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Three-Dimensional Observation of Immediate Behavioral Response of Benthic Freshwater Copepods to Hydro- and Thermopeaking

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PhD Thesis

**Three-Dimensional Observation of Immediate Behavioral Response
of Benthic Freshwater Copepods to Hydro- and Thermopeaking**

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‘Ich kann, weil ich will, was ich muss’
Immanuel Kant

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ABSTRACT

Drift of stream benthic invertebrates is a fundamental ecological process that is increasingly influenced by anthropogenic impacts due to e.g. hydropower operation, and by variability, due to e.g. climate change. The intermittent energy production by high elevation dam hydropower plants is responsible for periodic alterations of stream velocity and temperature, known as hydropeaking (HP) and thermopeaking (TP), respectively. These alterations have adverse impacts on the ecosystems downstream and are responsible for biological deficits due to passive washing away of invertebrates during high flow, the so-called “catastrophic drift”. Drift also occurs due to active behavior when organisms actively enter the water column to find better conditions downstream. It has been very difficult to distinguish between passive and active (behavioral) effects until recently because of the lack of appropriate measurement techniques to study behavior of fine temporal and spatial scales and near or in the sediment layer of rivers. The mechanisms for population persistence are fundamental for understanding and predicting environmental impacts but the behavioral traits thereof are still not well understood. This thesis investigates the behavior of benthic invertebrates exposed to HP and TP waves in the laboratory using three-dimensional high speed particle tracking (3D-PTV). We developed, designed, built and successively improved an experimental setup with an acrylic glass flume of 2 meter length, a closed water circle and a transparent sediment bed consisting of polyacrylamide spheres with an optical refractive index similar to water. This sediment bed mimicked a natural prealpine gravel river, with the possibility to observe animals inside the sediment bed. We selected the cyclopoid copepod *Eucyclops serrulatus* as a representative species. Cyclopoid copepods are a very important link in aquatic and riverine food webs and live in the free water, on the sediment surface but also inside the sediment bed. We reconstructed the trajectories of many copepods and quantified their velocity and vertical distribution under statistically steady state flow conditions. Copepods were able to swim in the entire flume and moved readily in the sediment bed. They moved by successive jumps and often against the flow. We recorded the swimming of copepods at different flow conditions at statistically steady states. We isolated the behavioral component of their motion by subtracting the local flow velocity. We showed that copepods perform active counter-current swimming to limit downstream drift. The counter-current swimming effort increased with flow velocity and therefore downstream drift remained moderate and did not vary strongly for the lower and middle flow rates tested and was reduced for the high flow rate. Furthermore, we described the reaction of *E. serrulatus* to changes in the flow velocity and temperature in the flume that are comparable to the alterations imposed by HP and TP in prealpine rivers. We investigated separately the effects of a sudden but steady variation in the flow velocity and in the water temperature on the small-scale swimming behavior by changing the discharge and by adding ice or hot water to the system, respectively. Copepods increased substantially their counter-current swimming effort in response to the increasing flow velocity. This behavioral response seems to occur above a threshold in flow velocity of approx. 40 mm/s and results in a reduction in their downstream transport. Copepods reacted differently to warm and cold variations in temperature. Decreasing temperature resulted in a substantial decrease in their swimming effort with a net downstream flux, whereas rising temperature had no clear effect on their behavior. Our setup enables the manipulation of chemo-physical parameters in a laboratory flume and a detailed observation of fine-scale behavioral processes of benthic invertebrates that could not be approached in field studies or through theoretical approaches. Our

results confirm the importance of behavioral traits in drift avoidance for species that lack an aerial stage and highlight the importance of understanding the behavioral traits that mediate the response of stream invertebrates to disturbances in the hydraulics and thermal dynamics of their environment.

ZUSAMMENFASSUNG

Der Drift von benthischen Fliessgewässerinvertibraten ist ein fundamentaler ökologischer Prozess welcher zunehmend durch anthropogene Einwirkungen aufgrund von Wasserkraftnutzung aber auch Schwankungen im Zuge des Klimawandels beeinflusst wird. Die variable Stromproduktion von stauseegespiessenen Hochdruckwasserkraftanlagen ist verantwortlich für die periodischen Änderungen von Fliessgeschwindigkeit und Temperatur im Unterstrom der Kraftwerke, bekannt als Schwall und Sunk. Diese Änderungen haben negative Auswirkungen auf die Ökosysteme im Unterwasser und sind verantwortlich für biologische Defizite aufgrund der passiven Ausschwemmung von Invertibraten bei hohem Abfluss während dem Turbinierbetrieb, dem sogenannten katastrophalen Drift. Drift kann aber auch durch aktives Verhalten entstehen, wenn Organismen aktiv in die Wassersäule schwimmen um unterstrom bessere Verhältnisse zu finden. Bis vor kurzem war es kaum möglich, zwischen aktiven (durch Verhalten) und passiven Effekten zu unterscheiden, da keine geeigneten Messtechniken bestanden um das Verhalten mit hoher Auflösung in Zeit und Raum und nahe oder sogar im Sediment von Flüssen zu studieren. Mechanismen für die Artbeständigkeit sind fundamental für das Verständnis und die Vorhersage von Umwelteinflüssen aber die Verhaltenszüge davon sind immer noch unklar. Diese Dissertation untersucht das Verhalten von benthischen Invertibraten während Schwall und Sunk im Labor mittels dreidimensionalem Partikelverfolgungsgeschwindigkeitsmesssystem (3D-PTV). Wir entwickelten, dimensionierten und konstruierten und verbesserten schrittweise einen experimentellen Aufbau mit einer 2 Meter langen Plexiglasrinne, einem geschlossenen Wasserkreislauf und einem transparenten Sedimentbett aus Polyacrylamidkugeln mit einem optische Brechungsindex ähnlich dem von Wasser. Dieses Sedimentbett ist einem natürlichen voralpinen Kiesfluss nachempfunden, mit der Möglichkeit Tiere im Sediment zu beobachten. Wir haben den cyclopiden Kopepoden *Eucyclops serrulatus* als repräsentative Spezies ausgewählt. Cyclopoide Kopepoden (Ruderfusskrebse) sind ein sehr wichtiges Bindeglied in aquatischen und Fliessgewässernahrungsnetzen und leben im freien Wasser, auf der Sedimentoberfläche aber auch im Sediment selber. Wir haben die Trajektorien von vielen Kopepoden rekonstruiert und haben ihre Geschwindigkeit und die vertikale Verteilung unter statistischen Gleichgewichtsbedingungen des Abflusses quantifiziert. Die Kopepoden konnten in der gesamten Rinne umherschwimmen und haben sich auch im Sediment fortbewegt. Das Schwimmen besteht dabei aus wiederholten Sprüngen, oft auch gegen die Fliessrichtung. Wir haben das Schwimmen von Kopepoden in verschiedenen Gleichgewichtszuständen des Abflusses aufgenommen. Wir haben den verhaltensbedingten Anteil der Bewegungen isoliert, indem wir die lokale Fliessgeschwindigkeit abgezogen haben. So konnten wir zeigen, dass Kopepoden aktiv gegen die Strömung anschwimmen um das Driften stromabwärts zu minimieren. Das Schwimmen gegen die Strömung hat mit zunehmender Fliessgeschwindigkeit ebenfalls zugenommen und daher konnten die Tiere ein Abdriften bei kleinen und mittleren Fliessgeschwindigkeiten in ähnlichem Umfang halten und bei grösseren Abflüssen reduzieren. Weiter haben wir die Reaktion von *E. serrulatus* auf Änderungen in der Fliessgeschwindigkeit und der Temperatur in der Rinne, wie sie bei Schwall und Sunk in voralpinen Flüssen auftreten, beschrieben. Wir haben separat untersucht, wie sich die Effekte einer plötzlichen aber konstanten Änderung in der Fliessgeschwindigkeit und der Wassertemperatur auf das Schwimmverhalten der Tiere auswirkt, indem wir den Abfluss geändert und dem Kreislauf Eis oder heisses Wasser zugeführt haben. Die Kopepoden erhöhten

ihre Schwimmfähigkeit gegen die Strömung mit zunehmendem Abfluss. Das Verhalten schien aber erst ab einer Strömungsgeschwindigkeit von ca. 40 mm/s aufzutreten und reduzierten so ihr Abdriften. Die Kopepoden reagierten unterschiedlich auf das Abkühlen und Erwärmen des Wassers. Das Reduzieren der Wassertemperatur resultierte in einer Abschwächung des Schwimmens gegen die Strömung und dadurch zu einem erhöhten Fluss an Tieren in Strömungsrichtung, während bei einer Erhöhung der Wassertemperatur kein klarer Effekt im Schwimmverhalten festgestellt werden konnte. Unser Versuchsaufbau ermöglicht die Kontrolle über physio-chemische Parameter in einer Laborrinne und die detaillierte Untersuchung von kleinskaligen Verhalten von benthischen Invertebraten, was in Feldstudien oder mit theoretischen Ansätzen nicht möglich war. Unsere Resultate bestätigen die Wichtigkeit von Verhaltenszügen für die Reduktion der Abschwemmung von Tieren ohne Flugstadium und unterstreichen die Wichtigkeit, Verhaltenszüge von Invertebraten in Fließgewässern zu verstehen und wie diese Reaktionen auf Störungen im hydraulischen und thermischen Umfeld auslösen.

Chapter *1*

INTRODUCTION

1.1 Motivation

Renewable energy sources are a highly disputed subject nowadays but have a strongly increasing demand. The most important pillar in the energy strategy of Alpine countries for renewable electricity is clearly the hydropower. It is the most important renewable electricity source worldwide and has a high importance in the national strategies for the global CO₂ balance (Bratrich et al. 2004) and the substitution of fossil fuels. The generation of hydropower also has a high economic value and plays a key role in the European electricity system with its possibility to regulate the electricity production in minute scale and react to the highly volatile energy demand and energy generation by other renewable energy producers. An important part of the energy production by hydropower is generated with high elevation dam hydropower plants. Furthermore these power plants can be equipped with pumps to use a temporary surplus of generated energy from other producers to refill the high elevation reservoirs with water and even increase their effect to counterbalance the energy system.

As one of the negative effects of hydropower generation, the intermittent electricity production is directly coupled with an intermittent release of water from the turbines into the receiving water body. Depending if the receiving water body is a lake, a river or a stream and on the amount of released water, the discharge in the receiving water body can be changed by a factor reaching values of small fractions to even a few tens (see e.g. Zolezzi et al. 2011; Baumann et al. 2012). This alteration of the discharge in alpine rivers by hydropower plants is called hydropeaking (HP). In the case of high elevation dam hydropower plants, the water is mostly taken from the hypolimnion, below the thermocline with nearly constant water temperature throughout the year. The water temperature of the receiving river varies with the seasons and also daily and is very often different than the one of the turbinated water. The release of the turbinated water therefore changes the temperature of the receiving river. In winter, this leads to a warming of the cold river, in summer the warm water of the river gets cooled. These abrupt alterations of the temperature by hydropower plants are called thermopeaking (TP) waves. In this way high elevation dam hydropower plants alter in the receiving river the discharge and the temperature and related variables as the water depth, the width of the river, the water velocity, the shear stress

on the bottom, the amount and the composition of suspended biologic and abiotic materials, the chemical composition and water temperature (Ward and Stanford 1979; Cushman 1985; Moog 1993; Webb & Walling 1993; Fette et al. 2007; Olden and Naiman 2010).

The water released from a high elevation dam with a different temperature than the receiving river increases the discharge and thereby the water level and the flow velocity. The hydrodynamic water wave travels faster as the temperature wave. This leads in the propagation downstream to a divergence of the two waves and at large enough distance downstream the two waves are experienced separately but in consecution (Toffolon et al. 2010, Zolezzi et al. 2011, Vanzo et al. 2016). These manmade alterations and the induced adverse effects on the riverine ecology are the subject of a multitude of research articles, which mostly focus on the long term impacts, the disappearing of local species and resulting alterations in the food web and affecting higher taxa (e.g. Cushman 1985, Moog 1993, Robertson et al. 1995, Céréghino et al. 2002, Jakob et al. 2003, Robinson & Uehlinger 2008, Gibbins et al. 2010, Robinson 2012, Hauer et al. 2017, Holzapfel et al. 2017). Net samplings and studies on short term animal processes during single events are available down to resolutions of minutes and even organisms as copepods with body lengths of around a millimeter are mentioned (e.g. Lancaster 1999, Imbert & Perry 2000, Bruno et al. 2012, Casas-Mulet et al. 2016, Schülting et al. 2016). With core samplers and Bou-Rouch samplers even animals inside the sediment bed can be sampled at a spatial resolution of some centimeters (Poole and Stewart 1976, Dole-Olivier and Marmonier 1992, Palmer et al. 1992, Holomuzki & Biggs 2000, Hahn 2005). Field studies thereby struggle with the trade-off between size and time resolution, as small sized animals cannot be observed directly but only as a sample over a collection period. As it comes to the sediment bed the situation is even more difficult due to the sampling methods. This leads to a lack of studies about the actual behavior of animals smaller than macroinvertebrates in the flowing water and the real-time behavior of animals inside the sediment is elusive in most cases.

1.1.1 *Cyclopoid copepods*

Among the community of animals in rivers, cyclopoid copepods play an important role in the food web and are a key element in lotic ecosystems (Reiss and Schmid-Araya 2008). Copepods belong to the sub-phylum of crustaceans, can be found in nearly every freshwater body, are extremely abundant and form a major part of most ecological communities in the groundwater, the benthic interstices and in the free water column (Boxhall & Defaye 2007). Cyclopoid copepods constitute an important order including widespread taxa. They are one of the most abundant and successful groups in freshwater ecosystems (Park et al. 2005) and can be found in most prealpine grave-bed rivers, from the free water down into the sediment (Brunke and Gonsler, 1999). Out of the cyclopoid copepods in Europe, *Eucyclops serrulatus* is with around 1mm in body length one of the most common (Maier 1990) species and can be very abundant. Figure 1 shows photographs of two individuals of *E. serrulatus*, with an ovigerous female in panel (A), out of the population extracted from the river Töss and cultured in our laboratory.

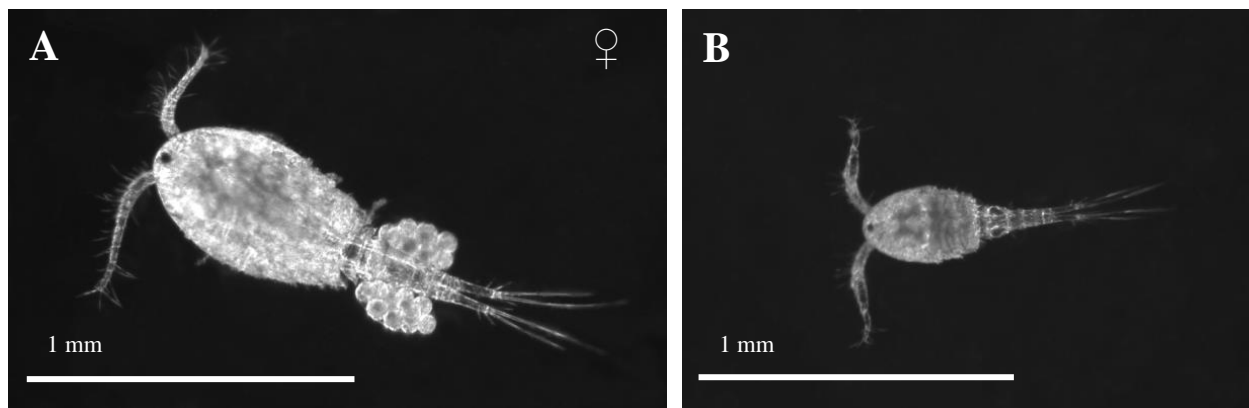


Figure 1. Two images from our cultures of *Eucyclops serrulatus*, with a female in panel (A). Pictures by author.

Despite their small size, copepods are reputed to perform very strong and directed displacement jumps. Studies suggest that these jumps enable copepods to perform self-locomotion despite directed currents and strong hydrodynamic conditions (e.g. Genin et al. 2005). Copepods are also known to respond behaviorally and immediately to variations in the physical and chemical properties of their environment.

Although *E. serrulatus* can be abundant and is an important link in lotic food webs, the information on their behavioral ecology as benthic copepod remains scarce. This species can be found in water bodies, from lentic habitats and lotic environments to sediment interstices with depths ranging to more than one meter (e.g. Rundle and Hildrew 1990; Robertson et al. 1995; Di Lorenzo et al. 2015). The available literature about *E. serrulatus* mostly covers the description of anatomic characteristics for taxation and therewith the mapping (e.g. Alekseev and Defaye 2011), effects of temperature, diets and toxins on their life history (see e.g. Maier 1990, Nandini and Sarma 2007, Di Lorenzo et al. 2015) or their genetics (e.g. Tinson and Laybourn-Parry 1985, Christie 2015). Existing studies about the reaction of copepods to HP and TP waves (see Bruno et al. 2010, Bruno et al. 2012 and Carolli et al. 2012) focus on net samples taken from the free water, have a rather coarse time resolution of two minutes and are done as drift samplings with meshes. These sampling results suggested a reduced abundance by increasing flow velocity and a strong increase of individuals in the water during temperature waves. An observation of the actual behavior to distinguish e.g. passive dislodgement and active behavior has not been performed.

Benthic copepods living in streams are continually exposed to flowing water and subject to drift. To act against downstream drift to stabilize in a preferred habitat several strategies are possible, such as upstream flight of adults to oviposit or upstream-biased dispersal. One possibility also for species with no aerial life stage is a random or directed upstream movement near or in the sediment bed (Poff and Ward 1992; Speirs and Gurney 2001; Humphries and Ruxton 2002). However, this actual behavior is only observed in some cases on the sediment surface and for bigger invertebrates, but not for benthic species or copepods.

The aim of this dissertation is to get an insight into the ecology and behavior of benthic copepods in a lotic environment and to their reaction on HP and TP waves, with *E. serrulatus* as an important representative species for the meiofauna of prealpine rivers. As an observation in nature is not yet possible, we decided to perform flume experiments in the laboratory, with

controllable physical conditions and a transparent sediment bed that allows for monitoring of organisms in the sediment interstices. The observation of the fast swimming animals in a relatively large volume is achieved by a camera system consisting of four cameras for stereoscopic imaging, with a high recording rate and a strong laser illumination. We find that *E. serrulatus*, as a representative species for benthic cyclopoid copepods in rivers, mostly moves near the sediment bed but frequently explores the free water as well as the interstices in the sediment. The swimming consists of consecutive, short, but intense jumps with high velocities compared to their body size. With increasing flow velocity, above a threshold of around 40 mm/s, the copepods increase significantly their counter-current swimming, which could represent a possible strategy for drift avoidance. As a reaction to cold TP waves, the copepods reduce their active upstream swimming which results in an increased mean drift velocity. There is no clear trend visible for warm TP waves.

1.1.2 *Experimental design*

The experiments for this dissertation are performed under hydraulic and thermal conditions that are in the range of those of event characterizations (see e.g. Zolezzi et al. 2011, Vanzo et al. 2016) of prealpine rivers affected by hydropower plant operation, but with the advantages of a laboratory flume with parameters that are uniform, well defined and therefore reproducible. The site we collected the animals from is the prealpine river Töss in the north of Switzerland, with water temperatures ranging over the year from 0 to 21°C and changes during single days of up to around 5°C. The chosen temperatures for the experiments between 11° and 19°C are therefore in a suitable range for *E. serrulatus* that are encountered in nature. Kumazawa (2000) reported that suitable culturing conditions can be realized at temperatures as high as 23 – 27°C. In our experiments we acclimatized copepods to a given baseline temperature before starting the experiment (see Chapter 4) and we observed no differences in the behavior of the copepods at constant temperatures, which suggests that copepods react to changes of temperature rather than a given temperature level. A natural river reach typically consists of fast velocity zones alternated by pools and bedform features such as gravel bars can be present. This generates a variety of flow regimes and water depths with rather uniform flow in the center of the stream and regions with very slow flow towards the river banks. We sampled the animals in the pebble gravel bars with shallow waters and low flow velocities similar to the conditions in the experiments where the achievable Reynolds number is limited. An inherent difficulty in hydraulic experiments with living components is scalability. Physical properties can be reproduced accurately at smaller scales provided that similarity laws are fulfilled, meaning that the ratios between relevant forces should be the same between the field and the laboratory model. The dominant forces in fluvial hydraulics are gravity, inertia and friction implying two dimensionless numbers, namely the Froude (i.e. the ratio between gravitational and inertial forces) and the Reynolds (i.e. the ratio between inertial and friction forces) numbers. It is difficult to reproduce both Froude and Reynolds similarity in the laboratory. Lower Reynolds numbers than in the field are used often in experiments implying scale effects in the ratio between friction and inertial forces. However, as mentioned above, the conditions that we realized in the experiments are representative of shallow lateral flows with low velocity. While scaling of physical variable is possible, it is not possible to scale a living organism. However, in lateral sections of a river the water table can be very shallow and therefore, again, our experiments will mimic well the size ratio between, e.g. depth of the water column and organism size for these lateral and slow river sections.

Another factor to consider in the experiment is how to mimic the disturbances introduced by hydropower operation due downstream river reaches, namely hydro- and thermopeaking. Actual magnitudes of discharge and flow velocities can vary greatly depending on hydropower management strategy and the downstream and spanwise location in the river. Previous works indicated that for living biota gradients (i.e. the magnitude of the temporal change of a physical variable) is the most relevant factor determining the response because it governs, e.g. the time available for refuge finding etc. In our hydropeaking experiments we gradually varied the magnitude of the discharge and reproduced different intensities of the gradient that are representative of gradients found in real hydropeaking cases. An additional important factor for the feasibility of the experiments was the successful culturing of the copepods in large numbers. The cultures could be maintained stable at low animal density, but to get a sufficient number of animals for the experiments, the supplied nutrients had to be increased and enriched with the algae *Scenedesmus* sp. This required an intensified maintenance effort and led to occasionally unstable conditions with a possible collapse of the culture. For the reproducibility of the experiments and the exclusion of life stage effects only animals with similar swimming abilities have been used (adults and late-stage copepodids with a prosome longer than 300 μm , sorted out of the culture with iron sieves). After the experiments, as many individuals as possible have been collected and transferred back to the culture, but it was inevitable that a large number of them died in the procedure of the experiments, e.g. when removing the sediments. This resulted in a reduction of adult individuals in the culture and a recovery time of at least two weeks for new experiments. In order to get statistically meaningful measurements, the total recording time during single experiments was set to the maximum achievable time of around 30 min to track as many individuals as possible. Several replicates (i.e. a minimum of 2 and a maximum of 10 depending on the quality and number of individuals that could be tracked) were recorded for the different experimental conditions, both for the hydropeaking and the thermopeaking experiments.

