universität wien

MASTERARBEIT

Titel der Masterarbeit

Linking Behavior and Hydraulics in Riverine Fish Larvae: Rheoreaction and Movement Patterns of Early Stages of *C. nasus* under different Flow Conditions

verfasst von

Bernhard Zens, BSc

angestrebter akademischer Grad

Master of Science (MSc)

Wien, 2015

Studienkennzahl lt. Studienblatt:	A 066 833
Studienrichtung lt. Studienblatt:	Masterstudium Ökologie
Betreut von:	ao. Univ.-Prof. Dr. Hubert Keckeis

Table of Contents

Abstract

The dispersal of fish larvae in rivers results from water-movement but also from larval behavior, a factor that has been widely neglected. This study examines the swimming activity and orientation and associated patterns of movement in larvae of a characteristic rheophilic species, the nase (*Chondrostoma nasus).* This study was designed to determine different, dispersal-relevant types of movement and to quantify their application in different flow-velocity gradients. Using an experimental approach, I analyzed individual swimming trajectories of larvae based on their swimming speed as well as their orientation in relation to the current vector. Furthermore, frequencies of stops and turns as well as displacement of fish larvae were quantified. Based on these parameters I discriminated distinct types of movement. In order to simulate conditions of natural habitats of fish larvae, the experimental flume mesocosm offered characteristic features of shoreline morphology as well as a velocity gradient. Experiments were carried out at three different velocity scenarios representing under-, near- and over-critical flow conditions with respect to swimming abilities of larvae and were conducted at daylight and during the night. Information on current direction and velocity at any location in the flume was obtained from a 3-D hydrodynamic numerical model based on Acoustic Doppler Velocimetry (ADV). The quantification and analysis of larval fish movement revealed that downstream migration is strongly governed by active behavioral components under all tested flow scenarios. In contrast, totally passive transport by the current was rare, indicating the high importance of behavior in the dispersal of early life stages of riverine fish.

Key words

early developmental stages, *Chondrostoma nasus*, swimming trajectories, orientation, displacement, dispersal

Zusammenfassung

Die Ausbreitung von Fischlarven in Flüssen ist die Folge von Wasserbewegungen wird aber auch maßgeblich vom Verhalten der Tiere beeinflusst, einem bisher weitgehend vernachlässigten Faktor. Im Rahmen der vorliegenden Studie erfolgte die Erforschung der Schwimmaktivität und der Orientierung und den sich daraus ergebenden Bewegungsmustern von frühen Entwicklungsstadien einer charakteristischen, strömungsliebenden Fischart, der Nase (*Chondrostoma nasus*). Hauptziel dieser Untersuchung war es, die verschiedenen, für die Ausbreitung der Fischlarven relevanten Faktoren unter variablen hydraulischen Bedingungen zu erfassen und zu quantifizieren. Im Rahmen einer Serie von Experimenten in einer Strömungsrinne wurden Ausbreitungspfade einzelner Individuen im Hinblick auf ihre Schwimmgeschwindigkeiten und ihrer Orientierung in Bezug auf Strömungsvektoren, untersucht. Zusätzlich erfolgte die Quantifizierung der Schwimmaktivität, die Erfassung der Häufigkeiten von Richtungsänderungen und deren und Winkel und der Ausbreitungsstrecken der Larven. Anhand dieser Parameter, war die Analyse und Unterscheidung unterschiedlicher Bewegungsmuster der Fischlarven möglich. Das Gerinne der verwendeten Strömungsrinne wies typische gewässermorphologische Charakteristika von Uferbereichen von Fließgewässern auf, wodurch ein Strömungsgradient vorhanden war. Bei den Experimenten kamen drei unterschiedliche Strömungs-Szenarien zur Anwendung, die in Bezug auf die Schwimmleistung der Fischlarven unterkritische, kritische und überkritische Strömungsbedingungen repräsentierten. Die Experimente wurden bei Tageslicht und auch bei Nacht durchgeführt. Um Informationen über die exakte Strömungsrichtung und -geschwindigkeit an jedem beliebigen Punkt der Fließrinne zu erhalten, wurde auf ein 3-D Modell zurückgegriffenen, welches anhand Ultraschall-Doppler-Profil-Strömungsmessungen erstellt wurde. Die Erfassung und Quantifizierung der unterschiedlichen Bewegungsweisen der Jungfische zeigte, dass die flussabwärts gerichtete Wanderung von Fischlarven unter den getesteten Strömungsbedingungen zu einem großen Anteil von aktiven Verhaltensweisen geprägt ist. Im Gegensatz dazu, trat völlig passive Verfrachtung von Fischlarven durch die Strömung verhältnismäßig selten auf.

