* contributed to nocturnal periodicity in the experiment, or more specifically whether *D. baumanni* contributed to the peak in drift observed at dawn (i.e., 04:00–06:00 h). As with the first rmANOVA, two time periods were analyzed (02:00–4:00 h and 04:00–06:00 h), and we were interested in the

time × predation interaction. In addition, a paired *t*-test was used to determine whether *B. tricaudatus* exhibited nocturnal periodicity in the absence of benthic predators, or more specifically whether the ratio of *B. tricaudatus* drift in control channels from 02:00–04:00 h to 04:00–06:00 h differed from 1:1.

The third rmANOVA was used to test for the time × predator interaction in Observation Basin. Like in the previous rmANOVAs, there were two levels of time (day and night), and two levels of predation (present and absent). Differences were that the nocturnal periodicity question was not addressed because there was not a dawn peak in the natural drift pattern, and the "day" and "night" time periods were different ("daytime" included drift from 13:30 to 19:30 h, and "nighttime" from 19:30 to 05:30 h) because of seasonal changes in day length and an earlier start time for blocks. Otherwise the analysis was identical. Like the Convict Creek rmANOVA testing for the effect of *D. baumanni* on diel periodicity, the test was conservative because more baetids drifted from the predator channels during the day than from the control channels during the day (in this case significantly more).

Results

Baetid drift exhibited diel periodicity with nocturnal maxima at the four streams sampled (Table 1). *B. tricaudatus* in Convict Creek (fish-bearing) had a nighttime drift rate 112 times higher than daytime (night/day drift ratio = 95). Baetids in the fishless stream draining Observation Basin had a nighttime drift rate 3.4 times higher than daytime (Fig. 2; Table 1), baetids in the fishless stream at Goethe Basin had a nighttime drift rate 3.8 times higher than daytime (Table 1), and baetids in the fishless stream
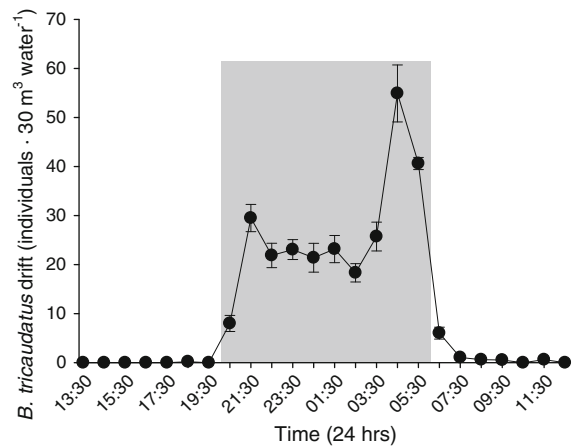

Fig. 1 Mean ± SE of *B. tricaudatus* drift in Convict Creek (fish-bearing) for the 1-h period preceding indicated times. For example, the point at 19:30 represents the mean drift from 18:30 to 19:30. The *shaded* region represents the period of time between sunset and sunrise
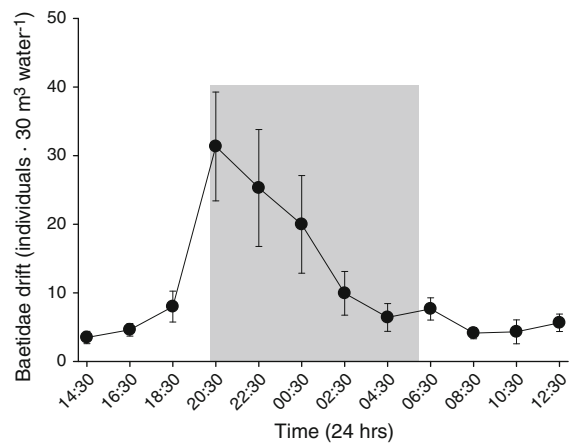