1.2 Summary

This dissertation is divided into three main chapters. The following chapters 2, 3 and 4 are three scientific publications, containing the main findings of my scientific work. Chapter 5 gives a synthesis of the findings of the three publications and a suggestion for future research. The chapters 2, 3 and 4 start each with the title and citation of the scientific publication, a list of the authors and their contributions, an abstract of the article and an introduction and motivation to the addressed problems separately.

Chapter 2 presents the culturing of the animals, explains the experimental setup and how the different parts were developed. It constitutes the basis for all experiments conducted in the thesis (Chapter 3 and 4). The Chapter 2 provides a characterization of the hydraulic conditions, describes the measurement technique, explains the data acquisition and analysis and shows first results obtained from the tracking of many copepods. The model species, which is the widespread benthic cyclopoid copepod *Eucyclops serrulatus*, was sampled in the river Töss, near Winterthur in North Switzerland. Given that the number of animals in the nature is fluctuating depending on seasonal food sources and predators, we culture the species in the laboratory by testing different methods and diets described in literature and adapt them to successfully culture several thousands of individuals over 2 years in a constant culture. The experimental setup consists of an acrylic glass flume with a water recirculation system. The discharge is controlled with a ball valve and measured with a flow meter. The water is supplied from a high elevation reservoir, fed by a pump

from the low level reservoir. To provide the animals a natural-like river bed and to get an optical access without disturbance and an insight into the not yet seen actual behavior of invertebrates inside the sediment bed, we use polyacrylamide spheres and manage to create a transparent gravel bed. To establish a natural-like flow that is reproducible and well defined, we measure the flow filed above the sediment bed with a particle image velocimetry (PIV) system and adapt bricks, meshes and voluminous webs at the inlet and the perforation of the vertical gate at the outlet of the flume to obtain uniform flow conditions for different discharges. To detect and track the animals, we use a three dimensional particle tracking velocimetry (3D-PTV) system from our group to study flow turbulence. The system consists of a planar rig with four synchronized cameras to obtain stereoscopic images with a high frame rate. In-house built software detects many animals at the same time and links them between consecutive images, resulting in the trajectories of the animals. We reconstruct the trajectories of many copepods and we quantify their velocity and vertical distribution under statistically steady state flow conditions. Copepods are able to swim in the entire flume and move readily in the sediment bed. They move by successive jumps and often against the flow. This behavior may contribute to drift avoidance in natural streams. Copepods are preferentially found within the interstices of the sediment bed and within a narrow layer at its surface, in agreement with the epibenthic nature of this species. Our setup enables the detailed observation of fine-scale behavioral processes that could not be approached in field studies or through theoretical approaches before. It offers the opportunity to better understand the impacts of physical conditions on the behavioral processes that drive invertebrate ecology in streams.

In **Chapter 3**, the counter-current swimming of copepods at different, steady state flow conditions is presented. The same experimental setup as in chapter 2 is used, but is improved in some points. An additional recirculation of copepods flushed from the flume is installed to establish a constant number of individuals in the flume. We use 3D-PTV to track flow tracers and copepods which allows for a statistical comparison of passive particles versus motile organisms. We reconstruct the three-dimensional trajectories of the widespread cyclopoid copepod *Eucyclops serrulatus* moving freely in the water column and in the transparent sediment bed of the laboratory flume. The flow velocities tested are low to moderate to allow the animals an active moving and to overcome the flow. We isolate the behavioral component of their motion by subtracting the local flow velocity. We show that copepods perform active counter-current swimming to limit downstream drift. The counter-current swimming effort increases with flow velocity and therefore downstream drift remains moderate and does not vary strongly for the low and middle flow rates tested and is reduced at the high flow rate. We show that copepods explore the water column at low flow velocity, but stay close to and perform frequent incursions into the sediment bed at higher flow velocities. This active behavioral response to changing flow conditions, combined with frequent stops in the substratum where flow velocity is negligible, may confer lotic copepods the ability to reduce downstream transport. These results confirm the importance of behavioral traits in drift avoidance for species that lack an aerial stage, and the interest of studying taxon-specific responses to varying hydraulic conditions.

In **Chapter 4** we describe the reaction of benthic cyclopoids to changes in the flow velocity and temperature in the flume, mimicking the alterations imposed by high elevation dam hydropower plants in pre-Alpine rivers. We investigate separately the effects of a sudden variation in the flow velocity and in the water temperature on the small-scale swimming behavior of a widespread species by changing the discharge and by adding ice or hot water to the system. We track the copepods with the 3D-PTV system and quantify the kinematics of their motion. Copepods

increase substantially their counter-current swimming effort in response to the increasing flow velocity. This behavioral response seems to occur above a threshold in flow velocity of approx. 40 mm/s and results in a reduction in their downstream transport. Copepods react differently to warm and cold variations in temperature. Decreasing temperature results in a substantial increase in their swimming effort with a net downstream flux, whereas rising temperature has no clear effect on their behavior. This chapter highlights the importance of understanding the behavioral traits that mediate the response of stream invertebrates to disturbances in the hydraulics and thermal dynamics of their environment.

The following chapters 2, 3 and 4 of this thesis are based on the below listed publications:

Chapter 2:

D. Sidler^{1,2,3,4,5}, F.-G. Michalec^{1,3,4,5,6}, M. Detert^{4,6}, and M. Holzner^{1,4,6} (2017) Three-dimensional tracking of the motion of benthic copepods in the free water and inside the transparent sediment bed of a laboratory flume. *Limnology and Oceanography: Methods*, 15(2), doi: 10.1002/lom3.10147

¹Study concept and design; ²Experimental setup design and construction; ³Data acquisition and analysis; ⁴Interpretation of data; ⁵Drafting manuscript; ⁶Critical revision

My contribution to this chapter was the collection of the animals, the testing and adaption and application of the culturing methods and the maintenance of the cultures. I designed and dimensioned the experimental setup with all featuring devices and parts, I was responsible for the acquisition of all materials and apertures and I assembled most of the parts of the experimental setup. I did all the experiments on the flow and the animals and successively added, changed and adapted features on the setup and to perform the experiments. I processed the different data from the experiments and wrote the coding algorithms to analyze them. The interpretation of the results and drafting of the manuscript were also my task. All authors have subsequently contributed to improve the paper.

Chapter 3:

D. Sidler^{1,2,3,4,5}, F.-G. Michalec^{1,4,5,6}, and M. Holzner^{1,4,6} (2017) Counter-current swimming of lotic copepods as a possible mechanism for drift avoidance. Submitted to *Ecohydrology*

¹Study concept and design; ²Experimental setup design and construction; ³Data acquisition and analysis; ⁴Interpretation of data; ⁵Drafting manuscript; ⁶Critical revision

I contributed to this chapter with the improving of the culturing methods and the maintenance of the cultures. I performed the experiments and continuously improved features of the setup and of the processes to perform the experiments. It was my role to process the data from the experiments and write the coding algorithms to analyze the measured data. I interpreted the resulting findings and drafted the article. All the listed authors of the publication have improved the submitted article.

Chapter 4:

D. Sidler^{1,2,3,4}, F.-G. Michalec^{1,3,4,5}, and M. Holzner^{1,3,5} (2017) Behavioral response of the freshwater cyclopoid copepod *Eucyclops serrulatus* to hydropeaking and thermopeaking in a laboratory flume. Submitted to *Journal of Freshwater Ecology*

¹Study concept and design; ²Data acquisition and analysis; ³Interpretation of data; ⁴Drafting manuscript; ⁵Critical revision

For this chapter my work consisted in the maintaining of the cultures for the experiments and the execution of the experiments. I designed the procedure of the experiments with all additionally needed tasks and materials. The data processing as well as the writing of needed scripts for the data analysis was my task. With my interpretation of the results I wrote the text for the scientific publication. With the help of all the contributing authors we could submit the manuscript.

In chapter 5 of the dissertation a synthesis of the three publications is presented to summarize the main findings and some suggestions for future research are given.

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Chapter 2

THREE-DIMENSIONAL TRACKING OF THE MOTION OF BENTHIC COPEPODS IN THE FREE WATER AND INSIDE THE TRANSPARENT SEDIMENT BED OF A LABORATORY FLUME

This chapter consists of a manuscript published in *Limnology and Oceanography: Methods*, 2017:

D. Sidler^{1,2,3,4,5,6}, F.-G. Michalec^{1,3,4,5,6,7}, M. Detert^{5,7}, and M. Holzner^{1,5,7} (2017). Three-dimensional tracking of the motion of benthic copepods in the free water and inside the transparent sediment bed of a laboratory flume. *Limnology and Oceanography: Methods*, 15(2), doi: 10.1002/lom3.10147

¹Study concept and design; ²Experimental setup design and construction; ³Experiment performance; ⁴Data acquisition and analysis; ⁵Interpretation of data; ⁶Drafting manuscript; ⁷Critical revision

Abstract

Benthic organisms in rivers are exposed to sharp variations in environmental conditions. Variations in e.g. shear stress and water temperature can result in rapid alteration in invertebrate drift. Catastrophic drift is associated with hydrodynamic thresholds. Active drift results from deliberate behaviors and occurs e.g. in response to changes in temperature. Cyclopoid copepods constitute a substantial part of the meiobenthos. The relationships between hydraulic conditions or temperature and copepod drift, and between their behavior and drift entry, transport and exit, remain not well defined. We present a laboratory flume fitted with a transparent sediment bed

that enables studying the behavior of benthic invertebrates both in the free water and inside the sediment. By means of three-dimensional particle tracking velocimetry, we reconstructed the trajectories of many copepods (*Eucyclops serrulatus*) and we quantified their velocity and vertical distribution under statistically steady state flow conditions. Copepods were able to swim in the entire flume and moved readily in the sediment bed. They moved by successive jumps and often against the flow. This behavior may contribute to drift avoidance in natural streams. Copepods were preferentially found within the interstices of the sediment bed and within a narrow layer at its surface, in agreement with the epibenthic nature of this species. Our setup enables the detailed observation of fine-scale behavioral processes that could not be approached in field studies or through theoretical approaches. It offers the opportunity to better understand the impacts of physical conditions on the behavioral processes that drive invertebrate drift in streams.

2.1 Introduction

Hydropower plays a key role in the European electricity system and still has an important development potential. It has, however, major impacts on aquatic ecosystems. Upstream water release from hydropower plants causes HydroPeaking (HP), defined by rapid stage variations both in the rising and decreasing phases. Increase in discharge raises bottom shear stress and induces catastrophic drift of macro-invertebrates (e.g. Mochizuki et al. 2006; Bruno et al. 2010). Drift is usually associated with substrate mobilization but considerable washout of organisms can also occur in non-scouring flows with limited sediment movement (Imbert and Perry 2000; Gibbins et al. 2007). Drift typically occurs during the first stages of the release before the peak discharge. Predisposition to drift varies between taxa depending on ecological, morphological and behavioral traits e.g. on the swimming ability of an organism and its degree of adaptation to resist to flow or to move toward refuge habitats (Imbert and Perry 2000). Consequently, discharge intensity or exposure duration must reach a taxon-specific threshold before the animals drift (Mochizuki et al. 2006). Holomuzki and Biggs (2000) illustrated the importance of behavioral adaptations; they observed that mayflies, caddisflies and mudsnails would respond to increased flows by moving to deeper, more stable sediment layers. Similarly, Palmer et al. (1992) observed significant downward migrations of copepods and chironomids during increased water velocity in flume experiments. Jakob et al. (2003) observed no significant increase in mayfly and stonefly drift following experimental floods, which can be attributed to the better morphological and behavioral adaptations of these organisms to higher flow velocities.

Analysis of drift composition following HP has revealed the prevalence of insect taxa whereas cyclopoid and harpacticoid copepods, which constitute a substantial part of the freshwater meiofaunal community, were poorly represented. In the field survey of Bruno et al. (2010), benthic copepods were more abundant in the constant drift (i.e. the passive drift below their critical entrainment threshold) than during HP, which suggests that these small organisms can find a refuge from the increase in shear stress. Possible strategies include dwelling deeper into the sediment, moving toward a nearby patch of slower flow velocity, or even actively swimming against the flow (e.g. Richardson 1992; Shang et al. 2008). Equally important to studying the departure stage of drift is studying how animals behave while drifting as well as their settlement preferences for substrate and streambed topography (reviewed for instance in Naman et al. 2016). Potential settlement sites are determined by both the physical constraints imposed by flow conditions relative to the swimming abilities of the organisms, and the decisions individuals

make based on species-specific preferences. Oldmeadow et al. (2010) for instance have reported on the influence of the local hydraulic environment created by bed substrate configuration on drift transience and settlement behavior in two species of mayflies. However, mechanistic studies of the relationships between hydraulics and behavioral or morphological adaptations and drift entry, transport and exit are unavailable for many taxa (but see Blanckaert et al. 2013) and especially copepods. Consequently, the general processes that govern invertebrate drift remain elusive, limiting the ability to quantify the role of drift in an ecosystem context and the predictive models of drift flux (Naman et al. 2016).

Each HP wave is typically associated with a certain degree of thermal alteration in a phenomenon termed ThermoPeaking (TP). The celerity of the two waves is different, and they tend to separate while propagating downstream (Toffolon et al. 2010). ThermoPeaking strongly increases the natural daily variations of temperature downstream. Carolli et al. (2012) have shown that TP alone could induce drift. Bruno et al. (2012) have demonstrated the synergetic effect of HP and TP on the macro-invertebrate community, and evidenced marked taxa-specific differences in the response of organisms to variations in bottom shear stress and temperature. Some taxa did not respond to the alterations, some taxa responded to both the discharge and thermal variations, and others responded to the thermal variations alone. In Bruno et al. (2012) and Carolli et al. (2012), time lags following TP were short; the authors suggested that the drift was behavioral i.e. resulting from organisms actively entering the water column or being dislodged as a consequence of increased activity on the streambed. Active drift resulting from deliberate behavior and aiming at escaping from unfavorable abiotic conditions was for instance observed in *Gammarus pulex*, *Baetis rhodani* and *Leuctra* sp. exposed to a pulse of insecticide (Lauridsen and Friberg 2005). Copepods seem to drift more intensely during TP (Bruno et al. 2012). For these organisms therefore, the thermal wave may represent a strong stressing factor. However, the behavioral mechanisms leading to their drift entry have yet to be investigated.

Copepods are known to respond behaviorally and immediately to variations in the physical properties of their environment. In the estuarine species *Eurytemora affinis* for instance, variation in flow velocity and salinity induces active swimming behavior that reduces flushing of individuals toward downstream areas (Schmitt et al. 2011). In the open ocean, certain species of pelagic calanoid copepods migrate to deeper and calmer environment in response to turbulence in the surface layer (Incze et al. 2001). The behavior of freshwater benthic copepods remains not well investigated, despite their ecological importance in lotic ecosystems (Dole-Olivier et al. 2000). One reason for this is the lack of appropriate tools that enable addressing behavioral processes at a suitable range of spatial and temporal scales.

Recent developments in tracking procedures have allowed the description and understanding of behavior at scales previously not possible, from small (e.g. Michalec et al. 2012) to large (e.g. Attanasi et al. 2015). Automated tracking of the behavior of small organisms in three dimensions allows the reconstruction of a large number of trajectories and the accurate measurement of the Lagrangian properties of their motion. Three-dimensional particle tracking velocimetry (3D-PTV) is a flow measurement technique originally developed to study turbulent flows and recently applied to study the individual motion of swarming midges (e.g. Kelley and Ouellette 2013) and planktonic copepods (e.g. Michalec et al. 2012, Adhikari et al. 2015). The method is based on the visualization of moving particles and the acquisition of sequences of stereoscopic images from which the position of a large number of particles and their trajectories in three dimensions can be recovered.

Laboratory flume experiments offer the advantages of controlled, repeatable conditions, and the opportunity to make detailed observations of fine-scale processes that could not be approached in field studies or through theoretical approaches (Muschenheim et al. 1986). Flumes are commonly used in benthic ecology, for instance to study the small-scale movements of invertebrates in response to flow disturbances and to the influence of bed topography (Lancaster 1999; Lancaster et al. 2006), the role of turbulence in the dislodgment of stream invertebrates (Blanckaert et al. 2013), or how the hydraulic environments conditioned by a step bedform influence the behavior of drifting mayflies (Oldmeadow et al. 2010). Flume measurements involving copepods, however, remain comparatively rare. Foy and Thristle (1991) demonstrated that the interstitial harpacticoid copepod *Leptastacus rostratus* burrows deeper into the sediment in response to increased flow; this observation supports the view that active behavior plays a significant role in the ecology of benthic copepods. Similarly, Shang et al. (2008) reported on vigorous countercurrent swimming followed by downward movements and active substrate attachment by the estuarine epibenthic calanoid copepod *Pseudodiaptomus annandalei* in response to increasing flow velocity.

The research presented in this paper was conducted (i) to test the feasibility of using 3D-PTV to track organisms in three dimensions in the free water section and in the transparent sediment bed of an experimental flume, and (ii) to ensure that the hydrodynamic conditions within our flume are comparable with reference values found in the literature and can mimic conditions encountered by organisms in natural streams. We pay particular attention to the flow conditions near the channel bed, because not only the mean flow velocity but also the magnitude and temporal pattern of turbulent velocity fluctuations that extend onto the streambed affect benthic organisms living on an heterogeneous substrate (Blanckaert et al. 2013; Pepper et al. 2015). Several studies have used refractive index-matched materials in channels or flow cells to achieve optical accessibility during particle image velocimetry (PIV) measurements in fluids of various refractive indices (e.g. Northrup et al. 1991; Huang et al. 2008; Patil and Liburdy 2013). Examples are diverse and encompass for instance polymethyl methacrylate in sodium iodine solution to understand the flow field around spherical fuel pebbles (Hassan et al. 2008), transparent borosilicate glass beads in silicone oils to study the transition region between a porous layer and its overlying fluid layer (Goharzadeh et al. 2005), or glass spheres to measure the velocity profile within a porous bed below rough turbulent open channel flow (Pokrajac et al. 2007) and to study the interaction between the turbulent flow in an open channel and the turbulent flow within its permeable bed (Pokrajac and Manes 2009). In this study, fluid velocities were measured by PIV and the motion of copepods by 3D-PTV, which allowed us to investigate the swimming behavior and vertical distribution of these small animals against the spatial distribution of time-averaged flow properties. We tracked the motion of many epibenthic cyclopoid copepods under statistically steady state flow conditions. The measurements presented here constitute a reference case and will serve as a basis for future experiments in transient conditions i.e. during changes in temperature and discharge replicating those observed during HP and TP. It is our hope that these measurements will help build a better understanding of the impacts of physical disturbances on fine-scale behavioral processes in the freshwater meiofaunal community.

2.2 Methods

2.2.1 Model organisms

Copepods were collected using a Bou-Rouch pump from the prealpine, unregulated Töss River near Winterthur (Switzerland). The reach consists of riffles, pools and gravel bars. Only riffles were sampled to select organisms living in comparatively fast-flowing sections. A large volume of water was pumped from the coarse-grained sediment and filtered to separate benthic organisms from small pebbles and large detritus. Collected organisms were transferred into large containers filled with water from the sampling site, and quickly brought back to the laboratory. We followed the method described by Szlauer (1995) to recover copepods from the layer of fine sediment. The method is based on the degradation of a small quantity of added milk by bacteria living in the sediment. The bacterial activity reduces the oxygen content of the water in the container, which forces the organisms to rise to the surface. Individual *Eucyclops serrulatus* were collected from the surface layer of the containers and separated from other benthic organisms via manual sorting under a microscope. *Eucyclops serrulatus* (Fischer, 1851) is a relatively small-bodied (around 1 mm in size when adult) widespread epibenthic cyclopoid copepod commonly found in lotic ecosystems (e.g. Robertson et al. 1995; Alekseev et al. 2006).

2.2.2 Culture conditions

The culturing method was adapted from Kumazawa (2000) and Park et al. (2005). Adult copepods were fed on a diet of *Chilomonas paramecium* collected from a small artificial pond. Nauplii and copepodids were fed on a diet of *Scenedesmus* sp, cultured in autoclaved water and in Conway medium, at 18 °C and under a 12L:12D light cycle. The flagellates fed on a mixture of microorganisms isolated from the same pond and cultured on an infusion of soya bean and on wheat grains. The infusion consisted of ten soya beans cut in half and boiled in 1 L of water for at least 10min. Wheat grains were boiled for a few seconds. After cooling and filtering, both the infusion and the wheat grains were added to the copepod cultures. The copepods, the flagellates and the microorganisms were kept together in 20 L buckets, at a constant temperature of 18 °C and under a 12L:12D light cycle. We used 5-10 g of dry wheat grains per bucket, replaced every third week. The soya bean infusion was replaced every other week. The buckets were opaque to reduce unwanted algae growth. The culture was maintained for over a year in these stable conditions.

2.2.3 Experimental setup

Experiments were conducted in a custom-made acrylic glass flume (Fig. 1). The flume features a flow dampening area (1) which is described in more detail in the next section, an adjustable vertical gate at the outlet (2), two groundwater exchange chambers mounted below a perforated ground plate (3), and a transparent sediment bed (4). The flume is 2 m long and 27 cm wide. The sediment layer is around 50 mm thick, and the water level above the sediment bed was adjusted to 11 cm. The bottom of the flume was fitted with a sediment bed consisting of several layers of transparent PolyAcrylAmide (PAM) grains of approximately spherical shape with a diameter of 8-12 mm and a density of 0.95 g/cm³. The optical refractive index of the spheres (1.349) is very close to that of water (1.333) and this allows the tracking of the copepods inside the sediment layer. Polyacrylamide is harmless to animals. As it has nearly the same density as water, the grains would float and drift downstream even at small flow velocities. Therefore, we used a metal

frame and a fishery net of 6 mm mesh size and 0.14 mm wire diameter to keep the spheres in position. To obtain a uniform sediment layer that allows for uniform flow conditions, the sections up- and downstream the PAM grains were fitted with glass beads of the same dimensions (5).

