Behavioral response of the freshwater cyclopoid copepod *Eucyclops serrulatus* to hydropeaking and thermopeaking in a laboratory flume

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ABSTRACT

The generation of electricity by hydropower plants meets the requirements for intermittent energy demand and for the production of renewable energy. However, the hydropower plant operation has important consequences for the biotic compartment of the river reaches downstream. Intermittent release of water causes sudden variations in discharge that increase bed shear stress and dislodge benthic organisms. Release from high-elevation reservoir can also affect the thermal regime of the river by causing sharp variations in water temperature. Because the hydrodynamic and thermal waves separate while propagating downstream, the benthic community is exposed to two distinct stressors that affect taxa differently based on their sensitivity and adaptations. We investigated separately the effects of a sudden variation in discharge or in water temperature on the small-scale swimming behavior of a widespread species of cyclopoid copepod in a laboratory flume that allows the tracking of organisms both in the water column and in a transparent sediment bed. We gradually varied the discharge or the temperature of the water to mimic the artificial changes caused by hydropower plants. We tracked copepods in three dimensions and quantified the kinematics of their motion. Copepods increased substantially their counter-current swimming effort in response to increasing flow velocity. This behavioral response seems to occur above a threshold in flow velocity of approx. 40 mm/s. It results in a substantial reduction in their downstream transport and hence opposes drift. Copepods reacted differently to warm and cold variations in temperature. Decreasing temperature resulted in a substantially lower counter-current swimming effort, which may therefore increase drift. Rising temperature had no clear effect on their behavior. Our study highlights the importance of understanding the behavioral traits that mediate the response of stream invertebrates to disturbances in the hydraulic and thermal regimes of their environment.

Introduction

Drift of benthic invertebrates in streams is a normal ecological process that influences their dispersal and the distribution of their populations. Drift is voluntary when organisms leave the sediment bed and drift downstream as part of their normal behavior and life cycle, or passive when organisms are dislodged from the substrate by hydrodynamic forces. Passive drift has received much attention in the context of natural flooding events (reviewed in Naman et al. 2016) but also and especially in

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relation to the release of water from reservoir hydroelectric power stations (e.g. Mochizuki et al. 2006; Bruno et al. 2010). It is indeed particularly prevalent in flow-regulated rivers due to the intermittent pattern of hydropower plant operation. Release of water causes sudden variations in discharge, termed hydropeaking (HP), which increases near-bed shear forces and results in organisms being passively dislodged from the substrate. Although HP can cause mobilization and transport of sediments, considerable drift can also occur in non-scouring flow without sediment movement (Gibbins et al. 2005; Gibbins et al. 2007). Repeated fluctuations in discharge ultimately result in depletion of the benthos (Irvine 1985).

Hydropeaking may also affect the thermal regime of rivers. The release of water from the hypolimnion of high-elevation, stratified reservoirs often causes sharp and intermittent temperature variations, termed thermopeaking (TP). Thermopeaking occurs because the temperature of the released water differs from that of the downstream water body (Toffolon et al. 2010). In Alpine areas, TP usually warms up the water of the stream in winter and decreases its temperature in summer. At large temporal scales, thermal regime alteration can cause, e.g. the disappearance of local species and an asynchrony between the development of organisms and the availability of resources (reviewed in Olden and Naiman 2010). At shorter scales, i.e. daily or sub-daily, hydropower plant operation exposes benthic organisms to abrupt, repeated variations in water temperature that cause them to drift (Carolli et al. 2012). Because drift entry occurs within minutes, the effect of TP is attributed to organisms actively entering the water column or being dislodged as a consequence of increased activity on the streambed (Carolli et al. 2012).

After a first phase of overlap, the hydro- and thermopeaking waves separate because they propagate downstream at different speeds (Zolezzi et al. 2011; Vanzo et al. 2016). This asynchronous propagation exposes the benthic community to two distinct stressors that affect taxa differently based on their morphological and behavioral adaptations to resist high flow velocity and on their sensitivity to temperature (Jakob et al. 2003; Bruno et al. 2013; Bruno et al. 2016). Morphological adaptations against dislodgment by the flow include, e.g. body shape, which influences susceptibility to drag, and structures enabling organisms to cling onto the substrate (Naman et al. 2017). Behavioral traits that mediate drift include deliberate entry into the water column, horizontal displacements toward patches of lower flow velocity, or active downward displacement in the sediment bed (Holomuzki and Biggs 2000; Verdonschot et al. 2014). While the redistribution of benthic invertebrates during HP and their abundance in drift have been relatively well studied, there is much less information on the small-scale physical and behavioral processes that lead to drift entry or avoidance during high flow events (Blanckaert et al. 2013). Similarly, the behavioral mechanisms underlying drift entry in response to temperature variations are as yet little known (Carolli et al. 2012).