Fig. 2 Mean ± SE of Baetidae drift in the stream draining Observation Basin (fishless) for the 2-h period preceding the indicated time. The *shaded* region represents the period of time between sunset and sunrise

on Darwin Bench had a nighttime drift rate 2.0 times higher than daytime (Table 1). The mean night/day drift ratio in the fishless streams during the seven 24-h periods was 3.0. In addition to diel periodicity, *B. tricaudatus* in Convict Creek exhibited nocturnal periodicity in its drift. Elevated drift rates occurred at both dusk and dawn (Fig. 1). In every fishless stream sampled, the three highest mean drift rates occurred during the first three nighttime sampling periods (e.g., Fig. 2).
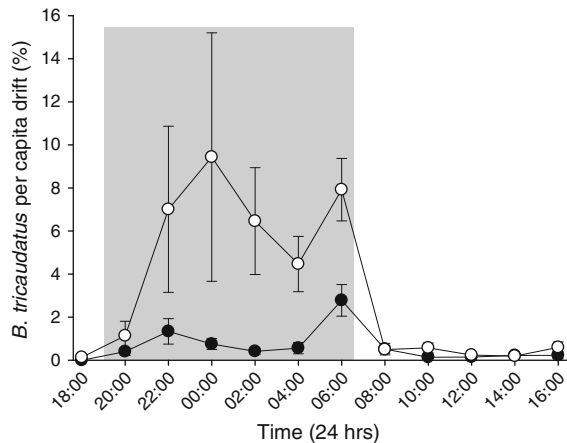
Table 1 Observed ratios of night/day drift at four locations

Location	Stream type	Observed night/day drift ratio
Convict Creek	Fish-bearing	95:1
Observation Basin	Fishless	3.4:1
Goethe Basin	Fishless	3.8:1
Darwin Bench	Fishless	2.0:1

Fig. 3 Mean ± SE of *B. tricaudatus* per capita drift (%) from channels in Convict Creek with (*open circles*) and without (*closed circles*) *D. baumanni* for the 2-h period preceding the indicated time. The *shaded* region represents the period of time between sunset and sunrise

Fig. 4 Mean ± SE of Baetidae per capita drift (%) from channels in Observation Basin with (*open circles*) and without (*closed circles*) invertebrate predators for the 2-h period preceding the indicated time. The *shaded* region represents the period of time between sunset and sunrise

D. baumanni presence influenced the diel periodicity of *B. tricaudatus* drift in the Convict Creek experiment (rmANOVA, test of the time × predation interaction, $F_{[1, 8]} = 31.05$, $P = 0.0005$; Fig. 3). An analysis of the simple effects showed that the presence of *D. baumanni* did not increase *B. tricaudatus* per capita drift during the day, while the presence of *D. baumanni* increased *B. tricaudatus* per capita drift at night by a factor of 5.1 (Tukey's HSD test). By causing more drift at night than during the day, *D. baumanni* presence contributed to the diel periodicity of *B. tricaudatus* drift.

In the same experiment, *D. baumanni* presence did not appear to contribute to the pre-dawn peak in drift of *B. tricaudatus* (rmANOVA, time × predation interaction, $F_{[1, 8]} = 1.37$, $P = 0.276$; Fig. 3). However, because of the asymmetric loss of individuals from the channels (by 02:00, 2.9% of the *B. tricaudatus* population had drifted from the control channels, but 20.6% from the *D. baumanni* channels), interpretation of an insignificant interaction is difficult (the test was performed because a significant dawn increase of drift in the *D. baumanni* channels over the control would have been meaningful). Therefore, a paired *t*-test was also performed to determine whether baetids showed a significant increase in pre-dawn drift independent of benthic predators (null hypothesis was that the mean drift in the control channels from 02:00 to 04:00 and from 04:00 to 06:00 were equal).
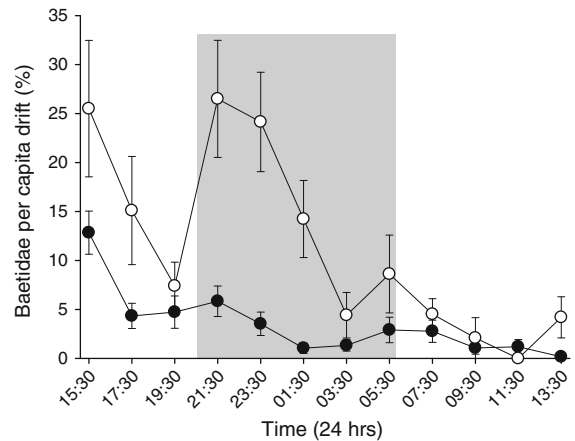
B. tricaudatus drift peaked at dawn independently of benthic predator presence (paired *t*-test, $t = 3.9$, $df = 5$, $P = 0.012$). *B. tricaudatus* drift from control channels was 5.0 times higher from 04:00 to 06:00 than from 02:00 to 04:00.