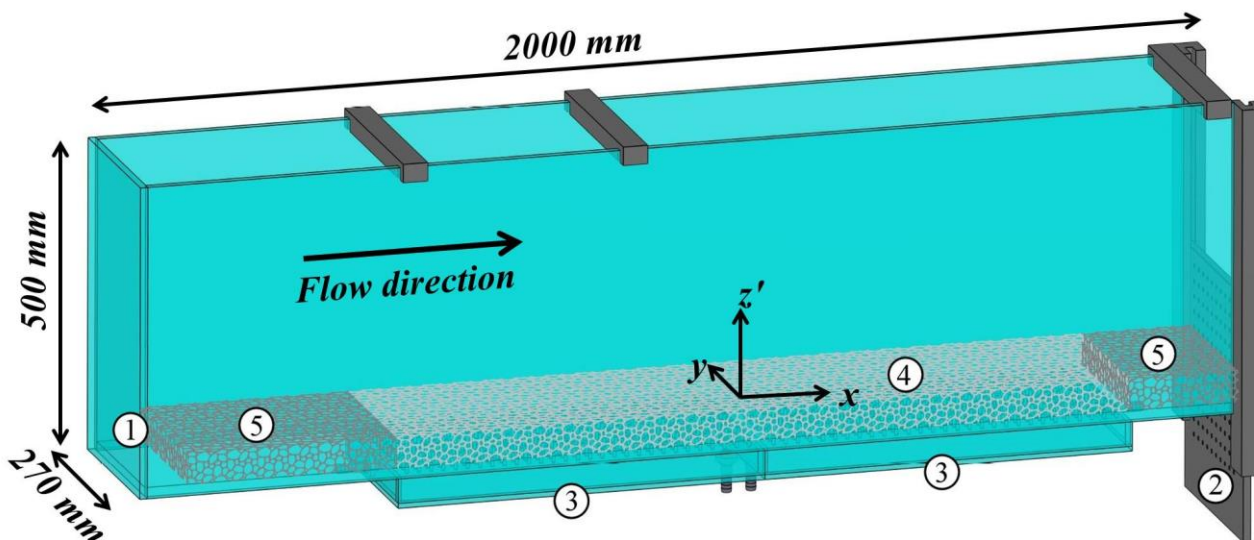


Figure 1. Detailed drawing of the acrylic glass flume. (1) Inflow dampening area, (2) Perforated vertical gate, (3) Groundwater chambers, (4) Refractive index matched sediment bed, and (5) Glass sediment sections. The flow is from left to right.

The flume is equipped with a circulating recharge system (Fig. 2). Water is pumped from the low-level reservoir (4) to the high-level reservoir (6) and released through a pipe into the upper part of the flume (1). Water flowing through and above the terminal plate is collected into a retention box (3) that serves to collect drifting copepods, and sent back to the low-level reservoir. A thin metal mesh mounted in front of the outlet in the retention box prevents the animals from entering the low-level reservoir. Constant discharge in the flume is reached via a surplus of water pumped to the high-level reservoir and an overflow system leading back to the low-level reservoir. This system generates a fixed water level inside the high-level reservoir with a stable pressure in the outlet. The flow rate in the flume was adjusted with a ball valve and monitored with a magnetic flow meter.

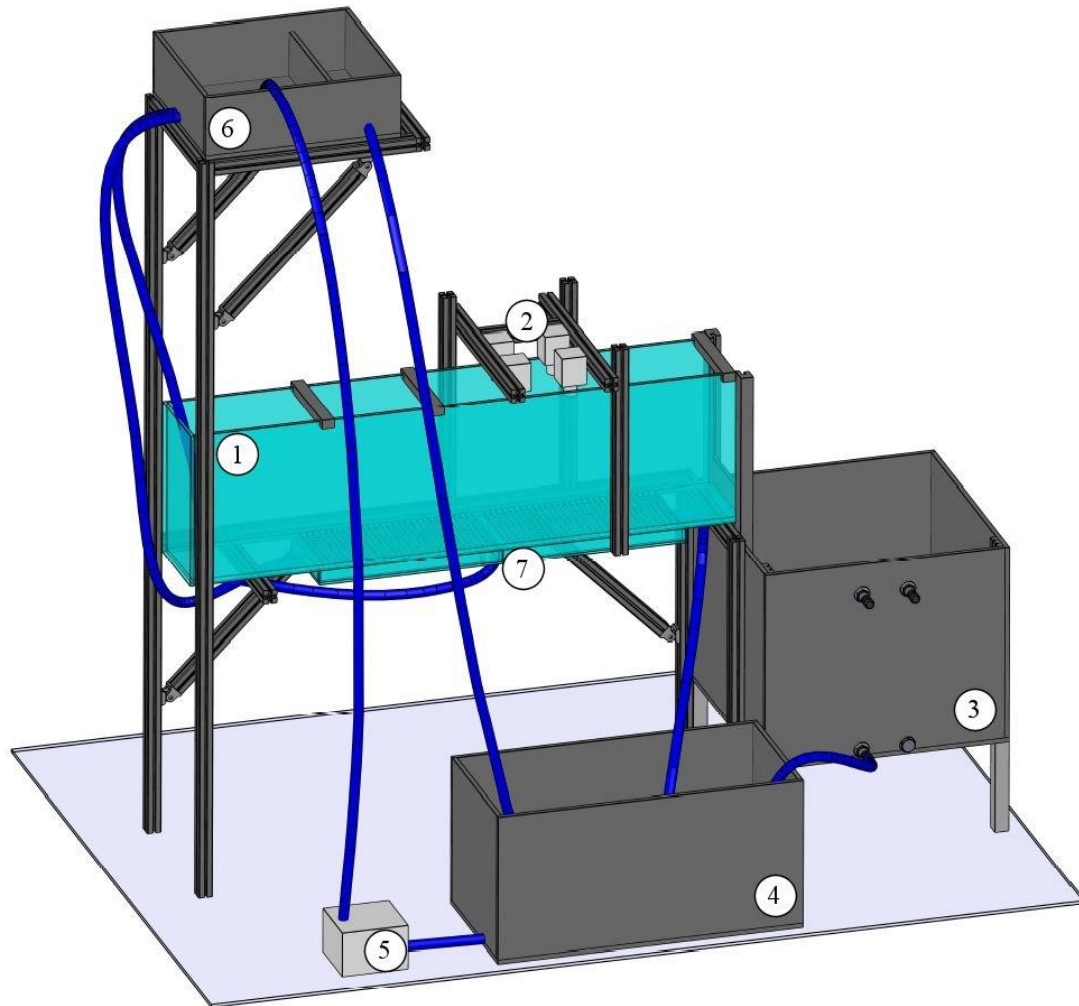


Figure 2. Drawing of the experimental setup showing the acrylic glass flume with (1) Flow dampening region, (2) Camera rig with four cameras, (3) Animal retention box, (4) Low-level reservoir, (5) Pump, (6) High-level reservoir, and (7) Groundwater exchange chambers. The flow in the flume is from left to right.

The flume features a permeable ground plate located beneath the sediment layer. This plate enables groundwater exchange via upwelling and downwelling zones. The exchange is operated through two separate chambers, which connect to the flume via drilled holes. From the high-level reservoir or a separate tank, it is possible to pump water into the groundwater chambers and from there across the sediment layer into the flume. This allows for the creation of exfiltration zones of groundwater with similar or different temperature than the flume water. Similarly, it is possible to create infiltration zones by pumping water from the flume through the groundwater chambers. As the two chambers are independent, it is also possible to achieve simultaneous groundwater infiltration and exfiltration in two subsequent downstream regions. A thin metal mesh is glued to the ground plate at the bottom of the flume to prevent the animals from migrating downward into the groundwater chambers.

2.2.4 *Hydraulic conditions*

To study the behavior of copepods in well-defined and reproducible hydraulic conditions, the in- and outflow conditions need to be carefully adjusted to mimic a longer flume with uniform flow conditions. The vertical adjustable gate controls both the flow condition at the outlet and the water depth in the flume. It features a multitude of holes that can be sealed with screws to adjust the outflow. The appropriate number and positions of opened and closed holes were determined in preliminary measurements to achieve a constant flow depth along the channel, and hence approximately uniform flow conditions. The water descending from the high-level reservoir to the flume leads to a flow velocity that is higher at the surface than at the ground. Consequently, the flume features in its upper section a flow dampening structure that enables an even distribution of the flow over its whole cross-section as the water passes a series of bricks, perforated sheets and voluminous filters. The perforated sheets feature different permeability coefficients at different elevations and are tailored to obtain a suitable inflow velocity profile. The inlet from the high-level reservoir to the low-level reservoir is a large tube (approximately 7 cm in diameter) ending 10 cm above the flume ground in the most upstream section of the flume. This upstream section is 10 cm long in the flow direction. It is bounded by the flow dampening structure that is approximately 20 cm long and which consists (from upstream to downstream) of (a) a perforated aluminum sheet with 3 mm diameter holes, (b) stacked bricks with uniformly distributed holes, (c) a thin iron mesh to prevent copepods from migrating upstream, (d) a second perforated aluminum sheet of the same dimensions, (e) a short open section of approximately 2 cm in length used to redistribute the discharge over the channel depth, (f) a plastic bee comb of 10 cm length, (g) a third perforated aluminum sheet, (h) a voluminous plastic webbing with large interstices, and (i) a voluminous plastic foam with pores of 1 mm diameter. The holes in the third perforated aluminum sheet were gradually blocked from the top to the bottom to force the discharge into a uniform flow profile. The flow velocity distribution was measured along the flume to check for the influence of the flow dampening structure. The velocity profiles measured in the test section could already be observed 50 to 60 cm upstream of the test section, which is located 100 cm downstream of the end of the flow dampening structure.

2.2.5 *Flow characterization*

We analyzed the hydrodynamic conditions in the flume by means of two-dimensional particle image velocimetry (PIV). We used a Photron FASTCAM SA5 camera with a recording frequency of 125 Hz at a resolution of 1024 by 1024 pixels, and for each measurement the recording duration was 50s. Neutrally buoyant polyamide tracer particles (VESTOSINT 1101, Evonik Industries) with a mean diameter of 100 μm and a density of 1.06 g/cm^3 were used as flow tracers. The choice of an appropriate diameter for seeding particles is a compromise between an adequate tracer response of the particles in the fluid (which requires small particles) and a high signal-to-noise ratio of the scattered light signal (which requires large diameters). Reasonably large tracer particles were desirable in our experiments because of the relatively large size of the observation domain. Our flow has a maximal mean velocity of around 100 mm/s. The resulting Stokes number (St) is $O(10^{-4})$, with $St = \tau_p/\tau_f$ and for our neutrally buoyant particles $\tau_p = d_p^2 \rho_p / (18\nu) \approx 5.5 \times 10^{-4}$. The flow time scale τ_f is given by the Kolmogorov time scale, estimated at $\tau_f = 0.12\text{s}$. This means that the particles behave as passive tracers in our flow. Moreover, our tracer particles are smaller than the Kolmogorov length scale, estimated at $\eta = h/(Re^{3/4}) \approx 0.3\text{ mm}$ for the highest flow discharge. The interrogation spot size used in the PIV program is 16 x 16 pixels corresponding to 1.6 mm, and the uncertainty in the displacement is

better than 0.1 pixels, which translates into an uncertainty of the velocity measurement of better than 5% (Raffel et al. 2007). Typical values for the turbulent velocity fluctuations are higher and between 10 to 20 % of the average velocity. Two halogen lamps placed above the observation area provided illumination. The lamps were mounted in a row in an elongated metal box with a slit at the bottom. This box was fixed above the flume. The light beam leaving the slit of the box was approximately collimated with a plano-convex glass lens (focal length of 15 cm) mounted over the entire length of the slit, producing a vertical light sheet parallel to the flow direction in the middle of the flume. The camera was mounted next to the flume and perpendicular to the light sheet, and took images through the acrylic glass wall (Fig. 3a).

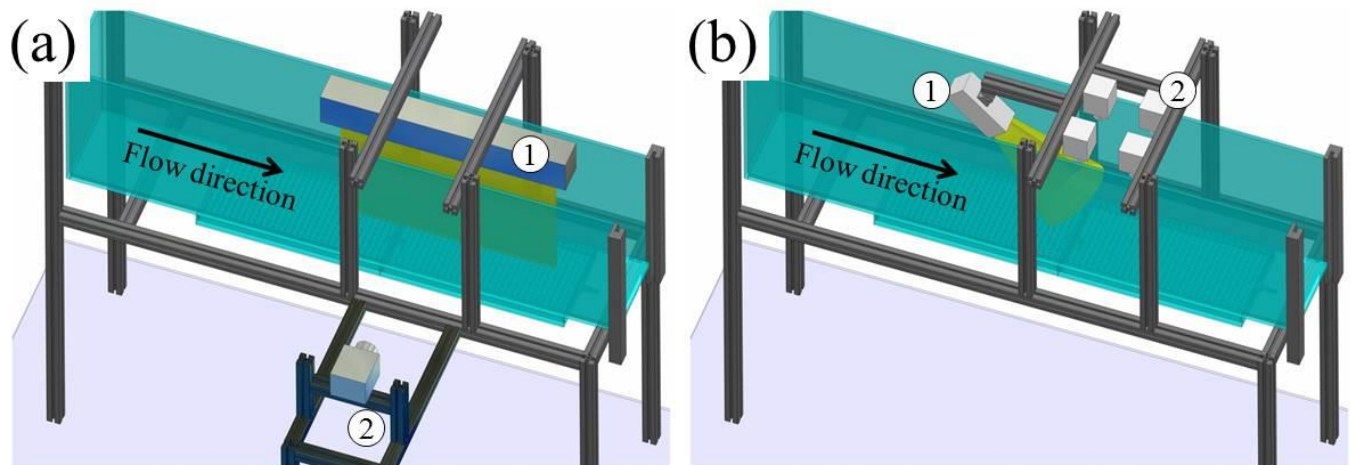


Figure 3. Schematic drawing of the experimental setup showing the position of the light source (1) and the position of the cameras (2) for the PIV (a) and PTV (b) measurements. Flow is from left to right.

The size of the observation window was 15 cm in the streamwise direction and 10 cm in the vertical direction. The thickness of the light sheet varied between 5 mm at the top and 10 mm at the bottom of the sheet. The difference in thickness between top and bottom is due to the relatively strong divergence of the light sheet. Because of this relatively large thickness, more particles will stay in the light sheet and hence in the interrogation window, improving the signal-to-noise ratio but causing a decrease in effective spatial resolution, because velocities are averaged in spanwise direction over the thickness of the light sheet. In our measurements the window size for cross-correlation was set at 1.6 mm (i.e. smaller than the thickness of the light sheet) to ensure that the maximal in-plane displacement of the particles in the images was less than a quarter of the size of the interrogation window. This may have resulted in out-of-plane loss of pairs. However, because the intensities of turbulence in our flume are relatively weak, the lateral displacements of the particles are not significant compared to the downstream displacement caused by the flow, and the out-of-plane displacements of the particles are much smaller than the required quarter of the light sheet thickness (e.g. Raffel et al. 2007).

Fig. 4 shows, as an example, two images from the PIV measurements. Panel (a) shows a sediment bed composed of glass beads, panel (b) shows a sediment bed composed of PAM spheres. Panels (c) and (d) show a close-up section of panels (a) and (b), respectively, in inverse

colors, after superposition of an image taken 16ms later. The non-moving parts are shown in black, and each corresponding particle pair is shown in green and purple.

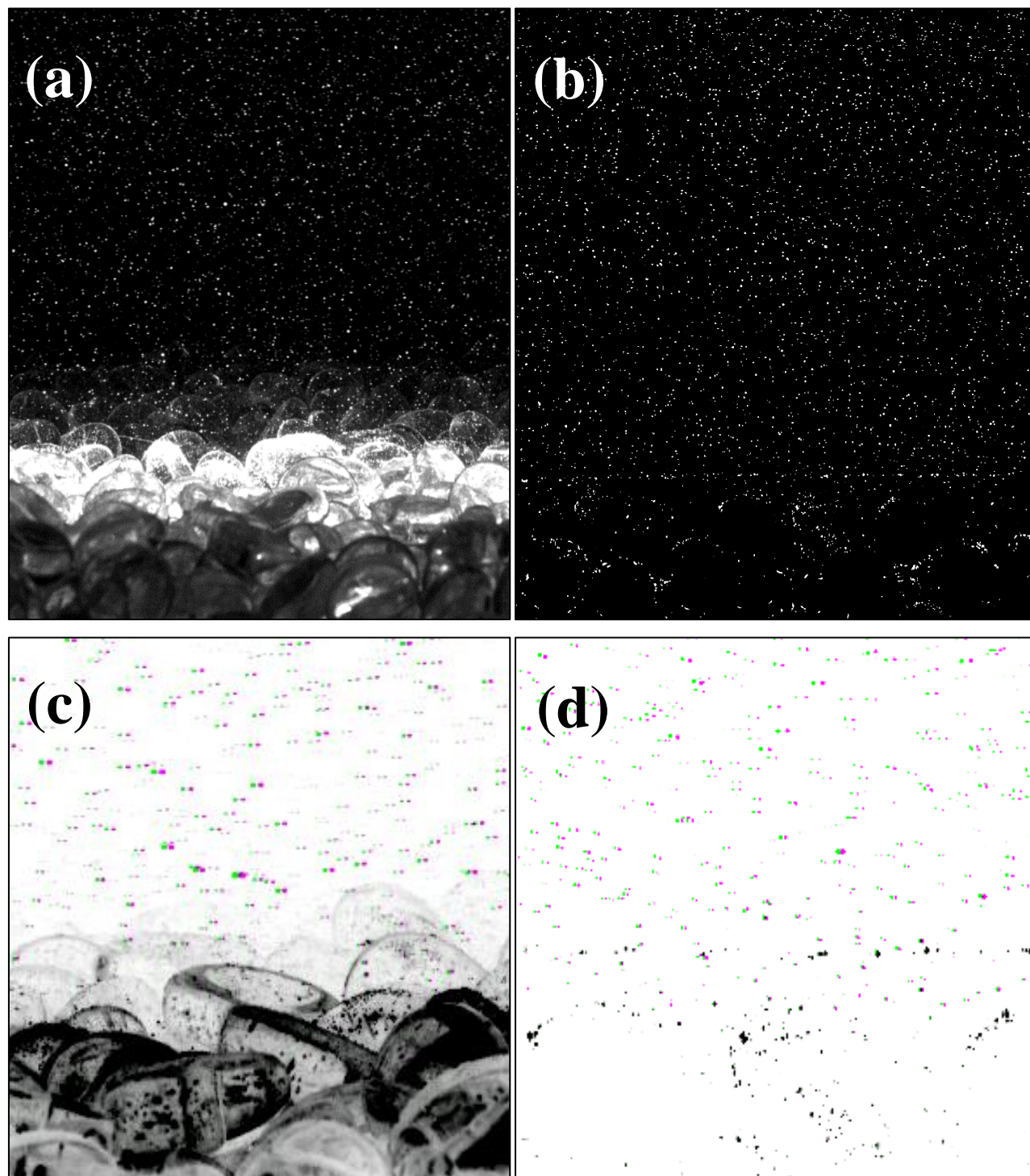


Figure 4. Images from the PIV measurements showing the sediment bed and the tracer particles. Image (a) shows a raw image from the sediment bed composed of glass beads; image (b) shows a raw image of the sediment bed composed of PAM spheres secured in the tightly adjusted retention net. Images (c) and (d) show a close-up section of images (a) and (b), respectively, in inverse colors and after superposition of an image taken 16ms later. Particle pairs colored in

green and purple. Flow is from left to right, and the investigation window has a side length of 12 cm.

Analysis was conducted by using PIVlab, a time-resolved digital particle image velocimetry tool for MATLAB (Thielicke and Stamhuis 2014). The PIV method calculates the mean displacement of particles in consecutive image pairs after background subtraction and high-pass filtering. The calibration for the transformation from pixel to real-world coordinates was implemented in the program via an image of an obstacle of known length. The background image was created by averaging the first 500 images and subtracted from all images in the sequence using MATLAB. An intensity capping was applied to improve image quality. We conducted measurements at three Reynolds numbers $Re = (U_{mean} \times h) / \nu = 1270, 3700$ and 7410 in steady state conditions (the parameters are defined in Table 1). The measured instantaneous flow velocity magnitude for a single time step is given in Fig. 5 as an illustrative example. Velocities are not distributed uniformly along streamlines as one would expect in the case of a laminar flow and patches of high and low velocities are clearly visible.