In this study, we quantify the behavioral response of cyclopoid copepods to hydro- and TP simulations in a laboratory channel that allows manipulation of flow discharge and temperature. We selected cyclopoid copepods because they are often found in prealpine gravel-bed streams (Richardson 1991; Brunke and Gonser 1999) and because this taxon represents an important element of lotic ecosystems (Reiss and Schmid-Araya 2008). In contrast to larval stages of insect taxa, whose swimming behavior and downstream drift have been particularly well studied (e.g. Lancaster 1999; Lancaster et al. 2006; Oldmeadow et al. 2010), information on the behavioral ecology of lotic copepods remains scarce. In this study, we take advantage of an advanced three-dimensional tracking technique to track the motion of many copepods swimming simultaneously and in three dimensions in the water column of a laboratory flume and in the interstitial spaces of its transparent sediment layer. Our study aims at understanding the relationship between hydraulic parameters or water temperature, self-locomotion, and drift entry in a widespread but often neglected component of the meiobenthos. We mimic the sudden variations associated with hydro- and thermopeaking, and track copepods moving freely under well-quantified flow and temperature conditions. We quantify the separate effects of changes in flow velocity and water temperature on their swimming behavior,

using metrics that capture the kinematics of their motion. We specifically address the following questions:

(1) What are the effects of a sudden increase in flow velocity on the swimming behavior and small-scale motion of the widespread cyclopoid copepod *Eucyclops serrulatus*? Previous laboratory measurements suggest that *Eucyclops* displays strong swimming abilities to maintain position at times of high flow (Richardson 1992). We have recently observed that this species (a) performs very frequent incursions within the interstices of the sediment bed where flow velocity is negligible, and (b) swims actively against the flow to reduce downstream transport (Sidler et al. 2017). In this study, we test for the hypothesis that these two behavioral traits render copepods less vulnerable to high flow velocities and less prone to drift entry.

(2) What are the effects of a sudden variation in temperature on the swimming behavior of *E. serrulatus*, and can we link drift entry to behavioral processes? Does this species respond similarly to cold and warm TP? We base our approach on previous measurements conducted on insect taxa and suggesting that (a) TP induces behavioral drift, (b) the sensitivity to temperature variation is taxon-specific, and (c) organisms showing an immediate reaction to sudden changes in water temperature rapidly drift because of their active entry in the water column or passive dislodgement due to increased activity on the streambed (Carolli et al. 2012; Bruno et al. 2013).

Methods

Model species

Our model species is *E. serrulatus*, one of the most common species of cyclopoid copepod in Central Europe (Alekseev et al. 2006). This species is found in various water bodies, from lentic habitats such as ponds and lakeshores to flowing environments, i.e. streams and rivers (Jersabek et al. 2001; Alekseev and Defaye 2011). Although sometimes considered as epigean (e.g. Richardson 1991; Richardson 1992; Di Lorenzo et al. 2015), this species is not restricted to the hyperbenthos and is often found in the epibenthos (Shiozawa 1986) and in the interstitial water of river reaches, at depth ranging from a few centimeters to more than one meter (e.g. Rundle and Hildrew 1990; Robertson et al. 1995; Di Lorenzo et al. 2015). We have observed in a previous laboratory study that this species preferentially moves at the surface of the sediment and performs very frequent excursions within the interstices of the sediment bed and into the free water column (Sidler et al. 2017). These behavioral features, i.e. the active exploration of both the water column and the sediment bed, make *E. serrulatus* an interesting model species to identify in lotic copepods the small-scale mechanisms responsible for drift entry or avoidance during rapid changes in the thermal and hydrodynamic properties of their environment.

We sampled copepods from the prealpine, unregulated Töss River near Winterthur (Switzerland), using a Bou-Rouch pump. This method was successfully used in previous studies to sample *E. serrulatus* and other sub-surface copepods at depths ranging from 30 to 150 cm (Fiasca et al. 2014). The reach consists of riffles, pools and gravel bars. Only riffles were sampled to select organisms living in comparatively fast-flowing sections. Interstitial water was pumped from the coarse-grained sediment at a depth of 5–50 cm and filtered to separate benthic organisms from small pebbles and large detritus. Copepods were transferred to the laboratory and cultured for several generations in a growth chamber. They were kept in 20 L buckets and at a constant temperature of 18°C. Like many other freshwater cyclopoids, *E. serrulatus* grazes on bacteria, algae, diatoms and protozoa from the riverbed biofilm community. In our cultures, they were fed with a mixture of *Paramecium*, *Chilomonas paramecium* and the algae *Scenedesmus*. *Paramecium* and *C. paramecium* were grown on wheat grains in soya bean infusion (Kumazawa 2000; Park et al. 2005; Sidler et al. 2017). *Scenedesmus* was grown in autoclaved water and in Conway medium, at 18°C and under a 12L:12D light cycle.