Invertebrate predators also contributed to the diel periodicity of baetid drift in the Observation Basin experiment (rmANOVA, time × predation, $F_{[1, 5]} = 12.51$, $P = 0.017$; Fig. 4). An analysis of the simple effects showed that invertebrate predator presence increased baetid per capita drift during the day and night (Tukey's HSD test); by a factor of 2.0 during the day and by a factor of 4.9 at night. An analysis of the 24-h observations of *Doroneuria* sp. foraging in Observation Basin showed that the taxon was more active at night than during the day (paired *t*-test, $t = 4.9$, $df = 4$, $P = 0.008$). The stoneflies changed location or orientation during 29.06% of the nighttime measurements and 0.05% of the daytime measurements.

In addition, we made two observations regarding differences in baetid behavior between the fish-bearing and fishless streams. First, in Convict Creek, 0.0% of *B. tricaudatus* introduced to the control channels drifted during the initial 2-h sampling period (Fig. 3), compared to 12.8% of baetids that drifted from control channels in Observation Basin (Fig. 4). Second, in the absence of benthic predators, *B. tricaudatus* exhibited crepuscular drift peaks in Convict

Creek (Fig. 3), but baetids in the three fishless streams did not (e.g., Fig. 4).

Discussion

The fish avoidance hypothesis (FAH) suggests that invertebrates drift at night to avoid predation by daytime-feeding fish (Allan 1978). The literature to date strongly supports the FAH, and indeed, the difference we observed between the initial drift measurements in the Convict Creek and Observation Basin experiments provides further support, as does the lower night/day drift ratios we observed in the fishless streams. However, several studies indicate that benthic predators also affect the timing of drift (Corkum and Pointing 1979; Malmqvist and Sjöström 1987; Huhta et al. 1999, 2000). Here, we present evidence that benthic invertebrate predators markedly increase nocturnal drift of baetids and suggest that a more complete explanation for the diel periodicity of invertebrate drift would include invertebrate predators.

The evidence from our study is threefold. First, baetids drifted most frequently at night in three fishless streams during seven 24-h periods in Sequoia-Kings Canyon National Park. Baetids exhibit a plastic response to fish, adjusting the timing of their drift behavior to be more nocturnal when fish are present (Douglas et al. 1994; Tikkanen et al. 1994; McIntosh and Peckarsky 1996). Therefore, because baetids were unlikely to be drifting nocturnally as an evolutionarily fixed response to fish, the FAH cannot explain the nocturnal drift patterns we observed in the fishless streams.

A second line of evidence is that the presence of *D. baumanni* increased the night/day drift ratio of *B. tricaudatus* in the Convict Creek experiment. This experiment corroborates the evidence provided by Corkum and Pointing (1979) and Malmqvist and Sjöström (1987) that an invertebrate predator can contribute to the diel periodicity of invertebrate drift. However, if *D. baumanni* restricted its foraging to the nighttime to avoid fish predation, fish would remain responsible for its contribution to the diel periodicity of *B. tricaudatus* drift (albeit both directly and indirectly). To address this possibility and to test the hypothesis that invertebrate predators caused the nocturnal drift we observed in the fishless streams, the experiment was repeated in Observation Basin.