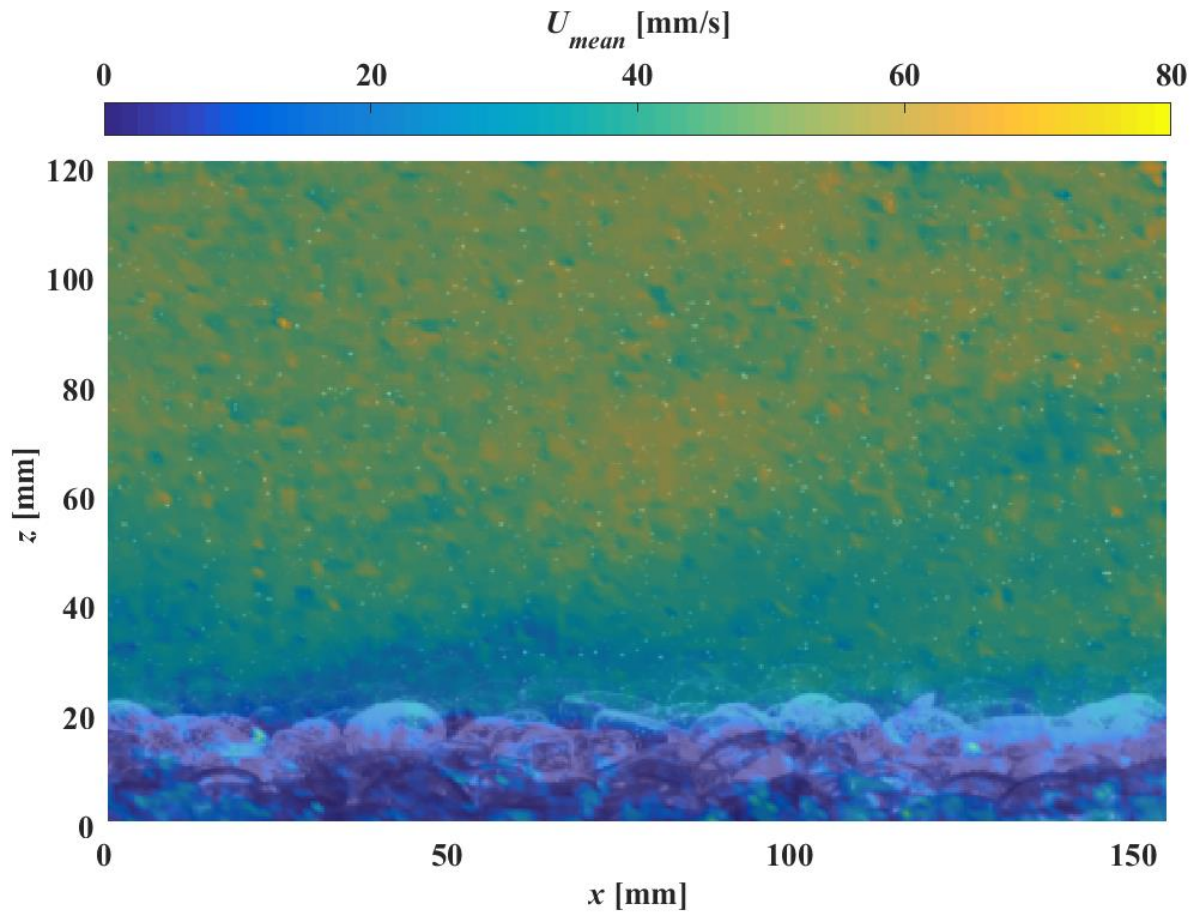


Figure 5. Instantaneous flow velocity magnitude obtained by PIV for a single time step and color-coded with velocity. Water flows from the left to the right. The surface of the sediment bed is located at about $z = 20$ mm.

2.2.6 *Experimental conditions and trajectory reconstruction*

Copepods were collected from the cultures with a 0.25 mm mesh size net and gently introduced into the flume through a 5 mm diameter pipe penetrating into the sediment bed 20 cm upstream of the PAM sphere section. Copepods were allowed to acclimate for 10 min to the experimental conditions in the flume. We collected drifting copepods exiting the flume in a plastic box located beneath the vertical gate. This retention box is equipped with a thin mesh that retains the copepods but lets the water flow through. Copepods were regularly re-introduced into the flume, between periods of recording, at the same position in the sediment bed, and allowed to acclimate for several minutes. We recorded the motion of copepods using four synchronized cameras (Mikroton EoSens) fitted with 60 mm lenses and recording at 60 Hz on two arrays of solid-state disks (DVR Express[®] Core 2 from IO Industries). The setup and measurement procedure are similar to those described in Michalec et al. (2015) and here, we briefly mention their main features. The four cameras were positioned at different angles and in a planar configuration (Fig. 3b). They focused from above on an investigation volume that was approximately 15 cm (x) \times 15 cm (y) \times 15 cm (z). A right-handed coordinate system is implied as illustrated in Fig. 1, where x is oriented positive in streamwise flow direction, y in transverse and z in vertical direction. $x = 0$ hold at the most upstream part of the measurement area, $y = 0$ defines a notional front wall level and $z = 0$ gives the zero-plane as found by a log-fit of the mean streamwise velocity to zero. We first calibrated the cameras to retrieve their relative orientations and their intrinsic parameters. We used a calibration block with 135 target dots whose coordinates in the three-dimensional space were precisely known. The calibration block was positioned inside the observation volume and under the same conditions (i.e. discharge and water depth) than during experiments, and pictures were taken. The spatial resolution in our measurements is around 10 pixels per millimeter for a plane imaged at the surface of the sediment bed. The accuracy for the detection of the center of mass of round calibration targets during the calibration procedure was estimated via the standard deviation of the measured image coordinates. This deviation was 2 μm , which corresponds to 0.14 pixels and to an estimated position accuracy of 0.014 mm for the two components perpendicular to the optical axis. By knowing the extrinsic and intrinsic parameters of the four cameras from the calibration procedure, it is possible to establish correspondences between the coordinates of the moving particles (i.e. copepods) in the four images. Particles were first detected within images by the application of a peakfitting routine after high-pass filtering, and their center of gravity were calculated with a weighted gray value operator (Maas et al. 1993). The three-dimensional coordinates of the particles were then derived for every time step by forward intersection, introducing their coordinates as unknowns in the augmented projection model (Maas et al. 1993, Malik et al. 1993). The corresponding particle coordinates in the object space form the point clouds at each time instant before the tracking is performed (Willneff 2003). The coordinates were linked between consecutive time steps, allowing the reconstruction of the trajectories of many organisms moving freely and simultaneously in the investigation volume (Willneff and Gruen 2002). The tracking procedure selects correct links for the individual particle from one time step to the next, using the information extracted from both image and object spaces. If a particle can be tracked in the object space over several consecutive time steps, its position in the next time step can be predicted assuming constant velocity or constant acceleration and back-projected onto the images of all cameras. The search in the image space after projection of the search volume from the object space either confirms the predicted location or leads to unmatched detections. For an optimal detection of the organisms in the sediment bed, the camera rig was placed on top of the flume to minimize the portion of the optical path crossing

PAM grains. The accuracy of the coordinates of copepods measured by the 3D-PTV system was higher in the free water than in the sediment bed, because of a very slight mismatch in the index of refraction between water and the transparent sediment, because of the curvature of the sediment grains and because of the complex shape of copepods. The root-mean-square error during spatial intersection obtained during the measurements with copepods was about 0.5 mm for the components perpendicular to the optical axis and about 1 mm along the optical axis. Nevertheless, the detection of the center of mass of the moving copepods was still precise enough to reconstruct long trajectories also in the subsurface region. The motion of *Eucyclops serrulatus* consists of periods of smooth displacement caused by flow transport, and sudden and frequent jumps that result in high values of velocity fluctuations. Our tracking algorithm estimates the next position of a particle based on the velocity and acceleration measured over the previous time steps, and on the assumption of a piecewise constant particle velocity or acceleration if previous positions are available. The search for suitable candidates is then performed both in the three-dimensional and image spaces by back-projection of the predicted position. Sudden jumps sometimes resulted in broken trajectories whose segments have been linked during post-processing using a spatio-temporal matching algorithm (Michalec et al. 2015). The efficiency of the matching algorithm is limited by the presence of multiple copepods nearby the final position of a broken segment. In our measurements the density of copepods was kept low but nevertheless, to avoid linking segments belonging to different trajectories, we limited the range of time steps and the distance over which our gluing algorithm would link positions. Trajectories were smoothed via a Savitzky-Golay filter (Wolf et al. 2012) with a span of 11 frames to improve the measurements of velocity and acceleration. The filter acts as a low pass filter and reduces noise efficiently (Lüthi et al. 2005). As our model species does not display phototaxis, illumination was provided by a strong mercury-vapor lamp placed on top of the flume beside the camera rig (Fig. 3b).

2.3 Results

2.3.1 Flow characterization

We show in Fig. 6 the horizontal component of the velocity, measured by PIV and averaged over the entire recording time and over the streamwise coordinates, for the three Reynolds numbers $Re = 1270$, 3700 and 7410 , corresponding to the three discharges of 0.33 L/s, 1 L/s and 2 L/s respectively. The three hydraulic conditions have a mean velocity averaged over the whole depth of 12 mm/s, 35 mm/s and 67 mm/s respectively. At the lowest Re , flow velocities are small and the behavior of copepods is relatively independent from the background flow motion. This condition corresponds to a mean velocity of 12 mm/s and will be used as a base-flow situation for planned hydropeaking experiments.

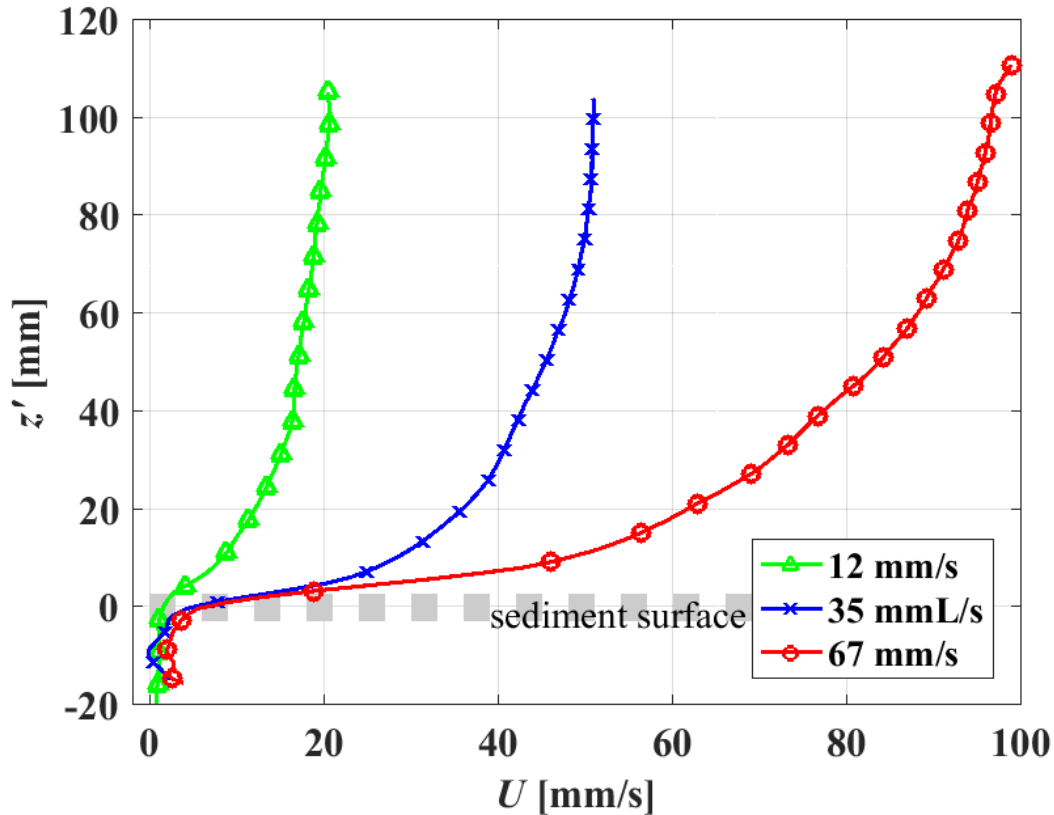


Figure 6. Vertical profiles of the mean horizontal component of the velocity measured by PIV over glass beads, for a mean velocity of 12 mm/s (green, triangle), 35 mm/s (blue, cross) and 67 mm/s (red, circle), first averaged over the duration of the recording and then over the size of the observation window.

The main hydraulic parameters obtained within our flume are given in Table 1. The origin of the vertical coordinates z' meets the condition of a logarithmic flow profile over a rough wall, which is given by the formula:

$$U = U_1^* / \kappa \times \ln((z - z') / k_s) + A_r \times U_1^*$$

where U is the time-averaged streamwise velocity component, U_1^* the shear velocity, κ the von Karman constant, z the vertical coordinate, z' the vertical coordinate with origin at the point where the log velocity profile becomes zero, k_s the equivalent sand roughness, and A_r a constant of integration. The constants κ and A_r were set to 0.4 and 8.5, respectively (Nezu and Nakagawa (1993), see also Graf and Altinakar (1998) and references therein). The values of U_1^* , z' and k_s are obtained by fitting a logarithmic profile to measured velocities (U) in the logarithmic profile region of the flow (i.e. between the sediment surface and $y'/h = 0.3$).

Table 1. Hydraulic parameters. Mean velocity (U_{mean}), channel width (B), water depth (h), discharge (Q), Froude number (Fr), Reynolds number (Re), equivalent sand roughness (k_s), shear velocity obtained by fitting measured velocities to a logarithmic flow profile (U_1^*), shear velocity obtained from the shear stress (U_2^*), and shear velocity obtained from the Reynold stress (U_3^*).

| U_{mean} [mm/s] | 12 | 35 | 67 |
|-------------------|----------------------------|------------------------|------------------------|
| B [m] | 0.27 | 0.27 | 0.27 |
| h [m] | 0.11 | 0.11 | 0.11 |
| Q [L/s] | 0.33 | 1 | 2 |
| Fr | 0.012 | 0.034 | 0.065 |
| Re | 1270 (transitional regime) | 3700 (fully turbulent) | 7410 (fully turbulent) |
| k_s [m] | 0.060 | 0.023 | 0.039 |
| U_1^* [m/s] | 0.0015 | 0.0044 | 0.0091 |
| U_2^* [m/s] | 0.0014 | 0.0041 | 0.0078 |
| U_3^* [m/s] | 0.0009 | 0.0026 | 0.0072 |

We further validate the quality of the shear velocity obtained from the logarithmic flow profile by considering that, for a uniform flow, the wall shear stress relates to the energy slope S_E and hydraulic radius r_{hyd} as:

$$U_2^* = (\tau_0/\rho)^{1/2} = (g \times r_{hyd} \times S_E)^{1/2}$$

where τ_0 is the shear stress, g the gravitational acceleration, r_{hyd} the hydraulic radius defined by the ratio of total percolated area over wetted perimeter, and S_E the energy slope. S_E is computed via the Gauckler-Manning-Strickler formula for uniform flows $v = k_{St} \times S_E^{1/2} \times r_{hyd}^{2/3}$ with k_{St} calculated to be $43 \text{ m}^{1/3}/\text{s}$ with the formula $k_{St} = 21.1/((d_{90})^{1/6})$ with d_{90} as the representative diameter of 90% of the grains. Finally, a third way of estimating the shear velocity is based on the Reynolds stress at the channel bed surface:

$$U_3^* = (\langle u \times v \rangle_{(z=0)})^{1/2}$$

where u and v are the horizontal and vertical velocity fluctuations, respectively, and the overbar represents the time average. The values for friction velocity and other relevant parameters are summarized in Table 1. Friction velocities U_1^* and U_2^* are generally in reasonable agreement, whereas U_3^* tends to be somewhat smaller, which could be due to the permeability of the sediment bed. We have used these three different approaches with the aim of improving the quality of our estimation of the shear velocity, as these methods usually result in slightly different

values. Since there is no clear best estimate, we take as a reference U^* the arithmetic mean of the three approaches.

To check for the presence of a developed logarithmic profile in the streamwise component of the velocity, we plotted the logarithm of the vertical axis z' normalized by the roughness coefficient (e.g. Graf and Altinakar 1998) against the time- and space-averaged velocities measured by PIV (Fig. 6). The linear regressions are calculated by taking into account the logarithmic part of the flow field only, and they agree well with the measured velocity values, even at the lowest Reynolds number where turbulence is not fully developed. Fig. 7 shows the velocity values for $z' = 0$ to $z' = 0.3h$. As the velocity close to the spheres does not reach zero because there is a flow inside the sediment bed, values of velocities that do not follow the logarithmic flow profile are excluded; black-filled markers indicate the velocity values used in our analysis. The coefficient of determination R^2 of the linear regressions are given for the three flow conditions. Fig. 6 and 7 show that the flow velocity inside the sediment bed does not decrease to zero, because the sediment bed forms a permeable rough wall that influences the flow properties near the wall and also in the free water column (see e.g. Manes et al. 2011 and references therein) as well. It was shown by Manes et al. (2011) that the influence of the sediment bed on flow properties depends on its roughness, thickness, and on the flow pattern that develops between the sediment grains. Large differences in the turbulence intensity or in the velocity fluctuations u and v are expected, especially towards the wall, when comparing with values obtained above an impermeable rough wall.

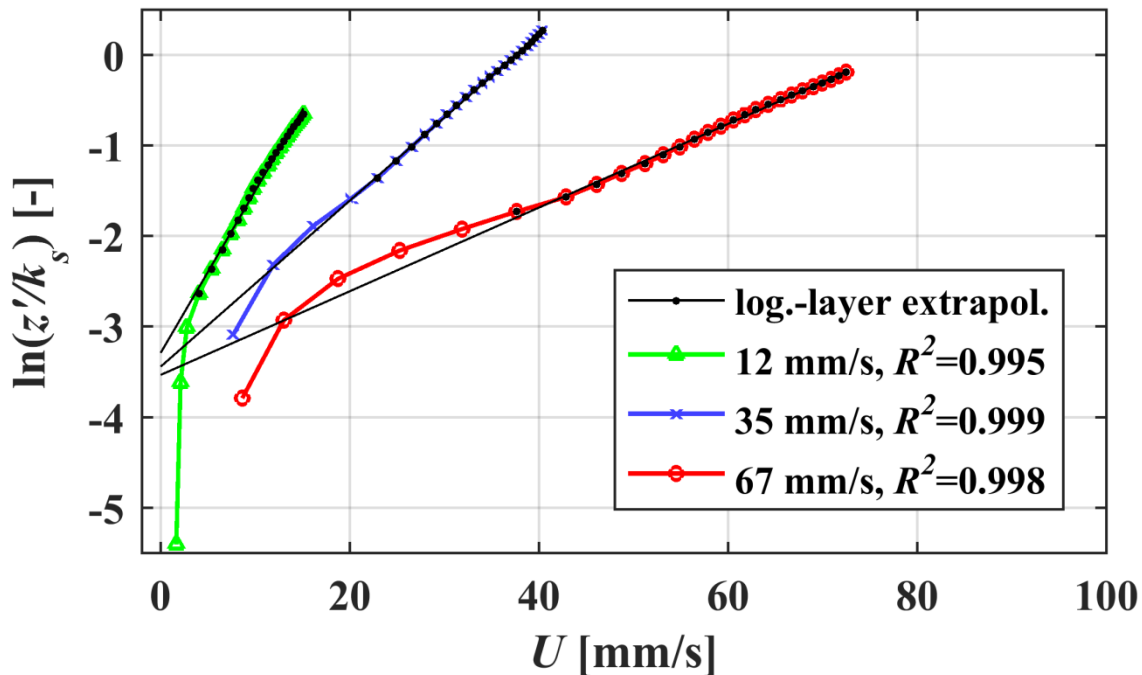


Figure 7. The natural logarithm of z'/k_s plotted against the time- and space-averaged horizontal velocity U in the logarithmic region, for the three hydraulic cases (12 mm/s: green, triangle; 35 mm/s: blue, cross; 67 mm/s: red, circle), with the respective linear regression shown as a solid line.

To compare our measured values with reference values presented e.g. in Manes et al. (2011), we plot in Fig. 8 the vertical profile of the root-mean-square velocity fluctuations. The lowest Reynolds number in the experiments of Manes et al. (2011) is 25700, the highest Reynolds number in our measurements is 7410 and the lowest is 1270. The permeability in our measurements (at the highest Reynolds number) can be approximated as $K = C \times (d_{10})^2 / 100 \times \mu / (g \times \rho) = 1.02 \times 10^{-7}$ (with the conductivity estimated from the grain size as $C \times (d_{10})^2$ with C as a factor often set to be 1 and d_{10} the diameter of the 10 percentile grain size, μ the dynamic viscosity of water, g the gravity acceleration and ρ the density of water) and agrees relatively well with the value obtained by Manes et al. (2011) at their lowest Reynolds number ($K = 1.6 \times 10^{-7}$). The data from our measurements at $Re = 7410$ (67 mm/s) agree quite well with the values of Manes et al. (2011). At the lower Reynolds numbers however, the differences between our measurements and the reference values are larger, our data showing differences up to a factor of 2. These marked differences of lower values can be explained by the low Reynolds numbers and by a flow that presumably involves non-turbulent / turbulent flow transition. Besides this stronger intermittency at lower Re , the flow conditions in our flume represent mean velocity and turbulence characteristics that are comparable to reference values in the literature.

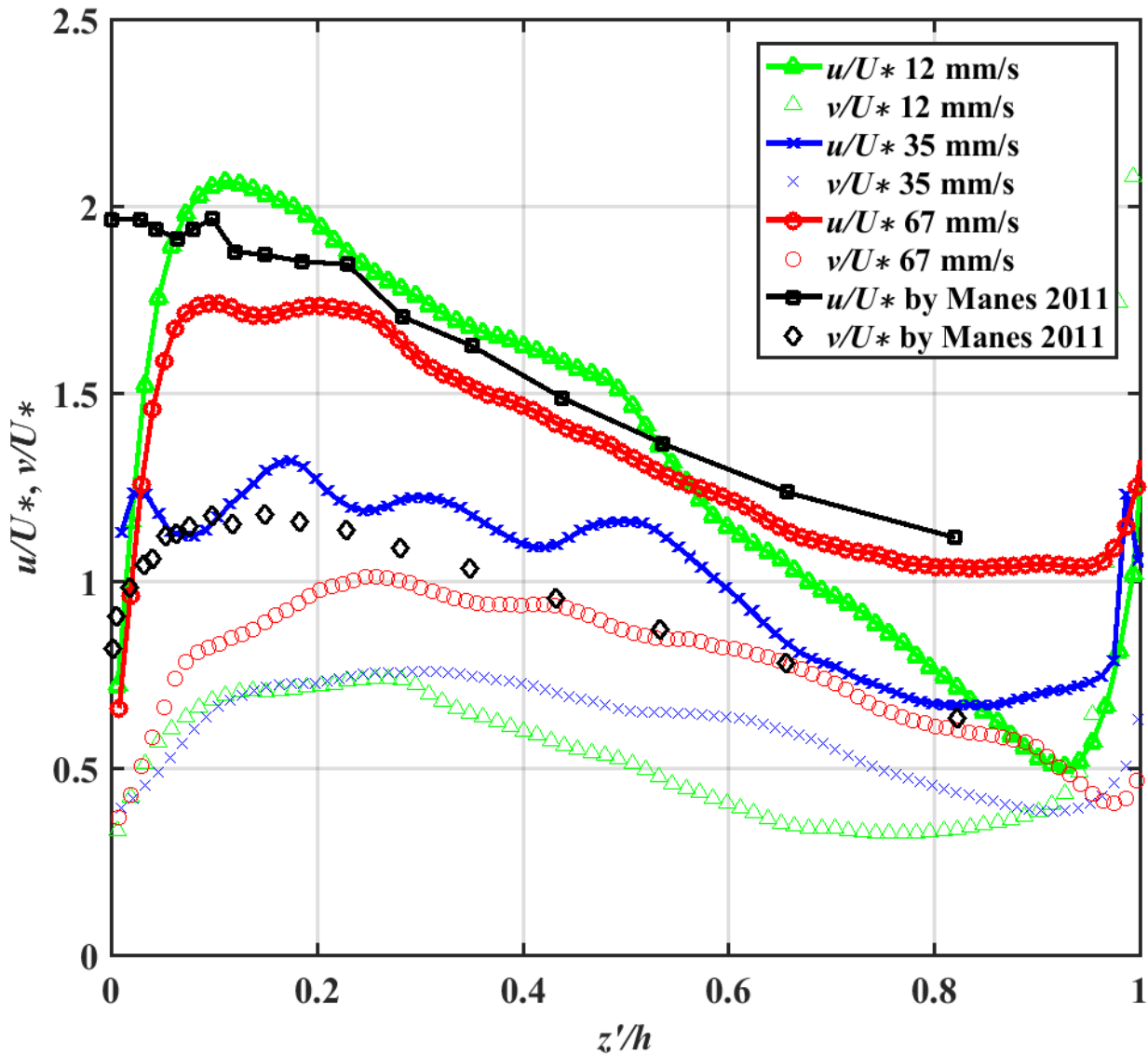


Figure 8. Horizontal and vertical velocity fluctuations u/U_* and v/U_* over the normalized height z'/h for the three hydraulic cases (12 mm/s: green, triangle; 35 mm/s: blue, cross; 67 mm/s: red, circle). The data from Manes et al. (2011) for their lowest Reynolds number of 25700 and a similar permeability is given as reference.