Experimental setup

We tracked copepods by means of three-dimensional particle tracking velocimetry, a quantitative flow measurement technique originally designed for studying turbulent flows (Maas et al. 1993; Malik et al. 1993; Willneff 2003) and recently applied to investigate the behavior of small organisms (Michalec et al. 2017). The experimental setup and methodology are described in detail in Sidler et al. (2017). Here we briefly restate the main features.

We performed measurements in a custom acrylic glass flume (200-cm long and 27-cm wide) fitted with a transparent sediment bed consisting of polyacrylamide spheres (Figure S1). The spheres are approximately 10 mm in diameter. They have nearly the same index of refraction as water, which allows the tracking of moving organisms in the near sediment bed. The setup includes a low- and high-level reservoir, a pump for water recirculation, and a valve with a flow meter to regulate the discharge. Uniform flow conditions are obtained via bricks, voluminous meshes and perforated plates located at the inlet and outlet of the flume. The water level in the flume was kept approximately constant (from 8 to 10 cm above the surface of the sediment bed) using a perforated vertical gate at the outlet of the flume. A laser (527 nm, pulse energy of 60 mJ) provided homogeneous illumination through the sidewall of the flume.

The recording system was composed of four synchronized cameras (Mikrotron EoSens) equipped with 60 mm lenses and recording on two arrays of solid-state disks (DVR Express® Core 2 from IO Industries). The cameras were mounted above the flume at different angles and in a planar configuration (Sidler et al. 2017). They recorded at a frame rate of 100 Hz and were focused on an investigation volume that was approximately 15 cm (x) × 15 cm (y) × 15 cm (z). The investigation volume is located far enough from the inlet and outlet and centered in-between the two sidewalls, where flow conditions are approximately uniform and where there is a developed logarithmic velocity profile (Sidler et al. 2017). The x-axis of the coordinate system is oriented along the downstream direction, the y-axis is oriented along the spanwise direction, and the z-axis points upward, with the origin located at the boundary between the sediment and surface flow region. The z-extent of the observation volume spans both the sediment layer (approx. 7 cm) and the flow depth (approx. 8–10 cm).

The hydraulic conditions in the investigation volume (both in the sediment bed and in the water column) have been previously characterized for base flow (case I) and peak flow (case II) conditions by tracking inert flow tracers (Sidler et al. 2017). Because the mean flow velocities along the spanwise (y) and vertical (z) axes are comparatively small, we focus our analysis on the streamwise velocity, i.e. along the x-axis. From the trajectories of tracer particles, we computed the time-averaged vertical profiles of the streamwise flow velocity component U_f and the profiles of the root mean square of streamwise velocity fluctuations u_f'. We indicate in Table 1 the mean flow velocity averaged over the entire depth of the investigation volume $<U_f>$, the Froude number, and the Reynolds number, for these two flow conditions. Flow case I corresponds to $<U_f>$ = 12 mm/s and flow case II to $<U_f>$ = 67 mm/s. We have previously observed that in our laboratory flume *E. serrulatus* distributes

Table 1. Flow parameters for the two flow conditions. $<U_f>$ is the streamwise flow velocity averaged over the entire depth of the investigation volume. Brackets indicate time-average. $<U_{f,i}>$ and $<U_{f,w}>$ are the depth-averaged flow velocity in the interface and in the water column, respectively. u_f' is the root mean square of the flow velocity fluctuations. *Fr* is the Froude number. *Re* is the Reynolds number. The water depth was kept at approximately 8–10 cm above the surface of the sediment.

Flow case	I	II
$<U_f>$ (mm/s)	12	67
$<U_{f,i}>$ (mm/s)	9.1	44.1
$<U_{f,w}>$ (mm/s)	19.5	92.1
max. U_f (mm/s)	20	97
u_f' (mm/s)	2.5	7.6
Fr	0.012	0.065
Re	1270	7410

preferentially at the surface of the sediment but also explores actively both the water column and the interstitial spaces of the sediment bed (Sidler et al. 2017). The flow velocity is very small in the sediment bed, increases logarithmically above the sediment surface layer and is maximal in the water column. We therefore divide our investigation domain into three compartments that correspond to these three regions of different flow velocities and different copepod distribution: the sediment bed, the interface, and the water column. The interface corresponds to a narrow layer where the abundance of copepods is the highest. It is one centimeter thick and centered at the surface of the sediment bed and at the origin of the vertical axis (Sidler et al. 2017). Table 1 indicates the depth-averaged mean flow velocity in the interface $<U_{f,i}>$ and in the water column $<U_{f,w}>$.