Schlüsselwörter

Frühe Entwicklungsstadien, *Chondrostoma nasus*, Schwimmpfade, Orientierung, Verfrachtung, Ausbreitung

Introduction

After successful spawning, egg development and hatching, the offspring of many fluvial fish species are found in the organismic drift downstream of spawning sites. Drift patterns of freshwater fish larvae are strongly stage- and species specific and may vary between rivers and years for the same species (Copp & Cellot 1988, Johnston et al. 1995, Reichard & Jurajda 2007). The young of some species also have shown evidence to avoid drift (e.g. *Squalius cephalus*, *Gobio gobio*, Brown & Armstrong 1985, Reichard et al. 2001), whereas others demonstrate a high tendency to drift. Regarding the species-dependency of drift, a classification was introduced by Humphries & King (2004). The authors differentiate between obligate, facultative or non-drifters according to the relation of their abundances in the drift and simultaneously in retentive habitats (i.e. inshore low-flow zones). Moreover, larval fish drift in rivers shows distinct seasonal and diurnal patterns. The seasonality of drift is assumed to be directly correlated with that of reproduction (Brown & Armstrong 1985). Concerning the diurnal course of drift patterns, peak densities during night have been reported from the large majority of studied freshwater fish taxa, environments and rivers (e.g. Pavlov et al., 1978, D'Amours et al., 2001; Johnson & McKenna, 2007). The observed nocturnality of downstream migration/drift is often assumed to be related to the illumination level and its influence on drift entrance (Reichard et al., 2002; White & Harvey, 2003). However, rapid transport or migration to and settlement in suitable nursery habitats are considered essential for survival of early life history stages in numerous riverine fish species, thereby ultimately affecting further year class-strengths (Pavlov, 1994; Keckeis et. al 1997; Mion et al. 1998). In this context, the phenomenon of larval fish drift in rivers is regarded as directed migration, during which larvae actively use the flow as a transport vehicle from spawning sites to downstream nursery habitats (Pavlov, 1994; Oesmann, 2003). At the same time, accidental/catastrophic drift probably acts as a major population sink via wash-out effects (Bischoff & Wolter, 2001) and likely represents a significant source of mortality to young fish especially during high floods (Mion et al., 1998). Despite this ambiguity, a phase of downstream displacement presents an integral part in the life-cycle of various fluvial fish species (Fuiman & Werner, 2009). Our insights into the spatial and temporal dynamics of fish larvae in rivers have relied almost exclusively on data generated

by drift-net and point-abundance sampling (Gallagher & Conner, 1983). These methods, or combination thereof, enable quantifying larval fish drift and the abundance of settlers (Lechner et. al., 2014). This enables conclusions to be drawn on spawning activity (by the time of occurrence), larval production (by means of abundance) and environmental conditions triggering the drift entrance of fish larvae and larval settlement (e.g. light intensity, turbidity, current velocity and discharge).