2.3.2 Three-dimensional tracking of copepods

With our setup it was possible to observe copepods swimming freely not only in the water column and above the surface of the sediment, but also inside the transparent sediment bed (Movie S1). We show in Fig. 9 a subset of trajectories of copepods swimming under a constant discharge of 0.33 L/s and a mean flow velocity of 12 mm/s in 3D and in Fig. 10 the same trajectories are shown in 2D. Most individuals were observed near or inside the sediment bed and for better visualization we restrict the vertical coordinates in Fig. 9 to 2 cm above the sediment bed (although trajectories have been obtained up to the water surface i.e. $z = 11$ cm).

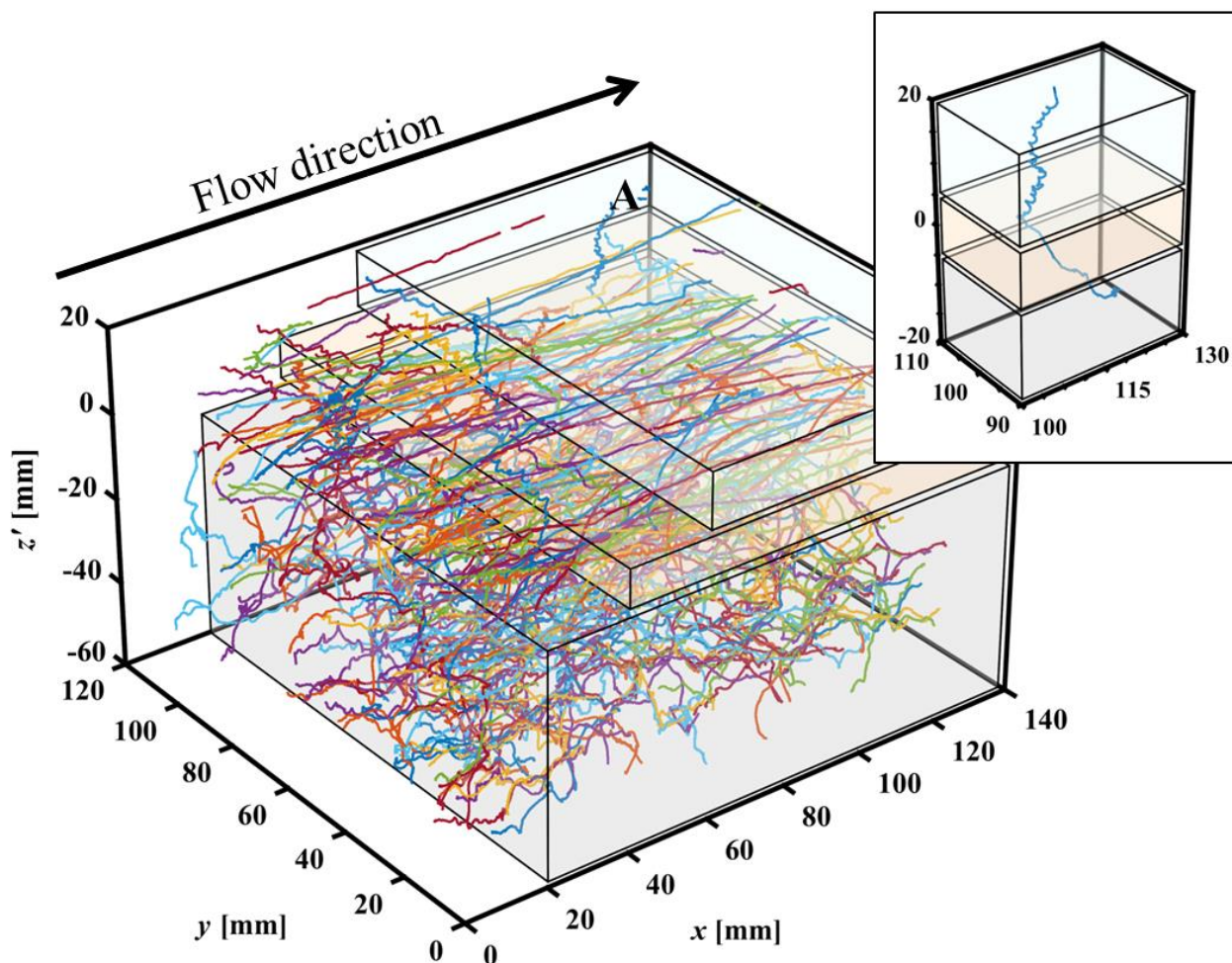


Figure 9. Subset of trajectories of copepods swimming under a constant mean flow velocity of 12 mm/s. Water flows from the left to the right (i.e. in the positive x direction). The surface of the sediment bed is located at $z' = 0$. The figure also shows the three compartments (sediment bed in gray; interface in orange; free water in blue). The inset shows a close-up view of trajectory A.

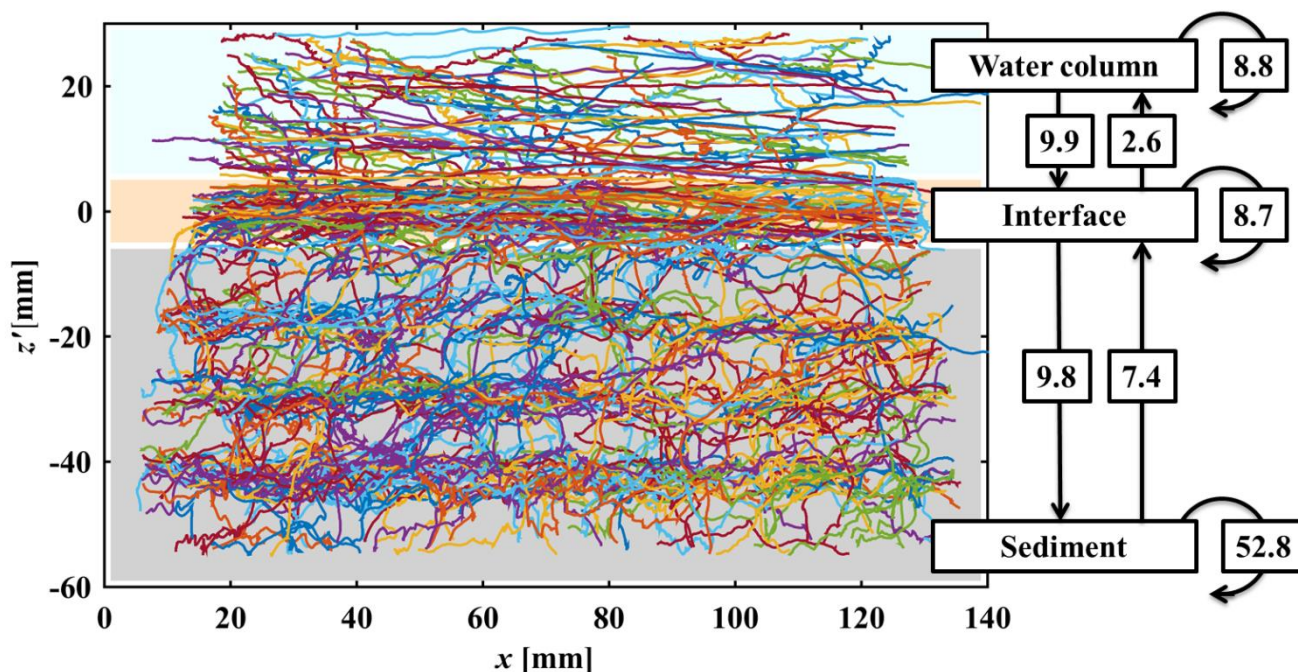


Figure 10. Two-dimensional projection of the trajectories, and percentages of trajectories starting and ending in each of the three compartments (sediment, interface and water column) over the entire recording duration. Flow is from left to right (i.e. in the positive x direction). The sediment layer appears in grey and the free water column in blue. The interface is as a transition zone of high copepod density, colored in orange and whose thickness approximates the diameter of a PAM sphere.

We obtained 32000 three-dimensional coordinates of copepods swimming in the free water and 350000 coordinates of copepods moving in the sediment bed, for a total of 3510 trajectories. Copepods were able to swim in the entire flume, and moved readily and in all directions both in the water column and inside the sediment bed. As an example of the active behavior of copepods that we observed during the measurements, we present in Fig. 9 the trajectory displayed by a copepod that moved upward while keeping its horizontal position constant (trajectory A). Horizontal maintenance despite flow motion was achieved by swimming actively against the flow via a series of successive jumps interrupted by very short sequences of passive drift.

By considering the bulk vertical distribution of the trajectories (Fig. 9 and Fig. 10) it is possible to define three compartments that correspond to the free water section ($z' > 5$ mm), to an interface region between the free water and the sediment bed ($-5 \text{ mm} > z' > 5 \text{ mm}$), and to the sediment bed ($z' < -5$ mm). The interface region corresponds to a relatively narrow layer whose thickness approximates the size of a PAM sphere (i.e. 10 mm). Within the interface region, the flow velocity is lower than in the free water, the PAM spheres protrude from the surface of the sediment layer, and copepods swim in a straight pattern both upstream and downstream before entering into the sediment layer via an interstice. Differences in the geometrical properties of the trajectories are illustrated in Fig. 10. As expected, trajectories in the sediment layer are more convoluted than trajectories in the free water, consequence of the motion of copepods in the interstices of the sediment bed, between the PAM spheres. We quantified the exchanges between these three compartments by considering the percentage of trajectories starting and ending in a

different or in the same compartment (Fig. 10). More than 50 % of the total trajectories are confined to the sediment bed. The percentage of trajectories confined to the free water and to the interface region is roughly similar. The percentage of trajectories starting in the free water and ending in the interface layer, and starting in the interface and ending in the sediment bed is also comparable. However, the percentage of trajectories leaving the water column to the interface and to the sediment bed is larger than the percentage of trajectories leaving the sediment to the free water. To reach a steady-state distribution of organisms in each compartment fluxes in or out need to balance and this is approximately the case, but a longer recording duration would be beneficial to balance the free water to sediment layer exchange. A net flux towards the sediment could also indicate that the flume is somewhat short and conditions upstream are such that more organisms are in the free water, while there is a marked preference of this epibenthic species for the sediment layer. That is, copepods drifting from upper regions of the flume exited their drift by actively swimming downward within our investigation volume. By considering the vertical distribution of copepods (Fig. 11) we show that the density of copepods inside the sediment bed is indeed much higher than in the free water, with peaks corresponding to the interstices between the layers of PAM spheres in the sediment bed. Because in our measurements the spheres were randomly packed together in loose random packing, these interstices are likely to be relatively spacious (Dullien 1992), allowing the copepods an easy and deep intrusion.

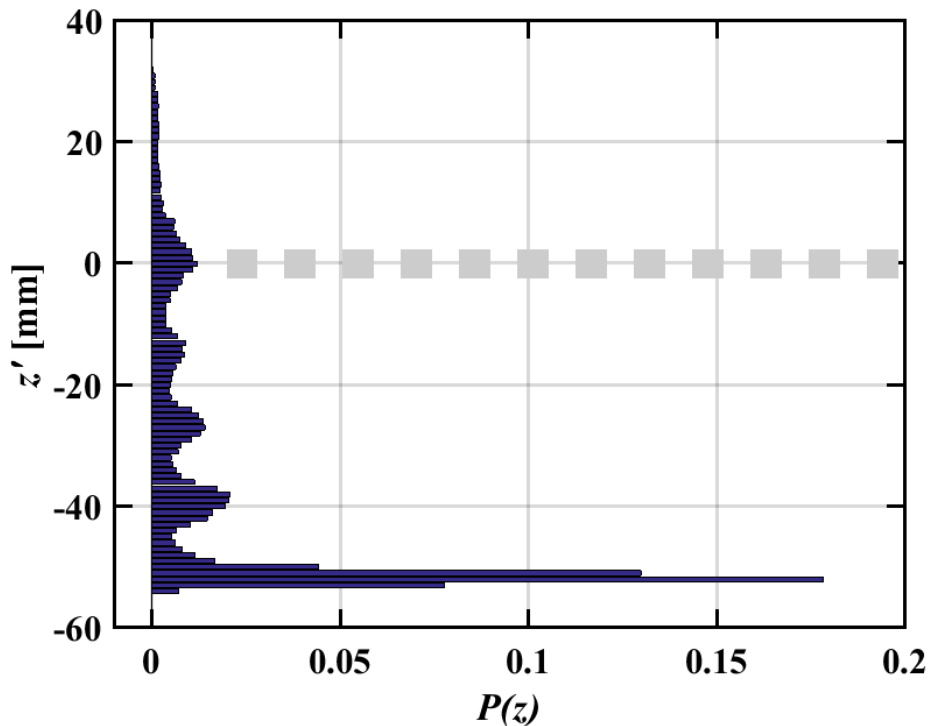


Figure 11. Histogram of the vertical distribution of copepods in the investigation volume. The surface of the sediment layer is located at $z' = 0$ and indicated by the grey dashed line. The multiples peaks correspond to regions of high copepod density i.e. to the interstices between the layers of PAM spheres.

Fig. 12 shows the streamwise component of the velocity and acceleration for one representative trajectory in the sediment layer. A positive velocity means a motion following the flow direction whereas a negative velocity indicates upstream swimming. For this copepod we notice maximal

velocities of about 40 mm/s and accelerations up to 800 mm/s², in agreement with the values typically reached by copepods during relocation jumps (e.g. Michalec et al. 2015). Fig. 12 also illustrates the intermittent properties of the motion of *E. serrulatus*, which swims by jumping. Periods of high frequency jumps do not occur over extended periods of time but can be very intense, with in some cases up to three to five jumps per second (Movie S1).

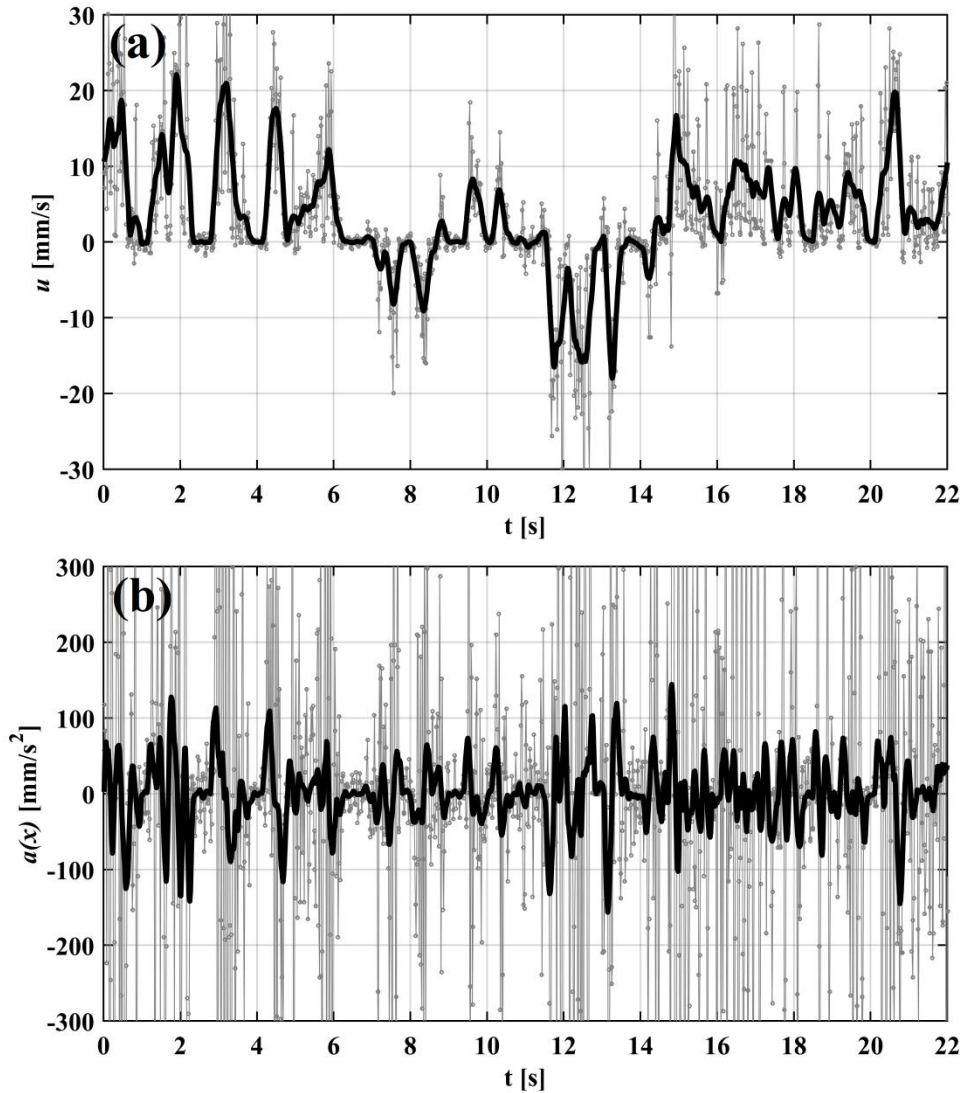


Figure 12. Time series of the streamwise velocity (a) and acceleration (b) of a single copepod, showing the very intermittent motion of these organisms. Grey dots with thin lines indicate raw values; solid lines in black indicate filtered values.

We further quantify the behavior of copepods in the three compartments by considering the probability density functions (PDFs) of their velocity. The relative horizontal velocity is computed by subtracting the first time- and then space-averaged streamwise flow velocity obtained from our PIV measurement (Fig. 6), as:

$$u_r(x,y,z,t) = u(x,y,z,t) - U(z)$$

where u_r is the relative velocity of the copepod in the three-dimensional space at time t , u is the velocity of the copepod measured by 3D-PTV at time t , and U is the time- and space-averaged streamwise flow velocity at the vertical coordinate z . The vertical and spanwise velocity components do not require correction, as mean flow velocities in these directions are negligible. The PDFs of the horizontal components of the velocity before (absolute velocity) and after (relative velocity) subtracting the mean velocity of the background flow are given in Fig. 13 and 14. The PDFs show a peak corresponding to low values of weak activity, and significant tails that indicate the relatively large probability of extreme values (i.e. strong jumps) in both surface and subsurface regions. The PDF of the absolute velocities in the sediment layer is symmetric and centered at 0 mm/s. The mean absolute velocity in this compartment is 0.6 mm/s, indicating that copepods are slightly drifting downstream in the sediment. The PDF of the absolute velocities in the interface region is centered at 0 mm/s with a mean velocity of 3 mm/s. The PDF of the absolute velocities in the free water shows a peak at 10 mm/s and has a mean of 11 mm/s. These results show that copepods are advected downstream by the flow in all three compartments.

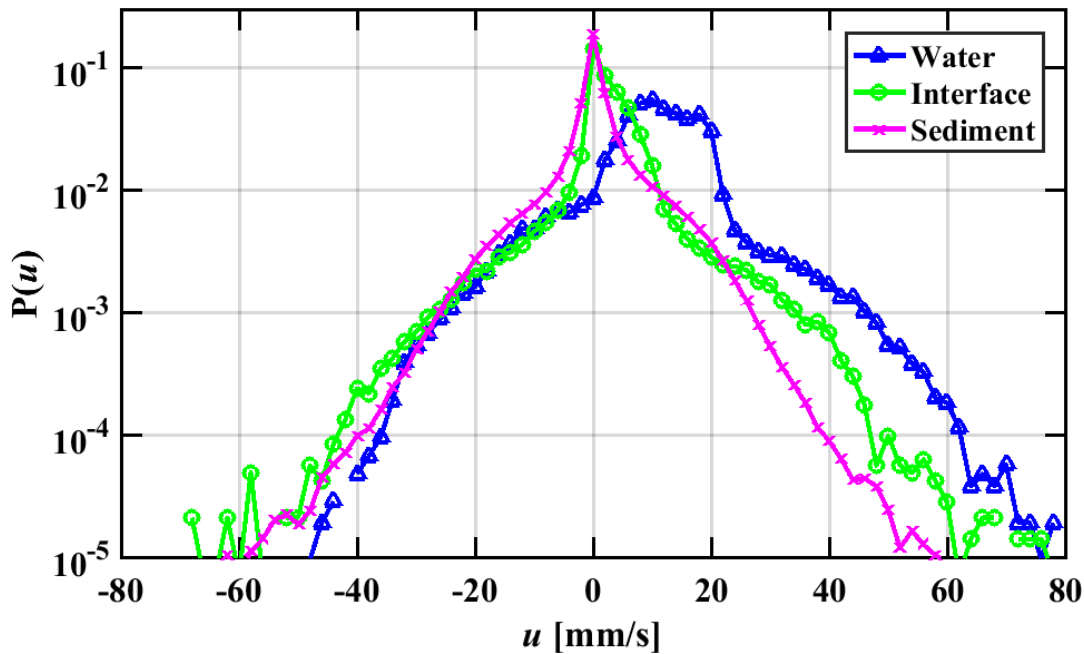


Figure 13. Probability density functions of the absolute streamwise component of the velocity for copepods moving in the sediment (purple, cross), in the interface layer (green, circle) and in the free water region (blue, triangle).