Discharge and temperature variation

Copepods (approx. 2000 individuals) were gently transferred into the flume, and allowed to acclimate to the recording conditions and to distribute in the entire flume for 30 min before the recording started. Because the swimming behavior of adult copepods is more vigorous than that of nauplii or copepodids, and also because the velocities reached during jumps scale with body length (Kiørboe et al. 2010; Bradley et al. 2013; Wadhwa et al. 2014), we used individuals of comparable size (adults and late-stage copepodids with a prosome longer than 300 μm) in all our measurements to ensure reliable behavioral observations. Flow case I was maintained during the duration of the acclimation period to expose copepods to realistic hydraulic conditions. At these low flow velocities, copepods distribute freely in the experimental flume in all directions and explore both the sediment bed and the water column (Sidler et al. 2017). The motion of copepods was recorded during HP which we realized by increasing the discharge from flow case I to flow case II over a time span of 5 min (*upward ramp*), keeping it at steady state for 4 min (*high flow*) and reducing it again to case I over a time span of 5 min (*downward ramp*) (Figure 1(A)). Although the flow velocities achieved at flow case II are relatively small compared to values recorded in situ (up to approx. 1 m/s, e.g. Gibbins et al. 2007), they allow the quantification of fine-scale behavioral processes that would be difficult to observe under stronger hydrodynamic conditions (Richardson 1992).

We simulated TP by warming or cooling, via the addition of warm water or ice, the temperature of the water pumped from the low-level reservoir and released in the flume via the high-level reservoir. Water temperature was measured every second both in the water column and in the sediment bed with temperature sensors (WTW Multi 3240) located immediately downstream of the flow dampening structure. Figure 1(B) shows that the variation in temperature occurred shortly after the addition of ice or warm water. The temperature in the water column decreased from 20°C to 14°C
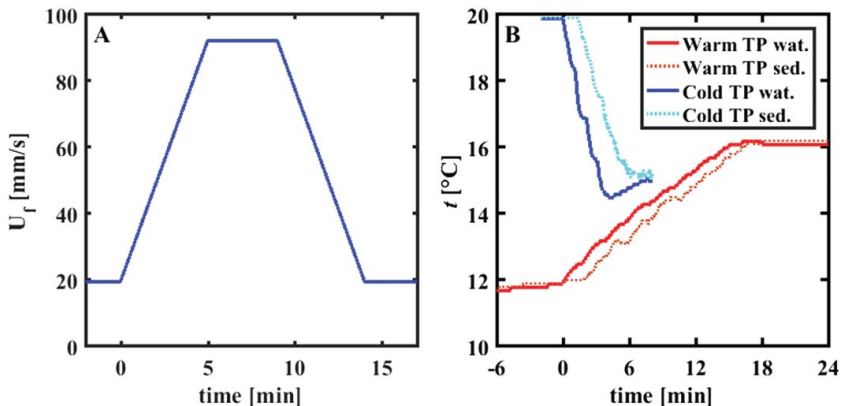

Figure 1. (A) Time evolution of the discharge. (B) Time evolution of the temperature recorded in the water column (solid line) and in the interstitial spaces of the sediment bed (dotted line) during the cold (blue) and warm (red) thermopeaking simulations.

in approx. 4 min during the cold TP simulation, and increased from 11°C to 16°C in approx. 15 min during the warm TP simulation. The temperature in the sediment paralleled that in the free water with a delay of approx. 1 minute. It is important to note that copepods have been acclimated to the initial temperature of the warm TP simulation several weeks before the measurements by maintaining the cultures at 11°C. We conducted TP simulation under flow case I to maintain a flowing environment that allows copepods to move freely in the experimental flume. During both HP and TP measurements, drifting organisms were collected in a retention box at the outlet of the flume, and regularly fed back into the flume via a separate recirculation system. This recirculation led to a nearly constant number density of copepods in the flume. We obtained 357522 coordinates for the HP measurement, 822978 coordinates for the cold TP measurement, and 536152 coordinates for the warm TP measurement.

The relative streamwise velocity of the copepods $u_{c,rel}$ was calculated as the difference between their streamwise velocity $u_{c,abs}$ in laboratory coordinates and the time-averaged flow velocity U_f at their instantaneous vertical position. The relative velocity of copepods is stronger than the turbulent velocity fluctuations for the two flow cases, and therefore mostly captures their active behavior: positive values indicate active downstream swimming, whereas negative values indicate active countercurrent swimming. We have previously shown that *E. serrulatus* swims preferentially at the surface of the sediment and explore the interstitial spaces of the sediment bed and the water column (Sidler et al. 2017). Its behavior remains constant in the sediment because flow velocities are very small and irrespective of the discharge. We therefore focus our analysis of the effects of water velocity in these two compartments: the water column and the interface layer between the sediment and the water column.