Despite these possibilities and the awareness of the important role of riverine larval drift as a linkage between spawning grounds and nursery habitats, there is a conspicuous lack of knowledge regarding relevant aspects of larval behavior associated with this processes (Reichard & Jurajda, 2007; Pavlov et al., 2008). Dispersal of fish larvae in environments characterized by currents (streams, rivers, estuaries and marine habitats) was often assumed to be a primary consequence of water movement (Wolter & Sukhodolov, 2008). The suspected dominance of the factor flow led many modelers to make the "simplifying assumption" by treating larvae as passive particles (Leis, 2007). Nonetheless, studies have demonstrated that not only the movement of water alone is responsible for dispersal outcomes in the larvae of fish (Leis, 2007) and invertebrates (Metaxas, 2011). It has been demonstrated that larval freshwater fish can detect fine-scaled stimuli induced by the current velocity and actively react to them from early larval stages onwards (Garner 1999, Stoll & Beeck, 2012). Once in the current, the behavioral mode and associated pattern of movement (i.e. active, active-passive, passive, sensu Pavlov, 1994) of fish larvae during downstream movement will ultimately determine their actual swimming trajectory, travel speed and destination. Knowledge about these features is indispensable when attempting to fully understand, model or predict dispersal patterns of fish larvae in rivers. Furthermore, a greater understanding of dispersal processes (i.e. the linkage between spawning and nursery habitats) will aid future conservation measures (e.g. habitat revitalization) targeting the growing number of populations of endangered riverine species. This strongly calls for detailed knowledge on the behavioral features of drift and/or movement with regard to larval fish dispersal in rivers.

In marine ecology, the importance of larval behavior for dispersal has been recognized during the past decade (Fiksen et al., 2007; Gallego et al., 2007; Leis, 2007) and the need to

"break the black-box of behavior" has been realized (Pineda et al., 2007). Fiksen et al. (2007), argue that the incorporation of behavioral attributes into models represents a "key step forward to improve our understanding of larval survival, growth and dispersal". Moreover, basic behavioral features of fish larvae have already been successfully incorporated into elaborate 3-D models of physical-biological interactions, which have increasingly become an integral tool for understanding larval fish dynamics in the sea (Gallego et al., 2007). It has been recognized that certain "long-term" behavioral changes are time-dependent and may considerably contribute to larval dispersion. These behaviors are associated with factors such as time of day, water temperature, salinity, food availability or ontogeny and act on time-scales of hours, days and weeks. Minimal knowledge, however, exists on behavioral adaptation operating on scales of seconds to minutes which responds to temporary "short-term" physical and biological triggers (Pineda et al., 2007) or to factors that change rapidly in space as fish larvae move (e.g. currents, rheogradients). Pineda et al. (2007) argue further that hypothesis on the contribution of behavior to larval dispersion must be developed and tested in order to achieve advances in modelling and in understanding transport processes of early developmental stages.

Essential behavioral aspects with relevance for dispersal outcomes in rivers are represented by the directional orientation (i.e. posture) and the movement rate (i.e. speed) of fish larvae in relation to the water flow. These features present integral components of a complex behavioral reaction caused by the water flow, termed rheoreaction (Pavlov et al. 2011). Additionally, rheoreaction also comprises a motivational component expressed as a preference to move in a certain direction in relation to the current, i.e. upstream (positive type of rheoreaction) or downstream (negative type of rheoreaction, Pavlov et al. 2011). During downstream movement, the orientation in relation to the current vector, and the relative speed of the fish larva in relation to the flow velocity, were successfully used to determine if movement/drift was performed actively, passively or in an intermediate style, termed active-passive (Pavlov et al., 2011). The significance for dispersal outcomes of these different types of downstream movement arises from the fact that larvae exhibit different speeds of dispersal depending on the drift mode.

This study provides an individually based, quantitative estimate of all dispersal-relevant patterns of larval fish movement, reflected as consequences of behavior (i.e. swimming activity and orientation). The manifestation of distinct movement patterns is compared among different hydraulic conditions, light levels and larval stages. Considering known ontogenetic differences among larval stages and reported diurnal differences in larval behavior, the following hypotheses were put forward:

(1) During larval ontogeny, swimming capacity improves and physiological costs of active locomotion decrease with growth; accordingly, movement patterns of older larval stages show higher proportions of active components compared to younger larvae.

(2) Vice versa, passive transport by the current more often occurs in earlier larval stages.

(3) Furthermore, active downstream migration occurs more frequently during the day, whereas passive migration dominates at night.