The PDFs of the relative velocity are given in Fig. 14 for the three compartments. Naturally, the subtraction of mean advection mostly affected the velocities of copepods swimming in the free water. The PDFs are generally rather symmetric and centered at 0 mm/s. An asymmetry can be noted for the relative streamwise velocity at the tails, with heavier tails at negative velocities. This implies that copepods actively swim against the flow in the free water to maintain their streamwise position. The mean relative velocity is 0.6 mm/s in the sediment, 0.4 mm/s in the interface and -1.7 mm/s in the free water, the latter confirming some degree of motility against the flow. We also show in Fig. 14, as an example, the PDF of the vertical component of the velocity in the sediment layer. The PDFs of the vertical and spanwise velocity components in the three compartments have their peak at around 0 mm/s, are symmetric and nearly identical. In the

interface and especially in the free water section, the streamwise velocities are higher than the spanwise velocities. This suggests that *E. serrulatus* directs most of its swimming effort into upstream swimming, potentially to avoid downstream advection by the flow.

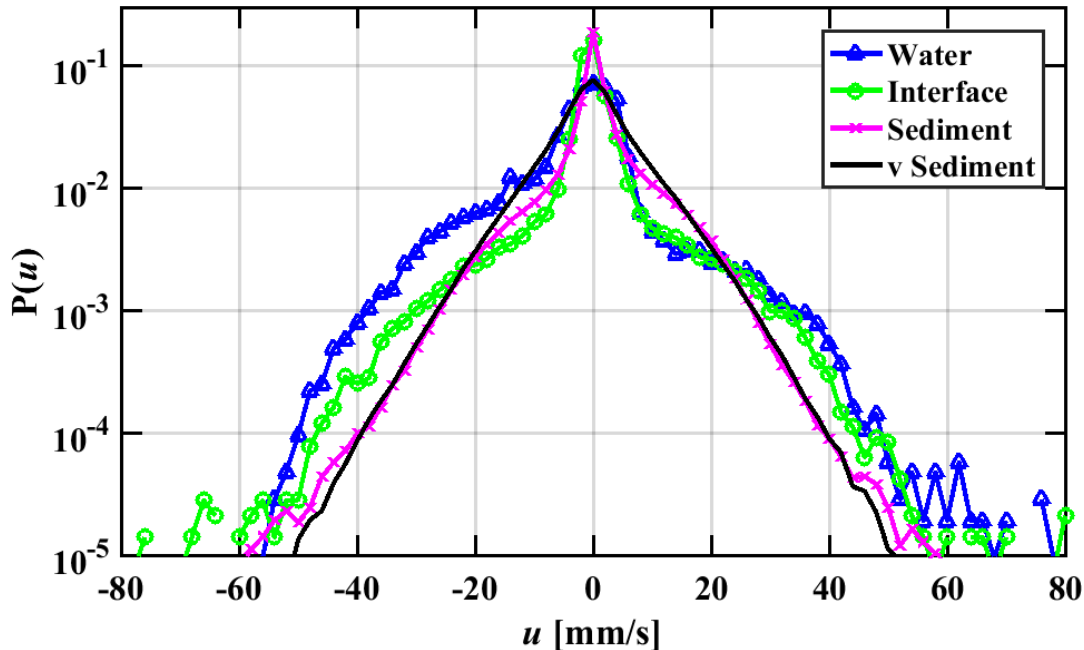


Figure 14. Probability density functions of the relative streamwise component of the velocity for copepods moving in the sediment (purple, cross), in the interface layer (green, circle) and in the free water region (blue, triangle) and PDF of the vertical component of the velocity in the sediment layer (black, solid line).

2.4 Conclusion

We have developed a new method to study the behavior of benthic copepods both in the surface and in the subsurface flow of a flume with a permeable and transparent sediment bed. The setup allows the manipulation of ecologically relevant physical parameters e.g. temperature, flow rate and groundwater exchange. The flow generated in our flume is reproducible, and can be described as a uniform flow field comparable to those found in the literature. By means of 3D-PTV, we were able to detect and track the motion of many benthic copepods swimming freely in the free water and inside the sediment layer. We obtained trajectories of sufficient length for a robust quantification of copepod velocity, acceleration and vertical distribution. Copepods were able to swim in the entire flume without massive loss of organisms due to drift. They showed strong substratum preference and moved readily and in all directions in the sediment bed.

This report lays the groundwork for future experiments in controlled and repeatable conditions. For instance, it will be possible to study the fine-scale displacement of copepods between streambed patches of different flow velocity, to quantify their activity in response to thermal variations and increase in shear stress, and to investigate the role of self-induced motion on drift entry, drifting distance and settlement process. Multiple lines of evidence indicate a substantial role of behavior in the ecology of benthic organisms and in the processes generating or

terminating drift (Malmqvist 2002; Naman et al. 2016). Our setup enables the detailed observation of behavioral processes that could not be approached in field studies due to logistic constraints, and provides opportunities for taxon-specific mechanistic studies, with useful ecological applications such as more realistic models of drift flux.

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Chapter 3

COUNTER-CURRENT SWIMMING OF LOTIC COPEPODS AS A POSSIBLE MECHANISM FOR DRIFT AVOIDANCE

This chapter consists of a manuscript submitted to *Ecohydrology*, 2017:

D. Sidler^{1,2,3,4,5,6}, F.-G. Michalec^{1,5,6,7}, and M. Holzner^{1,5,7} (2017). Counter-current swimming of lotic copepods as a possible mechanism for drift avoidance. Submitted to *Ecohydrology*

¹Study concept and design; ²Experimental setup design and construction; ³Experiment performance; ⁴Data acquisition and analysis; ⁵Interpretation of data; ⁶Drafting manuscript; ⁷Critical revision

Abstract

Drift of stream benthic invertebrates is a fundamental ecological process. Several mechanisms have been proposed to explain the persistence of benthic populations despite this drift. For species that lack an aerial adult phase, one possible strategy involves active behavior: organisms perform small-scale counter-current displacements along the streambed. Because these fine-scale processes are difficult to approach in field studies, evidence for behavioral mechanisms against flow advection has remained scarce. We reconstruct the three-dimensional trajectories of the widespread cyclopoid copepod *Eucyclops serrulatus* moving freely in the water column and in the transparent sediment bed of a laboratory flume. In the different experiments the average flow velocity is 12 mm/s, 35 mm/s and 67 mm/s. We isolate the behavioral component of their motion by subtracting the local flow velocity. We show that copepods perform active counter-current swimming to limit downstream drift. The counter-current swimming effort increases with flow velocity and therefore downstream drift remains moderate and does not vary strongly for the different flow rates tested. We show that copepods explore the water column at low flow velocity, but stay close to and perform frequent incursions into the sediment bed at higher flow velocities. This active behavioral response to changing flow conditions, combined with frequent

stops in the substratum where flow velocity is negligible, may confer lotic copepods the ability to reduce downstream transport. Our results confirm the importance of behavioral traits in drift avoidance for species that lack an aerial stage, and the interest of studying taxon-specific responses to varying hydraulic conditions.

3.1 Introduction

Invertebrate organisms living in streams are continually subjected to downstream transport, termed drift. Drift is a fundamental ecological process in streams. It may reduce competition through density-dependent emigration, can influence spatial population structure by linking populations through dispersal, and determines the energy flux that provides habitats with the capacity to support populations of larger, drift-feeder organisms (Naman et al. 2016). Certain benthic species have planktonic developmental stages and are hence subject to downstream drift for part of their life. Drift can also result from accidental, passive dislodgement from the surface of the sediment bed: variations in velocity and in near-bed stress created by rough bed surfaces or by turbulence structures create turbulence sweeps that may exceed critical entrainment thresholds (Blanckaert et al. 2013). Drift is also caused by active entry in the water column in response to diverse cues such as presence of predators, food shortage, or unfavorable abiotic conditions. For instance, behavioral drift was observed during experimental floods when the temperature of the water released into the stream was different from that of the stream, and was attributed to organisms actively entering the water column or being dislodged as a consequence of increased activity on the streambed (Bruno et al. 2012; Carolli et al. 2012). Hence, constant drift results from passive or active entry in the water column, and occurs during normal hydraulic conditions. Constant drift ensures the regular occurrence in the drift of otherwise benthic species.

Without mechanisms that restore population density in the upstream part of the reach, advection will result in depletion, and the bulk of the population will move downstream. This reduction is due to the tendency of benthic invertebrates to drift downstream with the current. However, upper reaches remain populated despite an apparently considerable reduction in the number of organisms. The long-term persistence of populations subjected to continuous advection is termed *drift paradox*. The widespread assumption has been that upstream flight for oviposition by adult individuals, coupled with density dependence, compensates for the loss of individuals, and hence allows population persistence (Anholt 1995). This mechanism is valid both for flights that are directed upstream and for movements in random directions. However, it does not explain the persistence of benthic species that are commonly found to drift but lack an aerial adult stage. More recent studies indicate that small-scale random movements along the streambed of the order of centimeters, combined with density dependence, are sufficient to maintain populations of species with no aerial stage (Humphries and Ruxton 2002). These movements over relatively small distances may compensate for previous drift events, and may allow efficient recolonization of upstream sites. Hence, active locomotion over small scales appears to play a crucial role in the capability of populations to avoid excessive downstream advection in species that lack an adult dispersive stage.

Unlike constant drift, catastrophic drift relates to factors that depend directly on flow velocity. Catastrophic drift is defined as a rapid increase in passive drift. It results from mechanical dislodgement from the substrate caused by increase in discharge and high near-bed shear stress. Catastrophic drift is usually associated with substrate mobilization, but considerable washout of organisms can also occur in non-scouring flows with limited sediment movement (Imbert and

Perry 2000; Gibbins et al. 2007). Laboratory and field studies show that predisposition to drift varies greatly between taxa depending on ecological, morphological and behavioral traits: many species have evolved adaptations to avoid being swept downstream into habitats that are less favorable for their development and reproduction. For instance, certain species perform small-scale displacements and move toward refuge habitats e.g. to deeper and more stable sediment layers or to nearby patches of lower flow velocity (Borchardt 1993; Imbert and Perry 2000; Holomuzki and Biggs 2000). Other species have better morphological adaptations to resist high flow velocities (Jakob et al. 2003; Naman et al. 2017).

Downstream advection of benthic invertebrates has received much attention, in the context of constant drift, and also in relation to increased flow caused by natural spates and by the release of water from reservoir hydroelectric power stations. However, mechanistic studies of the relationships between hydraulic parameters, behavior and drift are unavailable for many taxa (Blanckaert et al. 2013), and our knowledge on the individual response and behavioral traits that may be responsible for population features remains scarce. Consequently, the general processes that govern invertebrate drift, including the behavioral mechanisms involved in drift avoidance and the ability of organisms to perform self-locomotion amid flow motion, remain elusive (Naman et al. 2016).

The present study investigates whether benthic cyclopoid copepods are able to limit downstream advection through active behavior. We selected cyclopoid copepods because they include widespread taxa in prealpine gravel-bed streams (Brunke and Gonser, 1999) and represent 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 behavioral traits in benthic copepods remains scarce, primarily because of the difficulties of tracking the motion of many organisms swimming simultaneously and in three dimensions both in the water column and in the interstitial spaces of the sediment layer. In this study, we take advantage of an advanced three-dimensional tracking technique to understand the relationship between hydraulics, active motion, and drift avoidance in a widespread but often neglected taxon.

Our model species is *Eucyclops serrulatus*, one of the most common cyclopoid copepod in Central Europe (Alekseev et al. 2006). This species inhabits various water bodies, from lentic habitats such as ponds and lakeshores to flowing environments such as streams and rivers (Jersabek et al. 2001; Alekseev and Defaye 2011). Although *E. serrulatus* is sometimes considered as an epigeic species (e.g. Di Lorenzo et al. 2015 and references therein), it is not restricted to the hyperbenthos, and is often found in the interstitial water of river reaches, at depth ranging from a few centimetres 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 features, i.e. the active exploration of both the water column and the sediment bed, make the widespread cyclopoid copepod *E. serrulatus* an interesting model species to identify the behavioral mechanisms used by lotic copepods to limit downward advection.

Despite their small size and limited swimming ability with regards to intense currents, copepods are reputed for their powerful motion, and for being able to perform directed displacements despite strong hydrodynamic conditions. Vigorous counter-current swimming was observed in marine planktonic calanoid copepods exposed to strong vertical currents (Genin et al. 2005).

Shang et al. (2008) reported on energetic counter-current swimming followed by downward movements and active substrate attachment in the estuarine epibenthic calanoid copepod *Pseudodiaptomus annandalei* in response to increasing flow velocity. We know much less about the behavioral response to flow in freshwater cyclopoid copepods living in streams, and about their capability of active swimming despite downstream currents.

We have previously shown (Sidler et al. 2017) that the cyclopoid copepod *E. serrulatus* swims by jumping, a feature shared with many species of calanoid copepods and which enables them to perform self-locomotion amid turbulent transport (Michalec et al. 2015). This observation prompted the present study, which tests for the hypothesis that self-locomotion and small-scale displacements, both in the interstices of the sediment layer and in the water column, enable lotic cyclopoid copepods to compensate for downstream drift. We recorded the motion of copepods swimming freely in a laboratory flume featuring a transparent sediment bed. Water discharge was varied to increase water velocity and near-bed shear stress. We performed measurements at three flow conditions with an average flow velocity of 12 mm/s, 35 mm/s, and 67 mm/s, and we quantified changes in their kinematics and vertical distribution.

3.2 Materials and method

Three-dimensional particle tracking velocimetry is a quantitative flow measurement technique originally designed for tracking particles in turbulent flows (Maas et al. 1993; Malik et al. 1993; Willneff 2003) and recently applied to study the motion of small organisms (Puckett and Ouellette 2014; 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.

Copepods were collected using a Bou-Rouch pump from the prealpine, unregulated Töss River near Winterthur (Switzerland). The reach consists of riffles, pools and gravel bars. Only riffles were sampled to select organisms living in comparatively fast-flowing sections. A large volume of water was pumped from the coarse-grained sediment and filtered to separate benthic organisms from small pebbles and large detritus. Copepods were cultured in the laboratory for several generations. The culturing method was adapted from Kumazawa (2000) and Park et al. (2005). Copepods were kept in 20 L buckets and 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 (Sidler et al. 2017).

We performed particle-tracking measurements in a custom acrylic glass flume (200 cm long and 27 cm wide) with a transparent sediment bed consisting of polyacrylamide spheres. The spheres are approximately 10 mm in diameter, and have nearly the same index of refraction as water. This allows tracking particles and organisms in the near sediment bed. The flume features a low- and high-level reservoir, and a pump for water recirculation. A valve with a flow meter regulates the discharge. Uniform flow conditions are obtained via a combination of bricks, voluminous meshes and perforated plates at the inlet and outlet of the flume. Homogeneous illumination was provided by a laser (527 nm, pulse energy of 60 mJ) through the sidewall of the flume. We used four synchronized cameras (Mikrotron EoSens) equipped with 60 mm lenses and recording on two solid-state disks arrays (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). This configuration is the most favourable for optical access to the copepods in the sediment, because it minimizes the distance travelled by optical rays through polyacrylamide spheres. The

cameras were operated at a frame rate of 100 to 250 Hz, and focused on an investigation volume that was approximately 15 cm (x) \times 15 cm (y) \times 15 cm (z). The investigation volume is located far enough from the inlet and outlet, and centred 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 cm). Copepods are mostly located near the sediment surface, and for clarity of representation we focus our analysis on a sub-region of the investigation volume given by $-3 \text{ cm} < z < 5 \text{ cm}$.

We conducted measurements at three flow conditions. For each condition, we first characterized the flow field in the investigation volume (both in the sediment bed and in the water column) by tracking neutrally buoyant polyamide tracer particles with a diameter of 100 μm and a density of 1 g/cm^3 . Because the mean flow velocities along the spanwise (y) and vertical (z) axes are very small (Sidler et al. 2017), we focus our analysis on the streamwise velocity i.e. along the x -axis. Hereafter we use the terms velocity and streamwise velocity interchangeably to characterize the motion of both tracer particles and copepods. From the trajectories of tracer particles, we computed the time-averaged vertical profiles of the streamwise flow velocity component U_f , the profiles of the root mean square of streamwise velocity fluctuations u_f' , and the profiles of the vertical gradient of the time-averaged streamwise velocity dU_f/dz . We indicate in Table 1 the mean flow velocity, the Froude number, the Reynolds number, the number of trajectory points, and the recording duration for the three flow conditions.

Table 1: Flow parameters and number of tracer and copepod coordinates for the three flow cases. $\langle U_f \rangle$ is the mean flow velocity. Fr is the Froude number. Re is the Reynolds number. The water depth was kept constant at approximately 10 cm above the surface of the sediment.

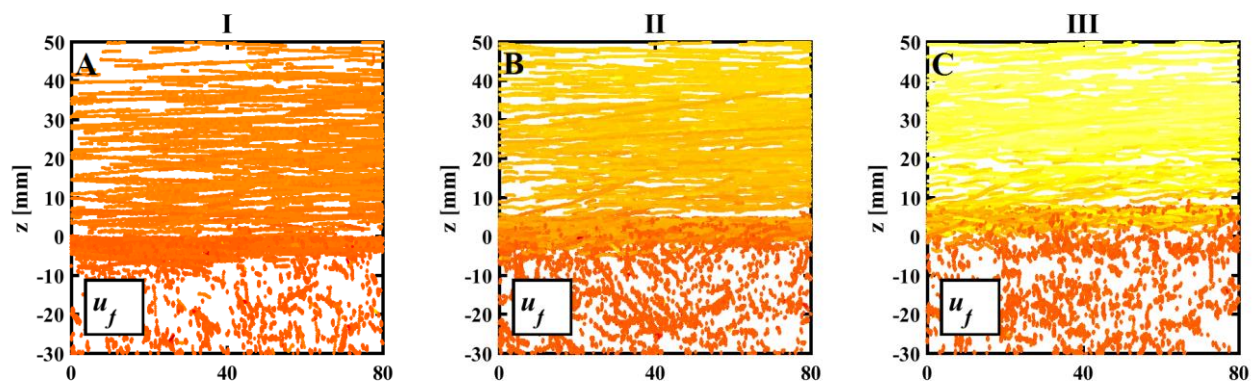
| Flow case | I | II | III |
|---------------------------------------|---------|---------|--------|
| $\langle U_f \rangle$ (mm/s) | 12 | 35 | 67 |
| $\max U_f$ (mm/s) | 20 | 52 | 97 |
| Fr | 0.012 | 0.034 | 0.065 |
| Re | 1270 | 3700 | 7410 |
| Number of copepod coordinates | 872546 | 1959107 | 119771 |
| Number of tracer particle coordinates | 2752541 | 2602439 | 722416 |
| Recording duration (min) | 37 | 15 | 14 |

Copepods 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. 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. The relative streamwise velocity of the copepods $u_{c,rel}$ was calculated as the difference between their absolute streamwise velocity $u_{c,abs}$ and the time-

averaged flow velocity U_f . The relative velocity of copepods is stronger than the turbulent velocity fluctuations, and therefore mostly captures their active behavior: positive values indicate active downstream swimming, whereas negative values indicate active counter-current swimming.

3.3 Results

We show in Fig. 1 the side views of three-dimensional trajectories of tracer particles (panels A to C) and copepods (panels D to I) for the three flow cases (left to right). The color coding of panels A to F displays the absolute streamwise velocity, whereas panels G to I show the relative velocity. Water flows from left to right i.e. along the positive x-axis, and the origin of the z-axis lies slightly below the surface of the sediment bed. Trajectories of tracer particles are for the most part straight and directed downstream (panels A to C). However, turbulence and possible weak secondary flows in the flume result in deviations from purely horizontal movements, which are more evident for cases II and III where turbulence is more pronounced. Trajectories of tracer particles in the sediment layer are much shorter compared to the surface flow region, because of the very small flow velocity in the pores between the polyacrylamide spheres. We show in Movie S1 that *E. serrulatus* swims actively toward the sediment bed when transferred into the experimental flume. Consequently, copepod trajectories were mainly recorded within and at the surface of the sediment, where the abundance of copepods was the highest. The difference in the higher information density and number of trajectories for the three flow cases is due to differences in the recording duration (longer time series were recorded for the low flow rate i.e. for case I). Contrary to tracer particles, the motion of copepods is not restricted to the downstream direction and is strongly intermittent, with frequent and sudden jumps separated by short periods of passive drifting and frequent changes in direction (Sidler et al. 2017). Copepods generally swam uniformly in all directions in the sediment bed for all three cases, while for the surface flow region downstream motion prevailed. For increasing flow speeds, downstream transport of copepods is enhanced. However, copepods do not behave as passive particles. Panels D to F show that they perform active counter-current swimming: their absolute velocity is typically lower than that of tracer particles, and copepods often manage to swim upstream despite advection. This active swimming against the flow is particularly visible when trajectories are color-coded with their relative velocity (panels G to I).