Results

Hydropeaking

We show in Figure 2(A) the velocity of copepods u_c and the mean flow velocity U_f in the interface layer and in the water column (velocities here and for all following results are averaged over segments of 1-minute duration). The mean flow velocity in the water column increases from approx. 20 mm/s to approx. 92 mm/s and returns to approx. 20 mm/s at the end of the experiment. The velocity of the copepods increases with the flow velocity but remains substantially lower than U_f during the entire measurement and in the two compartments. In the water column, the difference between the velocity of copepods $u_{c,w}$ and the flow velocity $U_{f,w}$ was maximal at the highest discharge and also very substantial: the active motion of copepods reduces their downstream velocity
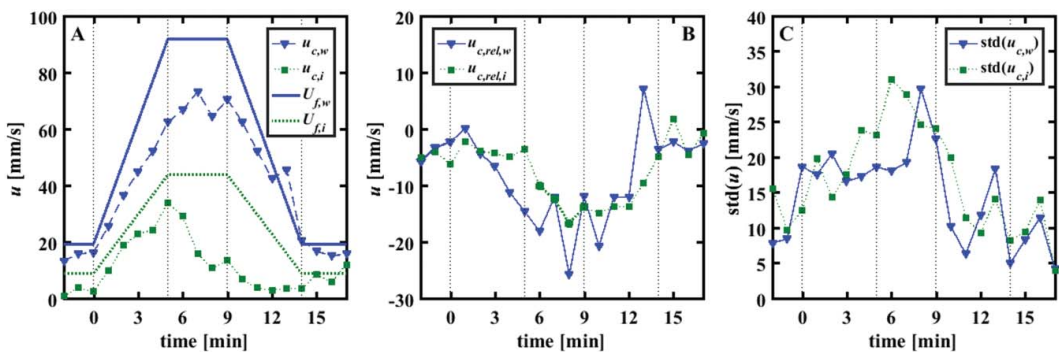

Figure 2. (A) Copepod absolute velocity u_c and flow velocity U_f in the water column (blue) and in the interface layer (green) during the hydropeaking simulation, averaged over periods of 1-minute duration. (B) Copepod relative velocity in the water column (blue) and in the interface layer (green). (C) Standard deviation of the absolute velocity of the copepods in the water column (blue) and in the interface layer (green). Vertical grey lines indicate the time onsets of periods of constant discharge and varying discharge.

by approx. 30 mm/s. In the interface layer, the velocity of copepods $u_{c,i}$ increases with the flow velocity $U_{f,i}$ but decreases substantially during steady state and only slightly during the downward ramp. Figure 2(B) shows the relative downstream velocity of copepods in the water column and in the interface layer. The relative velocity was approximately similar before HP in the two compartments. During the upward ramp the magnitude of $u_{c,rel,w}$ increases strongly, which indicates an increasingly vigorous counter-current swimming behavior, whereas $u_{c,rel,i}$ remains approximately constant. Copepods swimming in the water column were able to maintain their upstream swimming behavior over the duration of the high flow period. The relative velocity of copepods in the interface layer decreases substantially during high flow, in accordance with the trend observed with their absolute velocity. Figure 2(B) also indicates the existence of a surprising delay between the onset of the downward ramp and the decrease of $u_{c,rel,i}$. We quantify the intermittency in the motion of *E. serrulatus* via the standard deviation of its velocity (Figure 2(C)). The standard deviation shows large fluctuations but an overall trend of higher intermittency is visible during upward ramp and high flow both for $u_{c,i}$ and $u_{c,w}$.

Thermopeaking

During the TP simulation the flow velocity remained constant at flow case I. Therefore, any variation in the velocity of copepods indicates changes in the kinematics of their swimming behavior that are caused by the variation in temperature. As our results show no effect of temperature variation on the velocity of copepods in the sediment bed, we focus our analysis on changes in behavior that occur in the interface layer and in the water column.