The Observation Basin experiment supports the hypothesis that benthic invertebrate predators caused the nighttime drift peaks and represents the final line of evidence supporting the inclusion of invertebrate predators in explanations of invertebrate drift timing. Although it is conceivable that invertebrate predators were nocturnally active because, unlike baetids, they exhibit an evolutionarily fixed response to the threat of fish predation, observations made during the Convict Creek and Observation Basin experiments were inconsistent with this explanation. If invertebrate predators exhibited a fixed response to fish predation, predator behavior between fish-bearing and fishless streams should be similar. On the contrary, when introduced to channels, invertebrate predators in Observation Basin were slow to settle underneath rocks (sometimes taking more than 24 h), while in Convict Creek, *D. baumanni* individuals immediately moved underneath cobbles. Indeed, the significant daytime drift increase from the Observation Basin predator channels may be an artifact of insufficient predator acclimation time. The difference in predator behaviors indicates that they can detect fish presence and adjust their behaviors accordingly. Similarly, Huhta et al. (1999) observed that the activity of a stonefly predator was strongly affected by fish chemical cues (it was less active during the day and night when cues were present), but its foraging behavior was nocturnal whether fish cues were present or absent (although the same was not true for a second predator, *Rhyacophila nubila*). Therefore, rather than exhibiting a nocturnally fixed foraging pattern because of fish predation, we suggest that the invertebrate predators in our experiment had evolved to be most successful as nocturnal foragers (possibly due to the threat of fish predation or increased prey vulnerability at night) and forage at night regardless of fish presence.

A second result from the Convict Creek experiment was that baetids drifted from both control channels and channels with *D. baumanni*. The literature suggests that baetids may have drifted from control channels because of food limitation (Kohler 1985; Siler et al. 2001) and inadvertent drift entry (Elliott 1967), if for no other reason than injury sustained during collection. Therefore, it is tempting to draw conclusions regarding the relative importance of benthic predation and food limitation plus inadvertent drift. However, because stoneflies were provided three days to acclimate to channels, channel substrate, unexposed to

stream consumers, likely accumulated biofilm above ambient levels. Because increased food levels may have reduced drift caused by food limitation, conclusions regarding the relative importance of the causes of drift are unjustified based on these data.

The Convict Creek experiment did not support the hypothesis that the dawn and dusk peaks in *B. tricaudatus* drift were caused by increases in benthic predator activity. Because of the difference in population size between treatments by 02:00, the dawn test of the time × *D. baumanni* interaction provides only weak evidence that *D. baumanni* did not contribute to the pre-dawn peak in the drift of *B. tricaudatus*. However, the paired *t*-test provides strong evidence that *B. tricaudatus* exhibits a peak in drift independently of benthic predators. Non-reproducing prey are thought to minimize μ/f, both temporally (Metcalfe et al. 1999) and spatially (Gilliam and Fraser 1987), where μ equals instantaneous predation mortality risk, and f equals gross food intake rate (Gilliam and Fraser 1987). An alternative explanation for the nocturnal periodicity of *B. tricaudatus*, assuming drift from channels lacking *D. baumanni* was mainly caused by food limitation, is that *B. tricaudatus* minimized its μ/f ratio by feeding most actively at dusk and dawn. Therefore, assuming that food level does not vary significantly over a 24-h period, theory would predict *B. tricaudatus* in Convict Creek to feed most actively at dawn and dusk, thus minimizing exposure to nocturnally active invertebrate predators and diurnally active trout. In contrast, theory would predict that baetids in fishless streams should forage diurnally. Following the theoretical prediction, baetids appeared to exhibit crepuscular drift only in the fish-bearing stream, although replication at a multiple-stream scale would be required to address this hypothesis properly.