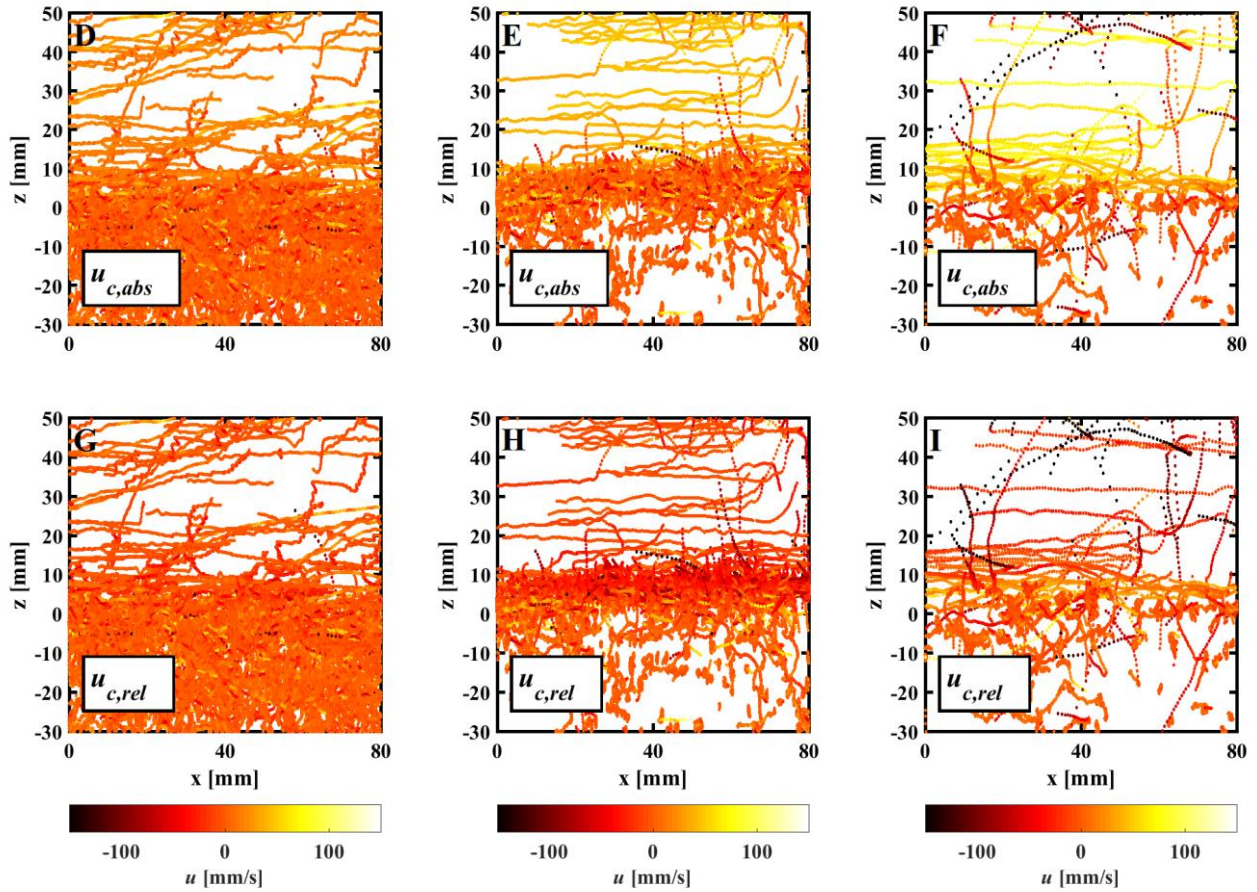
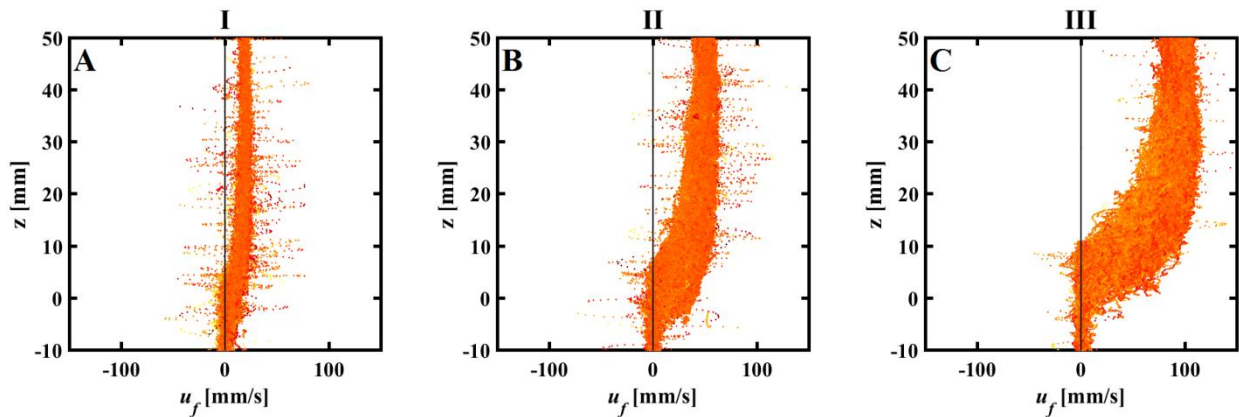


Figure 1: Side views of trajectories of tracer particles (panels A to C) and copepods (panels D to I) color-coded with the absolute streamwise velocity of the flow u_f or with the absolute streamwise velocity of the copepods $u_{c,abs}$ or with the relative velocity of the copepods $u_{c,rel}$. Flow is from left to right. In panels A to F, red indicates upstream motion and yellow indicates downstream advection. In panels G to I, red indicates counter-current swimming.



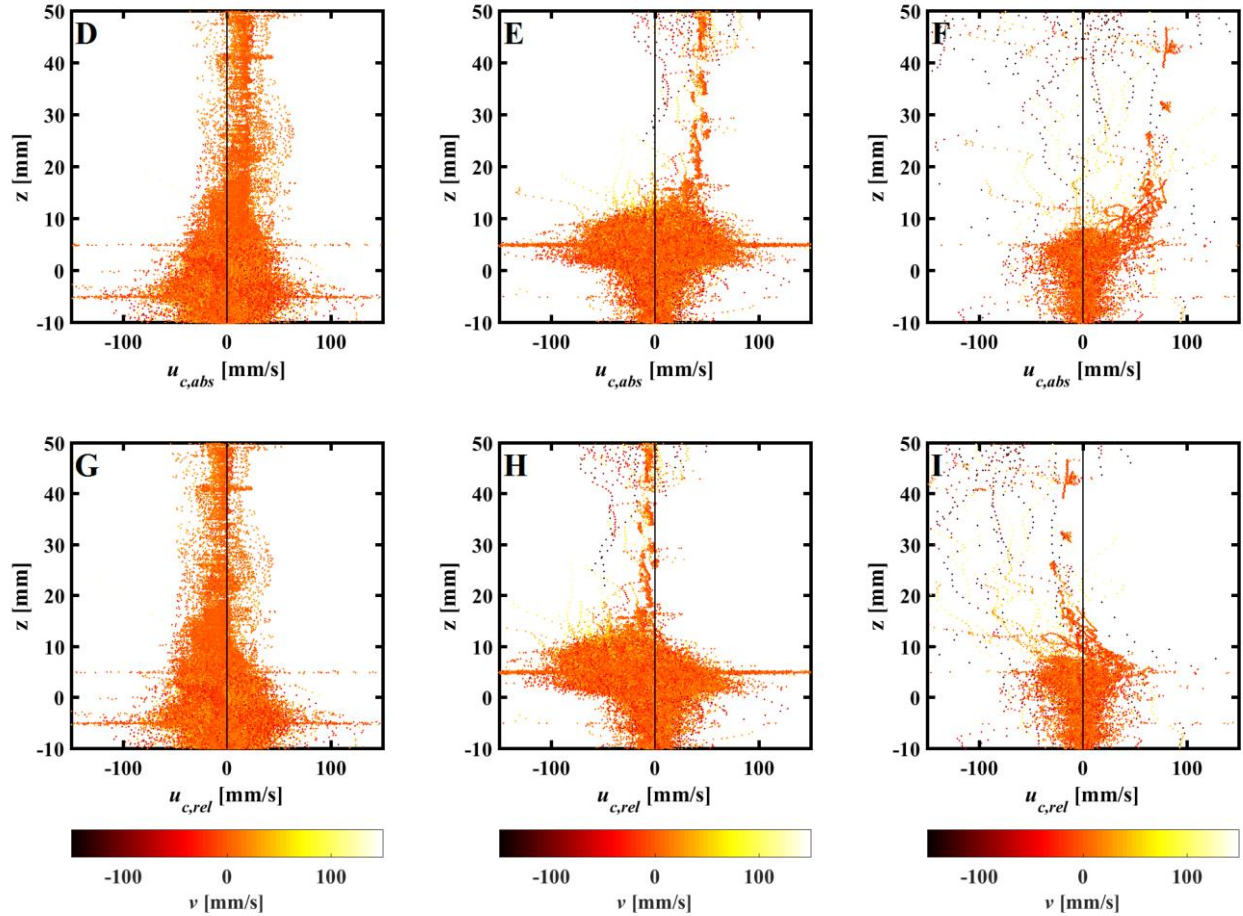


Figure 2: Streamwise velocity u_f (panels A to C), $u_{c,abs}$ (panels D to F), and $u_{c,rel}$ (panels G to I) for the three flow cases, color-coded with the vertical velocity component v .

We plot in Fig. 2 the flow velocity and the absolute and relative velocity of copepods against their vertical coordinates for all data. For u_f or $u_{c,abs}$ positive values indicate downstream motion. For $u_{c,rel}$ negative values indicate absolute copepod velocities that are lower than flow velocity and hence, active swimming against downstream transport. The plots are color-coded with the vertical velocity v . Panels D to F show that the distribution of $u_{c,abs}$ is only slightly shifted toward positive values at low flow velocity, but more strongly shifted toward positive values in cases II and III. However, strong upstream displacements still remain observable at cases II and III despite the high average flow velocity. These upstream displacements result in a highly variable velocity. They occur not only within the sediment where flow velocity is very low, but also in the water column despite local flow velocities of up to 100 mm/s. Panels D to F also show a much higher density of copepods in the sediment for the three flow cases, which confirms the preference of *E. serrulatus* for the sediment layer or its surface. Relative velocities are for the most part negative for the three flow cases (panels G to I). This confirms the active counter-current swimming behavior of this species. Moreover, $u_{c,rel}$ systematically increases as the flow velocity increases: copepods swim against the flow more forcefully in cases II and II than at low flow velocity, with relative velocities beyond 100 mm/s. The vertical velocity v increases as well, both in the upward and downward directions.

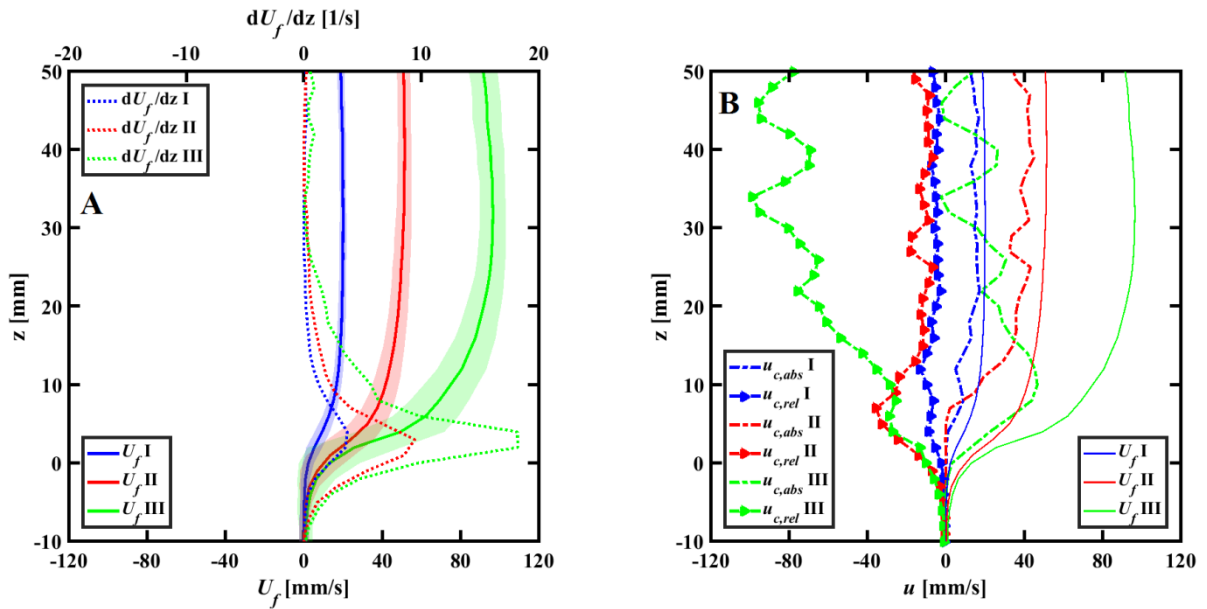


Figure 3: Vertical profiles of the streamwise velocity of tracer particles U_f and of the absolute $u_{c,abs}$ and relative $u_{c,rel}$ velocity of copepods averaged over time and horizontal planes. Panel A also shows the root mean square of the velocity fluctuations u_f' of the flow (as shaded areas) and the vertical profiles of dU_f/dz .

We show in Fig. 3 A the vertical profile of U_f for the three cases, with shaded areas corresponding to the root mean square of the velocity fluctuations u_f' . We also show the vertical profiles of dU_f/dz to identify regions of high mean velocity gradients. In Fig. 3 B we plot the vertical profiles of the average in time and over horizontal planes $u_{c,abs}$ and $u_{c,rel}$. The average absolute velocity of copepods is smaller than U_f across the entire water column for the three flow cases. The relative velocity of copepods $u_{c,rel}$ increases with U_f but the increase is not uniform along the vertical axis. At low flow velocity, the swimming velocity of copepods is approximately constant over the depth of the investigation volume. At medium flow velocity (case II) $u_{c,rel}$ is approximately constant in the water column and maximal at the surface of the sediment. At high flow velocity (case III) $u_{c,rel}$ increases strongly in magnitude and is maximal in the water column.

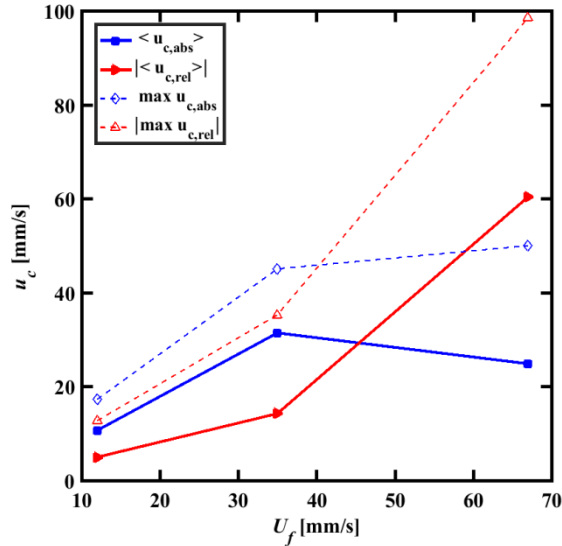


Figure 4: Absolute velocity of copepods $\langle u_{c,abs} \rangle$ (solid line, blue), absolute value the relative velocity of copepods $\langle |u_{c,rel}| \rangle$ (solid line, red), maximal value of the absolute velocity of copepods (dashed line, blue), and absolute value of the relative velocity of copepods (dashed line, red), versus the flow velocity U_f .

We summarize the main trends in Fig. 4 where we plot the mean and maximal values of the absolute velocity of copepods and the mean and maximal absolute values of their relative velocity against U_f for the three flow cases (case I: 12 mm/s; case II: 35 mm/s; case III: 67 mm/s). The positive values of $\langle u_{c,abs} \rangle$, where angle brackets denote the average over all trajectory points, indicate downstream transport for the three flow cases, but this transport is not simply proportional to U_f , and drift velocities are substantially smaller than U_f at high flow velocities (cases II and III). On the other hand, the relative velocity of copepods increases strongly with U_f . This forceful counter-current swimming results in an absolute velocity that is comparable at cases II and III. Hence, vigorous upstream swimming enables copepods to reduce downstream advection.

3.4 Discussion

Several hypotheses involving random or directed upstream displacements have been suggested as explanations of the drift paradox. Müller (1982) suggested that upstream movements for oviposition by adult insects compensate for downward drift. Field studies either confirmed or refuted this hypothesis, suggesting that the extent to which flight compensates for drift may vary among species and streams (Waters 1972). This hypothesis is also not applicable to species that do not have an aerial stage. Other behavioral mechanisms that may influence the persistence of populations in streams, valid both for species with and without a dispersive adult stage, include displacements toward refugia (i.e. areas of lower flow velocity), and organisms swimming in the water column and performing small-scale displacements at the surface of the streambed (Poff and Ward 1992; Speirs and Gurney 2001; Humphries and Ruxton 2002). For instance, *Gammarus*

pulex and larvae of *Baetis rhodani* can travel upstream several meters per day by crawling on the surface of the sediment (Elliott 1971).

In this study, we have quantified the swimming behavior of an important yet not well-studied taxon. Our results confirm the importance of small-scale movements for drift avoidance in benthic invertebrates. They show that counter-current displacements are able to compensate, to some extent, for downstream advection. Predisposition to drift depends, in part, on the swimming ability of an organism. Copepods are known for their vigorous swimming, which occurs via frequent and powerful jumps, and which can result in a persistent, directed motion despite strong hydrodynamic conditions (Michalec et al. 2015). For instance, calanoid planktonic copepods are able to maintain vertical position in coastal areas despite strong up- and downwelling currents (Genin et al. 2005). It is important to note that this behavioral feature is not restricted to calanoids: we observed an intermittent motion where short periods of passive advection alternate with frequent, powerful jumps that are directed upstream (Sidler et al. 2017) and which enable *E. serrulatus* to partially overcome advection by the flow. Similar results were obtained by Richardson (1992) who observed vigorous upstream swimming of the cyclopoid copepod *Eucyclops agilis* at flow velocities up to 7.5 cm/s. Our results also show that the active counter-current swimming of *E. serrulatus* increases with flow velocity, which indicates that this species can actively modulate its swimming effort in response to flow velocity.

We observed that copepods spent a large fraction of their time within the sediment bed, where flow velocity is negligible and where they move freely in all directions. Pachepsky et al. (2005) addressed the issue of persistence of populations of benthic organisms that reside mainly on the benthos and perform occasional displacements by entering the water column and drifting downstream. They presented a model of a population where organisms spend a fraction of their time immobile and a fraction of their time being advected downstream, and concluded that residence in the immobile state always enhances population persistence. Similarly, Shang et al. (2008) reported on vigorous counter-current swimming in the epibenthic estuarine copepod *Pseudodiaptomus annandalei* at low flow velocity (2.1 cm/s) and active substrate attachment to avoid dislodgment at higher flow velocities. Using marine benthic harpacticoid copepods sampled from an intertidal mudflat, Palmer (1984) evidenced active swimming toward the sediment in response to increasing flow velocity, followed by burrowing into or crawling on the surface of the sediment. Hence, the frequent incursions of *E. serrulatus* within the interstices of the sediment bed may contribute to the maintenance of their population in flowing environments.

This counter-current swimming behavior raises the question of how copepods perceive flow direction. Copepods are weakly inertial: they are slightly heavier than water and larger than the smallest turbulence length scale and hence, their Stokes number is not very small e.g. of order one at the highest flow rate tested (Michalec et al. 2015). Copepods detect velocity differences through mechanosensory setae on their first antennae (Buskey et al. 2012). Velocity difference may arise from (a) shear stress in the fluid, both in the turbulent velocity fluctuation field and the vertical mean velocity profile, (b) drag forces due to relative motion arising from inertia, and (c) differential rotation due to non-spherical shape and mismatch between center of gravity and center of mass of the organism. The latter for example gives phytoplankton the ability of directed vertical swimming (Roberts 1970). It has also been suggested that copepods may sense hydrostatic pressure as a means to orient in the vertical direction and swim against down- or upwelling currents (Genin et al. 2005). Our results show that copepods swim most actively against the mean current when the mean flow velocity is high i.e. their relative velocity is maximal when U_f is maximal (case III; Fig. 3 B). Their counter-current swimming behavior is not

particularly strong when turbulent velocity fluctuations are maximal or gradients of the mean flow velocity are maximal (at $z \approx 10$ mm; Fig. 3 A), which does not suggest a major influence of turbulent stresses (fluctuating shear, vorticity and drag stresses) or mean velocity gradients (mean shear and vorticity). The relative velocity of copepods appears to be maximal approximately in this interval in case II, but this observation is not corroborated by cases I and III. We therefore infer that the drag induced by the time-averaged flow velocity U_f is the main responsible stress leading to counter-current swimming. However, while drag stress allows perceiving a velocity difference (i.e. the relative velocity between the copepod and the local flow), it does not inform on the direction of the velocity (i.e. the sign of U_f). The mechanisms used by copepods to break Galilean invariance during advection constitute an intriguing topic for further study.

It is important to note that the ability of self-locomotion and to perform small-scale displacements despite downstream currents plays a role in the active in-stream dispersal of species during the short-term colonization of reaches following perturbation (e.g. Baumgartner and Robinson 2017). Motility of stream organisms, either by swimming or crawling, is indeed an important contributor to the small-scale colonization of empty patches (reviewed in Mackay 1992).

Considerable attention has been paid to the redistribution of stream invertebrates during high flow events, with a strong focus on the ecological effects of hydropower plant operation. There is much less information on the response of these organisms at short temporal and spatial scales, especially on copepods. We have considered here the effects of flow on the behavior of a key taxon. We show that both behavioral traits (active swimming and adaptation to flow velocity) and species traits (preferential distribution in the sediment bed or at its surface) are important mechanisms for drift avoidance. Anthropogenic stressors (e.g. waterborne pollution) that negatively affect the physiological state of copepods and their energy intake may limit their ability for counter-current swimming, with possible effects at the population level. Similarly, the deposition of fine sediment transported by turbinated water downstream of hydropower plants can reduce the interstitial space available for habitat (Bruno et al. 2009), and prevent organisms from seeking shelter in the sediment bed.