We show in Figures 3(A) and 4(A) the streamwise velocity of copepods in the water column and in the interface layer during the cold and warm TP measurements. The velocity before TP is roughly similar in copepods that have been acclimated to different temperatures (20°C for the cold TP and 12°C for the warm TP) for several weeks before the simulations. This suggests that our results are not affected by the temperature conditions of the culture. The velocity of copepods in the interface layer shows no clear trend both in the cold and warm TP simulations because of relatively large fluctuations that seem unrelated to variations in temperature. In the water column for the cold TP simulation, the downstream velocity of copepods increases during temperature change and decreases progressively to its initial value after reaching the final temperature. In the water column for the warm TP simulation, the velocity of copepods remains approximately constant over the course of
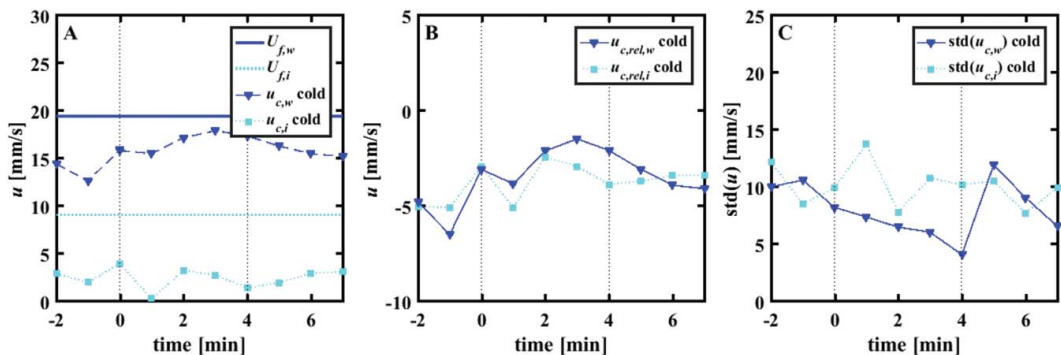

Figure 3. (A) Copepod absolute velocity in the water column $u_{c,w}$ and in the interface layer $u_{c,i}$ during the cold thermopeaking simulation, averaged over periods of 1-minute duration. The two horizontal lines indicate the flow velocity (which remains constant) in the water column and in the sediment layer. (B) Copepod relative velocity in the water column $u_{c,rel,w}$ and in the interface layer $u_{c,rel,i}$. (C) Standard deviation of the absolute velocity of the copepods in the water column (solid line with triangle symbols) and in the interface layer (dotted line with square symbols). Vertical grey lines indicate the time onsets of periods of constant temperature and varying temperature.
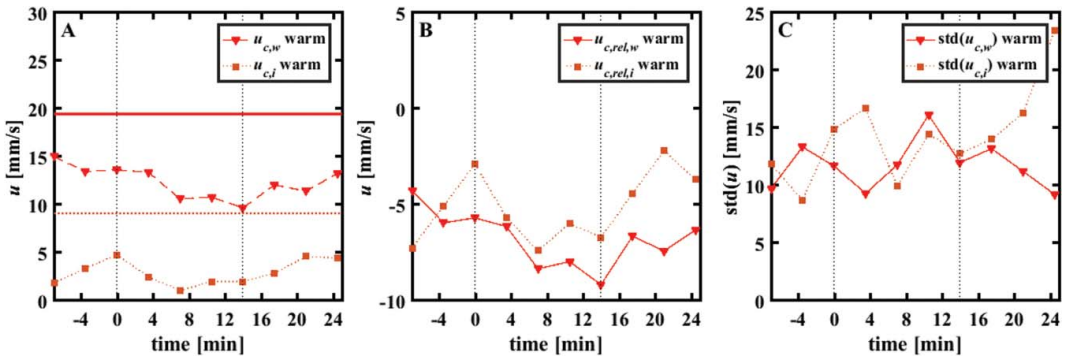
Figure 4. (A) Copepod absolute velocity in the water column $u_{c,w}$ and in the interface layer $u_{c,i}$ during the warm thermopeaking simulation, averaged over periods of 1-minute duration. The two horizontal lines indicate the flow velocity (which remains constant) in the water column and in the sediment layer. (B) Copepod relative velocity in the water column $u_{c,rel,w}$ and in the interface layer $u_{c,rel,i}$. (C) Standard deviation of the absolute velocity of the copepods in the water column (solid line with triangle symbols) and in the interface layer (dotted line with square symbols). Vertical grey lines indicate the time onsets of periods of constant temperature and varying temperature.

the measurements in one replicate but decreases during temperature change before returning to its initial velocity value after temperature change in the second replicate (shown in Figure 4(A)).

We show the relative velocity of copepods in Figures 3(B) and 4(B). Negative velocity values indicate counter-current swimming. During the cold TP simulation, the magnitude of these counter-current swimming velocities reduces during temperature change before increasing again at the final temperature both in the water column and in the sediment. The reduction of the averaged relative copepod velocity in the water during the cold TP ramp is also accompanied by a slight reduction of the copepod activity (shown in Figure 3(C)). No clear trend is visible during the warm TP simulation because the magnitude of the velocity variation observed before the temperature change is comparable to the magnitude observed during TP in both the interface layer and the water column. Similarly, the standard deviation of the velocity shows no clear pattern both in the water column and in the interface layer because of large fluctuations that appear unrelated to the temperature variation (Figure 4(C)).