Although our experiment in the fishless stream suggests that invertebrate predators caused the nocturnal drift pattern in Observation Basin, we do not suggest that the presence of invertebrate predators always induces nocturnal drift. Invertebrates in fishless streams often exhibit aperiodic drift (e.g., Flecker 1992; McIntosh et al. 2002), presumably in the presence of invertebrate predators. However, an aperiodic drift pattern should not be interpreted as evidence that invertebrate predators do not influence drift timing. Rather, because invertebrates may drift diurnally in the absence of invertebrate predators, we suggest that invertebrate predator's presence increases

the night/day drift ratio, perhaps shifting drift patterns from diurnal to aperiodic in some fishless streams, or nocturnal, as in Observation Basin.

Why drift was nocturnal in the three fishless streams we sampled but aperiodic in other fishless streams (e.g., Flecker 1992) is unknown. One possibility is that other fishless streams had invertebrate predator communities with smaller individuals. A relationship between predator size and strength of nocturnal behavior may exist, with smaller predators possibly being more aperiodic in their activity because their evolution would be less affected by visual predators. For this reason, and because the present study was restricted to invertebrate predators considered large enough to consume baetids, our conclusions should only be extended to large invertebrate predators (minimum length of predators used in experiments = 1.5 cm).

Our results indicate that a more complete explanation of the diel periodicity of invertebrate drift should include benthic predators. Therefore, we suggest that both visually feeding fish and nocturnally foraging benthic predators, when present, affect the timing of invertebrate drift; visually feeding fish by reducing daytime drift and benthic predators by increasing nighttime drift. Thus, in fish-bearing streams, both large invertebrate predators and fish are responsible for the diel periodicity of invertebrate drift.

References

Allan JD (1978) Trout predation and the size composition of stream drift. Limnol Oceanogr 23:1231–1237

Allan JD, Castillo MM (2007) Stream ecology: structure and function of running waters. Springer, Dordrecht

Corkum LD, Pointing P (1979) Nymphal development of Baetis vagans McDunnough (Ephemeroptera: Baetidae) and drift habits of large nymphs. Can J Zool 57:2348–2354

Culp JM, Wrona FJ, Davies RW (1986) Response of stream benthos and drift to fine sediment deposition vs. transport. Can J Zool 64:1345–1351

Culp JM, Glozier NE, Scrimegour GJ (1991) Reduction of predation risk under the cover of darkness: avoidance responses of mayfly larvae to a benthic fish. Oecologia 86:163–169

Douglas PL, Forrester GE, Cooper SD (1994) Effects of trout on the diel periodicity of drifting in baetid mayflies. Oecologia 98:48–56

Elliott JM (1967) The life histories and drifting of the Plecoptera and Ephemeroptera in a dartmoor stream. J Anim Ecol 36:343–362

Elliott JM (1973) The diel activity pattern, drifting and food of the leech *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae) in a lake district stream. J Anim Ecol 42:449–459

Elliott JM (2000) Contrasting diel activity and feeding patterns of four species of carnivorous stoneflies. Ecol Entomol 25:26–34

Elliott JM (2005) Contrasting diel activity and feeding patterns of four instars of *Rhyacophila dorsalis* (Trichoptera). Freshw Biol 50:1022–1033

Elliott JM, Humpesch U (2010) Mayfly larvae (Ephemeroptera) of Britain and Ireland: keys and a review of their ecology. Windermere: Freshw Biol Assoc 66:152

Flecker AS (1992) Fish predation and the evolution of invertebrate drift periodicity: evidence from Neotropical streams. Ecology 73:438–448

Gibbins C, Vericat D, Batalla RJ (2007) When is stream invertebrate drift catastrophic? The role of hydraulics and sediment transport in initiating drift during flood events. Freshw Biol 52:2369–2384

Gilliam JF, Fraser DF (1987) Habitat selection under predation hazard: test of a model with foraging minnows. Ecology 68:1856–1862

Gurevitch J, Chester S (1986) Analysis of repeated measures experiments. Ecology 67:251–255

Hinterleitner-Anderson D, Hershey AE, Schuldt JA (1992) The effects of river fertilization on mayfly (Baetis sp.) drift patterns and population density in an arctic river. Hydrobiologia 240:247–258