3.5 Acknowledgements

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Chapter 4

BEHAVIORAL RESPONSE OF THE FRESHWATER CYCLOPOID COPEPOD EUCYCLOPS SERRULATUS TO HYDROPEAKING AND THERMOPEAKING IN A LABORATORY FLUME

This chapter consists of a manuscript submitted to *Journal of Freshwater Ecology*, 2017:

D. Sidler^{1,2,3,4,5,6}, F.-G. Michalec^{1,3,5,6,7}, and M. Holzner^{1,5,7} (2017). Behavioral response of the freshwater cyclopoid copepod *Eucyclops serrulatus* to hydropeaking and thermopeaking simulations in a laboratory flume. Submitted to *Journal of Freshwater Ecology*

¹Study concept and design; ²Experimental setup design and construction; ³Experiment performance; ⁴Data acquisition and analysis; ⁵Interpretation of data; ⁶Drafting manuscript; ⁷Critical revision

Abstract

The generation of electricity by hydropower plants meets the requirements for intermittent energy demand and for the production of renewable energy. However, the temporal pattern of operation has important consequences for the biotic compartment of the receiving river. 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 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, an important but often neglected taxon of

the meiobenthos, in a laboratory flume that allows the tracking of organisms both in the water column and in a transparent sediment bed. We 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 velocity, 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.

4.1 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 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; Verdonshot 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:

- 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.
- 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).

4.2 *Material and method*

4.2.1 *Model species*

Our model species is *Eucyclops 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 epigeic (e.g. Richardson 1991; Richardson 1992; Di Lorenzo et al. 2015 and references therein), 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. 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 to 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, at a constant temperature of 18°C, and 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.

4.2.2 *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 (Fig. 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.

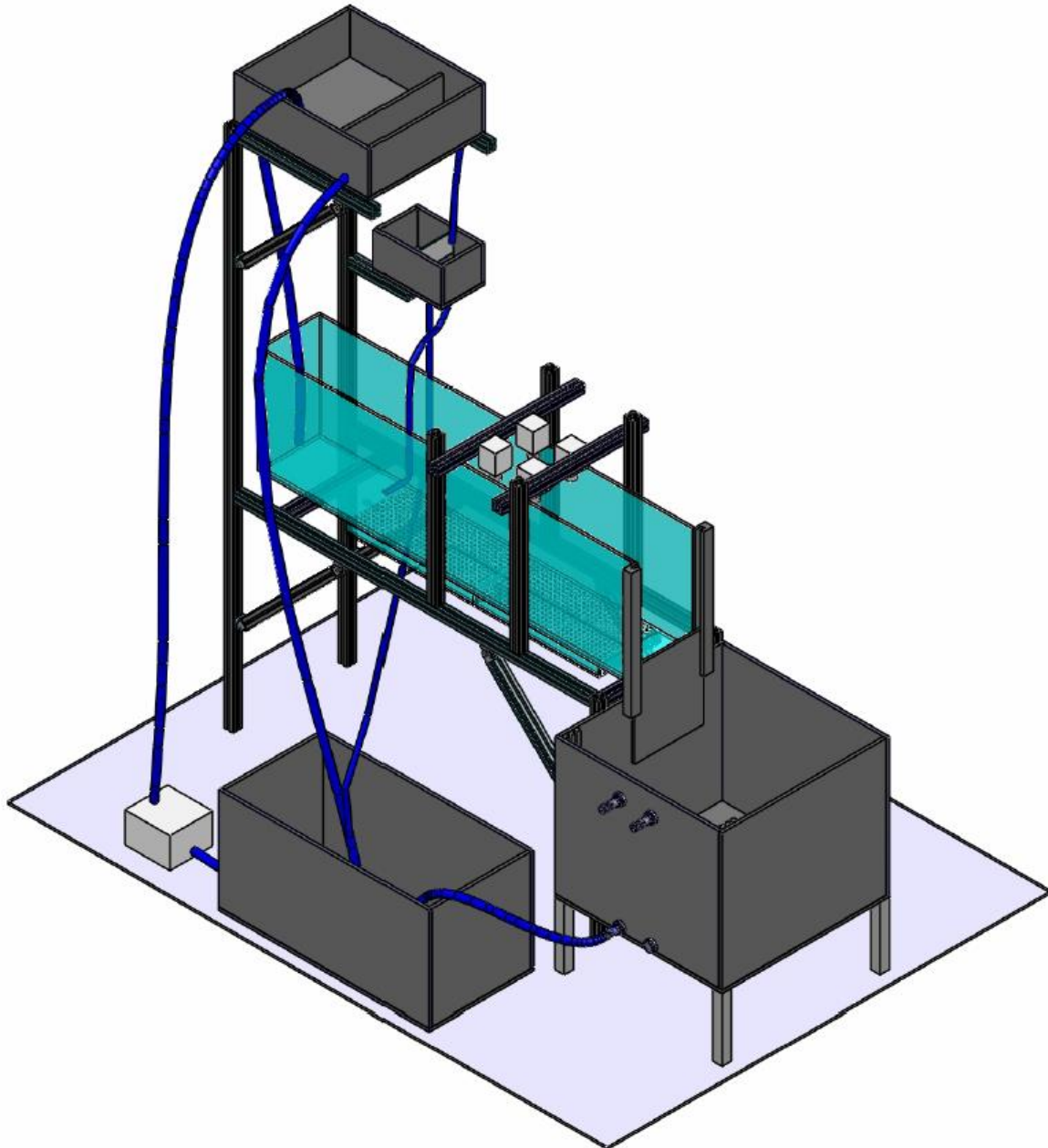


Figure S1. Sketch of the laboratory flume. The flume features a flow dampening area, an adjustable vertical gate at the outlet, and a transparent sediment bed. It is 2 m long and 27 cm wide. The sediment layer is around 50 mm thick. The flume is equipped with a circulating recharge system. Water is pumped from the low-level reservoir to the high-level reservoir and released through a pipe into the upper part of the flume. Water flowing through and above the

terminal plate is collected into a retention box that serves to collect drifting organisms, and sent back to the low-level reservoir. A thin metal mesh mounted in front of the outlet in the retention box prevents the animals from entering the low-level reservoir. Constant discharge in the flume is reached via a surplus of water pumped to the high-level reservoir and an overflow system leading back to the low-level reservoir. This system generates a fixed water level inside the high-level reservoir with a stable pressure in the outlet. The flow rate in the flume is adjusted with a ball valve and monitored with a magnetic flow meter.

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) \times 15 cm (y) \times 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 to 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 $\langle U_f \rangle$, the Froude number, and the Reynolds number, for these two flow conditions. Flow case I corresponds to $\langle U_f \rangle = 12$ mm/s and flow case II to $\langle U_f \rangle = 67$ mm/s. We have previously observed that in our laboratory flume *E. serrulatus* distributes 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 $\langle U_{f,i} \rangle$ and in the water column $\langle U_{f,w} \rangle$.

4.2.3 Discharge and temperature variation

Copepods 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. 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*) (Fig. 1A). 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).

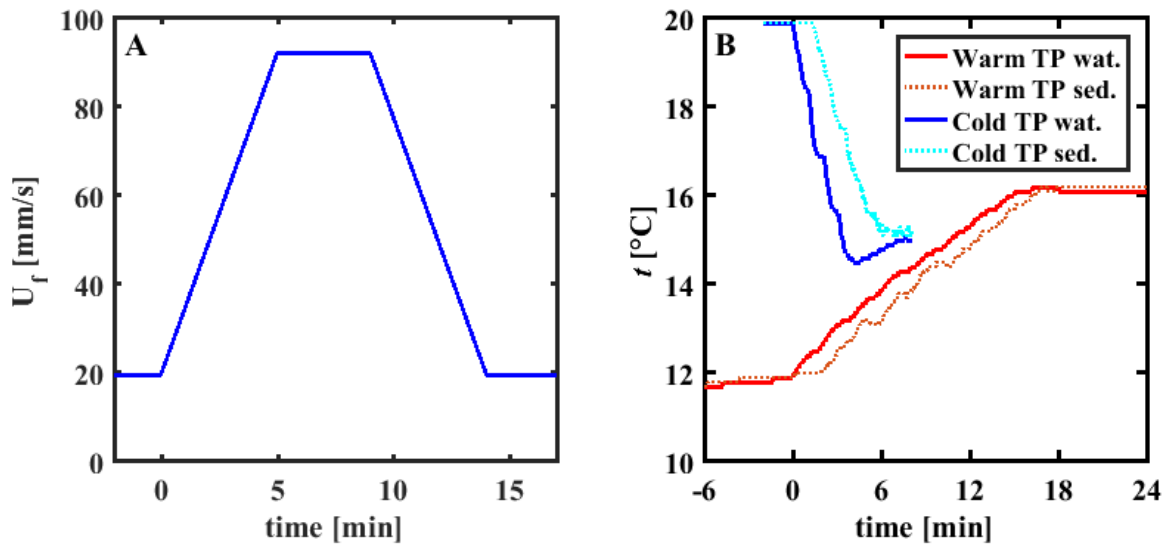


Figure 1. (A) Time evolution of the discharge. (B) Time evolution of the temperature recorded in the water column (solid line) and in the interstitial spaces of the sediment bed (dotted line) during the cold (blue) and warm (red) thermopeaking simulations.

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. Fig. 1B 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 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. one 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.

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 three flow cases, and therefore mostly captures their active behavior: positive values indicate active downstream swimming, whereas negative values indicate active counter-current 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.

4.3 Results

4.3.1 Hydropeaking

We show in Fig. 2A 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 one-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 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.

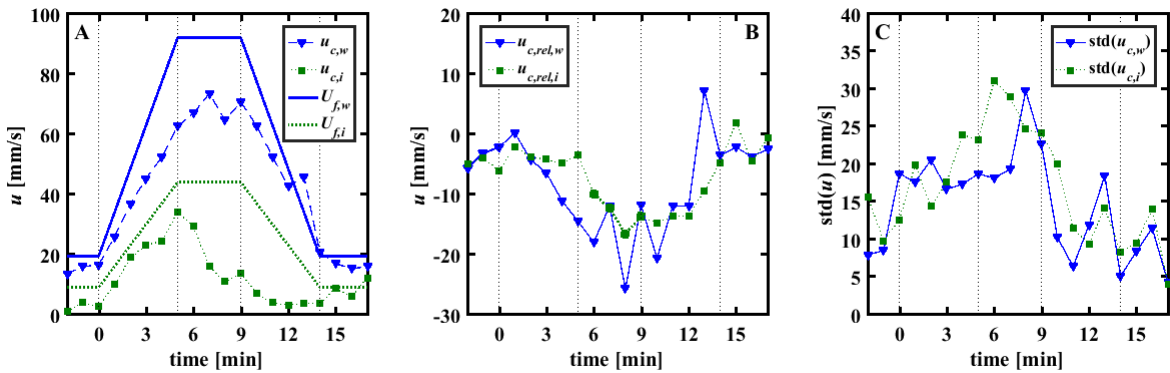


Figure 2. (A) Copepod absolute velocity u_c and flow velocity U_f in the water column (blue) and in the interface layer (green) during the hydropeaking simulation, averaged over periods of one-minute duration. (B) Copepod relative velocity in the water column (blue) and in the interface layer (green). (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.

Fig. 2B 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. Fig. 2B 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 (Fig. 2C). 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 $u_{c,w}$.

4.3.2 *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 Fig. 3A and in Fig. 4A 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 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 Fig. 4A).

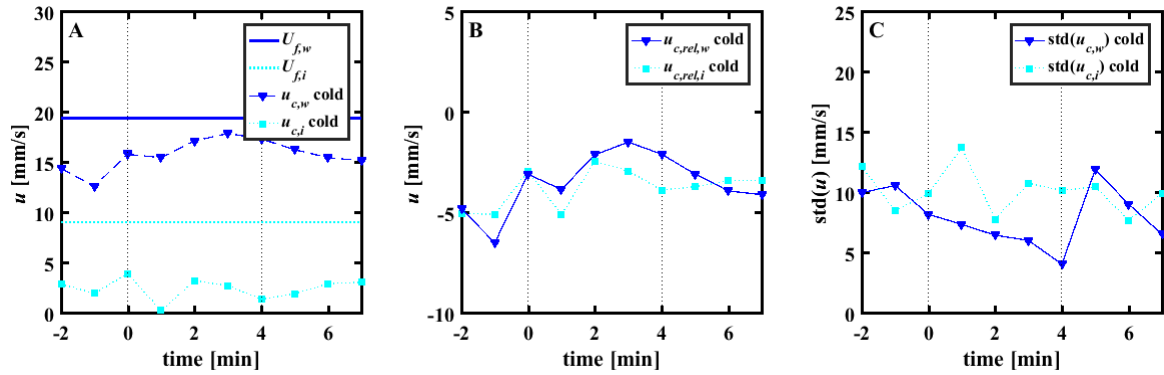


Figure 3. (A) Copepod absolute velocity in the water column $u_{c,w}$ and in the interface layer $u_{c,i}$ during the cold thermopeaking simulation, averaged over periods of one-minute duration. The two horizontal lines indicate the flow velocity (which remains constant) in the water column and in the 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 discharge and varying discharge.

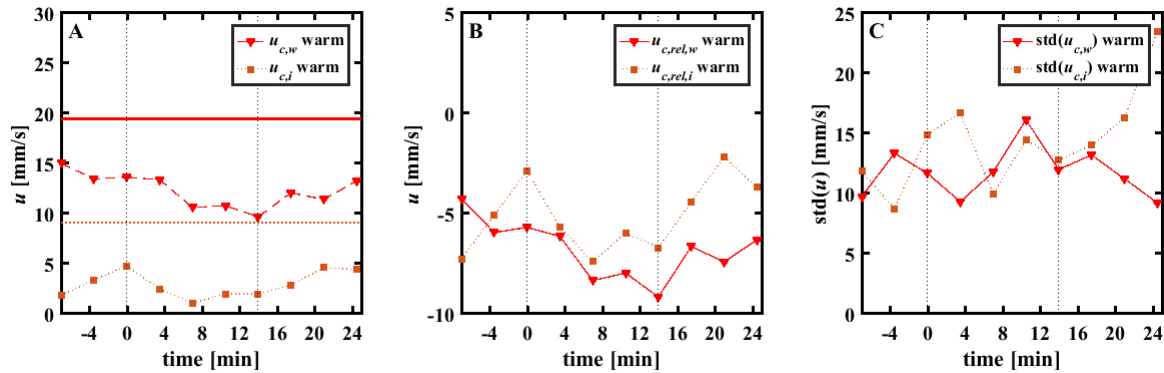


Figure 4. (A) Copepod absolute velocity in the water column $u_{c,w}$ and in the interface layer $u_{c,i}$ during the warm thermopeaking simulation, averaged over periods of one-minute duration. The two horizontal lines indicate the flow velocity (which remains constant) in the water column and in the 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 discharge and varying discharge.

We show the relative velocity of copepods in Fig. 3B and in Fig. 4B. 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. 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 (Fig. 4C).

4.4 Discussion

Downward drift is a common occurrence 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 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 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 to 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 result 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* and hence it was 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). However, since we conducted measurements using copepods from late developmental stages originating from the same culture, the difference between cold and warm TP reflects a difference in sensitivity rather than seasonal effects. Finally, the lack of 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.

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Chapter 5

SYNTHESIS

5.1 Conclusion

Hydropeaking and thermopeaking caused by the release of turbinated water from high elevation dam hydropower plants impose adverse conditions on the ecology of the receiving river. These negative effects of the most important renewable energy producer hydropower leaves a black spot on the clean record of this strongly promoted electricity source. The effects have been studied extensively and measurements are taken on national levels to mitigate them. However, due to the lack of measurement techniques to study animals in the sediment bed or at small scales, information on the ecology and behaviour of benthic invertebrates remained scarce. This significantly hampers our ability to devise models and predict future impacts of environmental impact.

Cyclopoid copepods living on and in the sediment bed of rivers form an important part of aquatic and riverine food webs, but despite their ecological importance not much is known about their behaviour and reaction to physical and chemical conditions and even less about alterations thereof.

The aim of this dissertation is to study the ecology and response of benthic invertebrates to physical alterations, namely waves in flow velocity and temperature, and get an insight into the behavioral reaction and adaptation of cyclopoid copepods to these alterations.

In the first part of this thesis, we have developed a novel method to study the behavior of benthic copepods both in the surface and in the subsurface flow of a flume. The optical access was achieved with a permeable and transparent sediment bed. This experimental setup allows the controlling and alteration of ecologically relevant physical parameters e.g. temperature, flow rate and groundwater exchange. The generated flow is reproducible, and can be described as a uniform flow field comparable to those found in the literature. With the use of a 3D-PTV system from measurements of turbulent flows, we were able to detect and track the motion of many benthic copepods swimming freely in the free water and inside the sediment layer. We obtained trajectories of sufficient length for a robust quantification of copepod velocity, acceleration and

vertical distribution. Copepods were able to swim in the entire flume, but showed strong substratum preference and moved readily and in all directions in the sediment bed.

In the second part, we have quantified the swimming behaviour of *Eucyclops serrulatus*, an important yet not well-studied benthic cyclopoid copepod. Copepods are known for their vigorous swimming, which occurs via frequent and powerful jumps, and which can result in a persistent, directed motion despite strong hydrodynamic conditions (Michalec et al. 2015). We observed that the copepods, even in the middle of the flume, can sense the flow direction and intensity and swim upstream with a higher velocity when the discharge was at a higher level. This counter-current swimming behaviour raises the question of how copepods perceive flow direction. Copepods are weakly inertial: they are slightly heavier than water and larger than the smallest turbulence length scale. Copepods detect velocity differences through mechanosensory setae on their first antennae (Buskey et al. 2012). Velocity difference may arise from (a) shear stress in the fluid, both in the turbulent velocity fluctuation field and the vertical mean velocity profile, (b) drag forces due to relative motion arising from inertia, and (c) differential rotation due to non-spherical shape and mismatch between center of gravity and center of mass of the organism. Our study shows no reaction, neither to the local turbulent velocity fluctuations nor to the gradient of the mean flow velocity, which does not suggest a major influence of turbulent stresses (fluctuating shear, vorticity and drag stresses) or mean velocity gradients (mean shear and vorticity). We therefore infer that the drag induced by the time-averaged flow velocity is the <main responsible stress leading to counter-current swimming. However, while drag stress allows perceiving a velocity difference (i.e. the relative velocity between the copepod and the local flow), it does not inform on the direction of the velocity. Our results confirm the importance of small-scale movements for drift avoidance in benthic invertebrates. They show that counter-current displacements are able to compensate, to some extent, for downstream advection.

In the final part, we have quantified the effects of flow velocity and temperature variations on the small-scale behavior of the freshwater cyclopoid copepod *E. serrulatus*. The experiments were conducted in a laboratory flume fitted with a transparent sediment bed, where the animals could explore the free water as well as the sediment. We used an advanced particle tracking technique and have reconstructed their trajectories in three dimensions and analyzed their swimming behavior. We show that copepods increase their counter-current swimming effort in response to increasing flow velocity. 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 result in a lower drift. During our measurements, *E. serrulatus* was mainly swimming 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. In Bruno et al. (2010) certain taxa were not or little affected by the increase in discharge because they inhabit the hyporheic habitat and hence were not exposed to shear stress. These authors observed that several taxa of 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* and hence it was 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. 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. 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. 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. Finally, the lack of 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.

5.2 Suggestions for future research

The presented experimental setup with a laboratory flume and a transparent sediment bed lays the groundwork for future experiments on benthic species, behavior and interactions in controlled and repeatable physical and chemical conditions. Important for the experiments is a quick and constant recirculation of the copepods to mimic upstream river sections. The transport of the copepods from the retention box at the flume outlet to the high level copepod box was done by hand and could be replaced with a piston or spiral pump or boxes mounted on a band-conveyor. The culturing method has also been optimized to a level with not much improving potential but needs a constant and careful attention for a maximal number of individuals without overstressing the cultures which could lead to unstable chemo-physical conditions and a collapse. The number of individuals is directly correlated with the invested effort and time, but also provokes instabilities.

The experiments could be generalized by extending the repetitions and parameter range, i.e. changing the duration, rate of change and magnitude of the hydraulic and temperature waves. A valuable extension based on the existing data, possibly complemented by additional measurements that capture simultaneously the 3D flow velocity near the copepods, would be a detailed investigation of behavior in relation to local conditions. It would be valuable to study behavioral responses to the surrounding turbulent flow and local temperature conditions to understand possible triggers of their behavioral changes and adaptations. Furthermore it would be interesting to study the fine-scale displacement of copepods between streambed patches of

different flow velocity, and to investigate the role of self-induced motion on drift entry, drifting distance and settlement process. Multiple lines of evidence already indicate a substantial role of behavior in the ecology of benthic organisms and in the processes generating or terminating drift (Malmqvist 2002; Naman et al. 2016). Our setup enables the detailed observation of behavioral processes that could not be approached in field studies due to logistic constraints, and provides opportunities for taxon-specific mechanistic studies, with useful ecological applications such as more realistic models of drift flux. Several hypotheses involving random or directed upstream displacements have been suggested as explanations of the drift paradox. Clear evidence for the existence of short but repeated upstream movements near the sediment of small taxa for the population persistence is still missing but could be studied with the presented flume experiments.

Besides the hydraulic condition and temperature, the turbidity and fine sediment transport seem to have a big influence on invertebrate drift (Wood and Armitage 1997, Larsen et al. 2009) and could also be studied in the presented setup. A technical problem thereby is the image recording system, as turbidity hinders the visibility of the animals. A solution could be the manufacturing of very small transparent polyacrylamide particles to mimic the transported fine sediment.

Another possible future direction could concern the influence of groundwater exchange patterns like described by Brunke (1998), with the same or even with different temperatures from the injected groundwater or groundwater infiltration. The designed experimental setup is already equipped with two independently controllable groundwater exchange chambers to have different groundwater exchange patterns simultaneously.

The finding that *E. serrulatus* probably is most affected by the time-averaged flow velocity as inducing factor for the counter-current swimming is surprising and intriguing. The mechanisms used by copepods to break the Galilean invariance during advection constitute a more detailed topic for further studies about copepod sensing the flow in a uniform or graded flow field and the interpretation of the received information. This would require much more work on a smaller scale to test different theories (like light sensitivity or the interpretation of acceleration and fine differences due to inertia) and the invention of suitable experimental setups (such as different illumination sources and acoustic imaging or flow generation with moving surfaces instead of discharge) and is not possible in the here presented setup.

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PUBLICATIONS

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