We show in Figure 5 a subset of trajectories recorded during the cold TP measurement. During the entire TP and HP measurements, both during the transients and under steady flow or temperature conditions, copepods alternated periods of passive drifting with active, predominantly
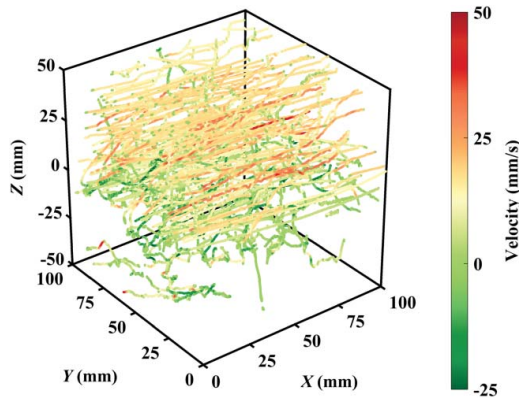
Figure 5. Subset of trajectories recorded during the cold thermopeaking measurement. Trajectories are color-coded with the streamwise velocity. Positive values indicate downstream motion. Negative values indicate active upward swimming. The mean flow velocity is 19.5 mm/s. Copepods alternate periods of passive drifting with active upstream swimming. The velocity of drifting copepods is determined by the velocity of the underlying flow when copepods remain passive, and by the combination of flow velocity and behavior when copepods perform active swimming.

counter-current swimming. The total velocity of drifting copepods is determined by the velocity of the flow when copepods remain passive, and by the combination of flow velocity and behavior when copepods perform active swimming. Because subsequent periods of passive drifting and active swimming occur independently between individuals, there exists a strong variability in the type of motion performed by different copepods at a given time instant. This variability is evidenced by the standard deviation of the velocity shown in Figures 2(C), 3(C) and 4(C). However, patterns that do not depend on inter-individual variability are visible when considering many copepods and averaging over a large number of observations. For instance, Figure 5 shows that the velocity of copepods drifting in the water column is on average lower than the mean flow velocity, indicating that self-locomotion on average opposes downstream flow transport, as evidenced in Figure 2(A). It also shows that upstream motion occurs predominantly within the interface layer, where the flow velocity is lower, and within the interstitial space of the sediment where flow velocity is almost negligible, even though vigorous upstream swimming was occasionally observed even in the water column.

Discussion

Downstream drift of lotic organisms is a common phenomenon and a key aspect of the population dynamics of benthic invertebrates in streams (Palmer et al. 1996). Its ecological importance has prompted much research, particularly in the context of catastrophic drift caused by the sudden increase in discharge following water release from hydropower plants. However, much of this research has focused primarily on understanding the causes and temporal patterns of drift (e.g. Robinson et al. 2004). The small-scale mechanisms responsible for the entry of benthic animals into the water column remain unresolved (Blanckaert et al. 2013). Drift is not necessarily a passive process caused by hydrodynamic conditions, but can be initiated by the organism, presumably to move away from areas with, e.g. limited food resources or where competitors or predators are present (Huhta et al. 2000; Heiber et al. 2003). Field studies suggest that variations in water temperature result in behavioral drift. However, no information is available on the mechanisms that lead to individual organisms leaving the sediment bed. In this study, we have quantified the effects of flow velocity and temperature variations on the small-scale behavior of the freshwater cyclopoid copepod *E. serrulatus* swimming freely in a laboratory flume fitted with a transparent sediment bed. Using an advanced particle tracking technique, we have reconstructed their trajectories in three dimensions and quantified changes in the kinematics of their motion.

We show that copepods increase their counter-current swimming effort in response to increasing flow velocity. In the field measurements of Bruno et al. (2010), peaks in drifting invertebrates occurred within 5–10 min after the beginning of the HP wave, and most of the invertebrates were washed out within the first 15 min of the water release. Drift abundance and composition returned to base-flow levels for most of the benthic taxa after 25 min. In our measurements, we observed no clear effect of variation in discharge on drift density, which may be explained by the finite size of our laboratory flume and the relatively short duration of our HP simulation. It was therefore not possible to observe a timeframe for drift onset. However, we observed that the increase in swimming effort occurred above a threshold in flow velocity of approx. 40 mm/s. The resulting counter-current swimming caused a substantial reduction in the downstream transport velocity of copepods, which may therefore results in a lower drift. During our measurements, *E. serrulatus* was mainly found at the surface of the sediment. It also explored the water column and performed very frequent incursions within the interstitial spaces of the sediment bed. Bruno et al. (2010, 2016) link different timeframes for the beginning of the drift to taxon-specific habitat preferences and behavioral adaptations. In their measurements, most taxa with no morphological or behavioral adaptations to high flow velocity began to drift quickly after the arrival of the HP wave. Indeed, field and flume measurements show that least-resistant taxa (e.g. *Baetis* spp. and Chironomidae) are rapidly removed from the surface of the sediment (Gibbins et al. 2005; Bruno et al. 2010, 2016). Species with adaptations (e.g. Simuliidae and Hydropsychidae) began to drift after a longer delay. Hence, habitat preferences and behavioral traits can strongly influence the effects of HP (Holomuzki and