Huhta A, Muotka T, Juntunen A, Yrjonen M (1999) Behavioural interactions in stream food webs: the case of drift-feeding fish, predatory invertebrates and grazing mayflies. J Anim Ecol 68:917–927

Huhta A, Muotka T, Tikkanen P (2000) Nocturnal drift of mayfly nymphs as a post-contact antipredator mechanism. Freshw Biol 45:33–42

Jenkins TM Jr, Diehl S, Kratz KW, Cooper SD (1999) Effects of population density on individual growth of brown trout in streams. Ecology 80:941–956

Johansen M, Elliott JM, Klemetsen A (2000) Diel fluctuations of invertebrate drift in a Norwegian stream north of the Arctic Circle. Nor J Entomol 47:101–112

Kohler SL (1985) Identification of stream drift mechanisms: an experimental and observational approach. Ecology 66:1749–1761

Kratz KW (1996) Effects of stoneflies on local prey populations: mechanisms of impact across prey density. Ecology 77:1573–1585

Malmqvist B, Sjöström P (1987) Stream drift as a consequence of disturbance by invertebrate predators. Oecologia 74:396–403

McIntosh AR, Peckarsky BL (1996) Differential behavioural responses of mayflies from streams with and without fish to trout odour. Freshw Biol 35:141–148

McIntosh AR, Peckarsky BL (1999) Criteria determining behavioural responses to multiple predators by a stream mayfly. Oikos 85:554–564

McIntosh AR, Peckarsky BL, Taylor BW (2002) The influence of predatory fish on mayfly drift: extrapolating from experiments to nature. Freshw Biol 47:1497–1513

Merritt RW, Cummins KW, Berg MB (2008) An introduction to the aquatic insects of North America, 4th edn. Kendall/Hunt, Dubuque

Metcalfe NB, Fraser NHC, Burns MD (1999) Food availability and the nocturnal vs. diurnal foraging trade off in juvenile salmon. J Anim Ecol 68:371–381

Müller K (1963) Diurnal rhythm in "organic drift" of Gammarus pulex. Nature 198:806–807

Peckarsky BL (1980) Predator-prey interactions between stoneflies and mayflies: behavioral observations. Ecology 61:932–943

Peckarsky BL, Cowan CA (1995) Microhabitat and activity periodicity of predatory stoneflies and their mayfly prey in a western Colorado stream. Oikos 74:513–521

Poff NL, Ward JV (1991) Drift responses of benthic invertebrates to experimental streamflow variation in a hydrologically stable stream. Can J Fish Aquat Sci 48:1926–1936

Reisen WK, Prins R (1972) Some ecological relationships of the invertebrate drift in Praters Creek, Pickens County, South Carolina. Ecology 53:876–884

Schulz R, Liess M (1999) A field study of the effects of agriculturally derived insecticide input on stream macroinvertebrate dynamics. Aquat Toxicol 46:155–176

Siler ER, Wallace JB, Eggert SL (2001) Long-term effects of resource limitation on stream invertebrate drift. Can J Fish Aquat Sci 58:1624–1637

Tanaka H (1960) On the daily change of the drifting of benthic animals in stream, especially on the types of daily change in taxonomic groups of insects. Bull Freshw Fish Res Lab Tokyo 9:13–24

Tikkanen P, Muotka T, Huhta A (1994) Predator detection and avoidance by lotic mayfly nymphs of different size. Oecologia 99:252–259

Tikkanen P, Muotka T, Huhta A, Juntunen A (1997) The roles of active predator choice and prey vulnerability in determining the diet of predatory stonefly (Plecoptera) nymphs. J Anim Ecol 66:36–48

Waters TF (1962) Diurnal periodicity in the drift of stream invertebrates. Ecology 43:316–320

Wiley MJ, Kohler SL (1981) An assessment of biological interactions in an epilithic stream community using time-lapse cinematography. Hydrobiologia 78:183–188