Biggs 2000; Jakob et al. 2003; Mochizuki et al. 2006). In Bruno et al. (2010) certain taxa were not or little affected by the increase in discharge (e.g. Harpacticoida and Nematoda) because they inhabit the hyporheic habitat and hence are not exposed to shear stress. These authors observed that several taxa of Harpacticoida and Cyclopoida were even more abundant in drift before the HP wave than during the wave, which suggests that these organisms move deeper into the sediment to escape from the increase in shear stress. In our measurements, we observed no clear effect of the increase in discharge on the vertical distribution of *E. serrulatus*. It was therefore not possible to conclude on the possible use of the interstitial spaces within the sediment bed as a refuge. However, the energetic counter-current swimming, coupled with frequent stops in the interstitial spaces of the sediment bed where velocity is negligible, may allow this species to reduce downstream transport, at least up to a level where flow velocity overwhelms their swimming ability.

In our measurements, copepods reacted differently to warm and cold variations in temperature. Decreasing temperature resulted in a substantially lower counter-current swimming effort, which may therefore increase drift. Rising temperature had no clear effect on their behavior. Carolli et al. (2012) and Bruno et al. (2013) conducted TP simulations in open flumes fed by an Alpine stream. They observed a higher drift, but with marked differences between taxa. Some taxa responded strongly to the variation in temperature, whereas others were unaffected. They also observed stronger effects during cold TP simulations than during warm TP simulations, particularly for harpacticoid copepods. In their measurements, and contrary to HP for which taxon-specific time thresholds of discharge exposure must be reached before the organisms start drifting, taxa responded quickly and started to drift within 2 min after being exposed to the thermal alteration. These authors conclude that benthic invertebrates exposed to temperature variations might require only a disturbance level and not an exposure time threshold to start drifting. Therefore, variations in water temperature trigger a drift that is probably behavioral and that may result from organisms actively entering the water column or being swept away by the flow because of increased activity at the surface of the sediment bed (Carolli et al. 2012; Bruno et al. 2013). Our results indicate a lower magnitude of the relative velocity (and hence a weaker counter-current swimming behavior) during the cold TP simulation, which may result in higher drift. However, we found no evidence of active drift entry and no evidence for copepods moving from the interstitial spaces to the surface of the sediment bed and being dislodged by the flow. Factors that may explain the difference in sensitivity between cold and warm TP are difficult to define, and the lack of previous observations on cyclopoid copepods makes the interpretation of our results difficult. Different species will respond differently to TP according to their thermal preference. In Carolli et al. (2012) for instance, larvae of Simuliidae, which have morphological adaptations to resist high flow velocity and hence are not strongly affected by the HP wave, strongly increased in drift during TP simulations. Organisms will also respond differently according to their developmental stage. Simulations conducted in the warm season by lowering water temperature produced stronger effects than simulations conducted in the cold season, but this difference was attributed in part to different densities of organisms in the benthos and to the relative abundance of different life stages, early instars having a higher propensity to drift (Carolli et al. 2012). Copepods change their swimming velocity when the temperature of their environment varies. The measurements of Moison et al. (2012) for instance show that the marine calanoid *Temora longicornis* swims faster and increases its activity in warmer water. In their study copepods were acclimated to different temperatures and the temperature did not vary during the recording. In our study, we conducted measurements using copepods acclimated to two different temperatures and we increased or lowered the temperature during the recording. Because we used individuals from the same development stages (late-stage copepodids and adults) that originated from the same culture, and also because the initial velocity before the temperature wave is comparable in cold and warm-acclimated copepods, the different response to cold and warm TP reflects a difference in sensitivity rather than seasonal effects. Finally, the lack of any observable effect in the warm TP cannot be explained by copepods hiding in the sediment layer, as the temperature in the sediment paralleled that in the water column.

Mitigating the ecological impacts of hydropower plant operation requires a better knowledge of the small-scale processes occurring in the early stages of the disturbance and leading to drift entry or avoidance. This study represents a contribution to the understanding of the effects of flow and thermal regime alterations on the benthic community. We expect that our results will assist and evoke further research to quantify the contribution of individual behavior to important ecosystem-wide processes in lotic environments.

Funding

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