Additionally, this study focuses on:

(4) Downstream displacement at under-critical, near-critical and over-critical flow conditions, thereby providing fundamental information for future modelling of dispersal patterns in a large river.

(5) The number of turns and stops during or between phases of movement. This is compared among the distinct flow-scenarios described above in order to assess the steadiness of swimming trajectories.

(6) The vertical position in the water column.

(7) Different larval stages of *Chondrostoma nasus* (Cyprinidae). This are compared with regard to their morphometric parameters, contributing to our knowledge of the species´ early development.

Materials & Methods

The species under study

In this study, larvae of the threatened nase (*Chondrostoma nasus*) were used as a model organism. This species from the cyprinid family is native to many large to medium-sized rivers in Central and Eastern Europe. Confined to moderate to fast-flowing waters with gravel substratum, the nase is a migratory species that spawns from March to May in smaller tributaries, which it usually does not inhabit during summer (Kottelat and Freyhof, 2007), or in suitable areas within the rivers´ main channel (Spindler, 1988; Keckeis et al., 1996). After the period of yolk-sac depletion during which larvae exhibit a benthic life style (usually 4 to 7 days post-hatch, Keckeis et al. 1996, Kamler et al. 1998), exogenously feeding larvae and juveniles are commonly found in the organismic drift downstream spawning sites (Reichard et al., 2001). Shallow inshore areas serve as important nursery habitats for larvae and early juveniles, which feed on small invertebrates (Reckendorfer et al., 2001). Larger juveniles and adults feed mainly on algae and invertebrates, which are scraped off rocks and gravel in fast current (Kottelat and Freyhof, 2007). Populations declined drastically during the past decades in many European river systems, most probably due to anthropogenic habitat degradation (Peňáz, 1996). The nase has become an important flagship species for conservation measures and has a high bio-indicative potential to assess habitat quality, ecological integrity and connectivity of river systems or sections (Schiemer et al., 2002, Szabó et al., 2002).

Artificial spawning & rearing of larvae

During their spawning migration, ripe male and female nase were collected by electrofishing from a tributary of the Danube River east of Vienna, the Schwechat River. An electrofishing power-generator (Honda®, 8.5 kW), supplying a single ring-shaped anode (30 cm diameter) with four to six amperes and approx. 400 volt direct current was used. The captured spawners were transported to the lab where they were hand-stripped and

fertilized promiscuously. Parental fish were then transported back to the Schwechat River and released alive. Eggs, and later fish larvae, were kept in a 150 l through-flow rearing tank with constant supply of filtered and well-aerated water at 11.7 ±0.7 °C. After the onset of exogenous feeding, appropriate food - life *Artemia salina* nauplii (Great Salt Lake Artemia Cysts, Sanders®) and dry food for freshwater fish larvae (Vipagran Baby, Sera®) - was provided ad libitum.

The experimental flume & the 3-D hydrodynamic numerical model

An artificial racetrack flume (Fig. 1) was constructed in the Hydraulic Engineering Laboratory of the University of Natural Resources and Life Sciences, Vienna (Glas et al., 2012). The oval-shaped flume featured basic properties of natural shoreline configuration of the main channel of the Danube River: the inner bank showed a slope (22° from horizontal) in the straight section of the flume, whereas the outer bank was designed vertically to resemble a cut bank and point-bar situation. Furthermore, this design induced the formation of a gradient in hydraulic parameters and available water depths (0 to 20 cm). An electric motor propelled a paddled belt drive which induced a clock-wise flow. A control module enabled regulating the induced current velocity as desired. A 3-D flow velocity field was measured in four layers using an Acoustic Doppler Velocimeter (ADV). In total, 1996 measurements were taken at each of the three different flow-scenarios and, together with a 3-D hydrodynamic model, fine scaled 3-D current velocity vectors for any position within the flume were obtained (for more details see Glas et al., 2012). For spatial reference, we used an observation grid with squares of 10 x 10 cm and an alphanumeric code, drawn on the bottom of the flume.

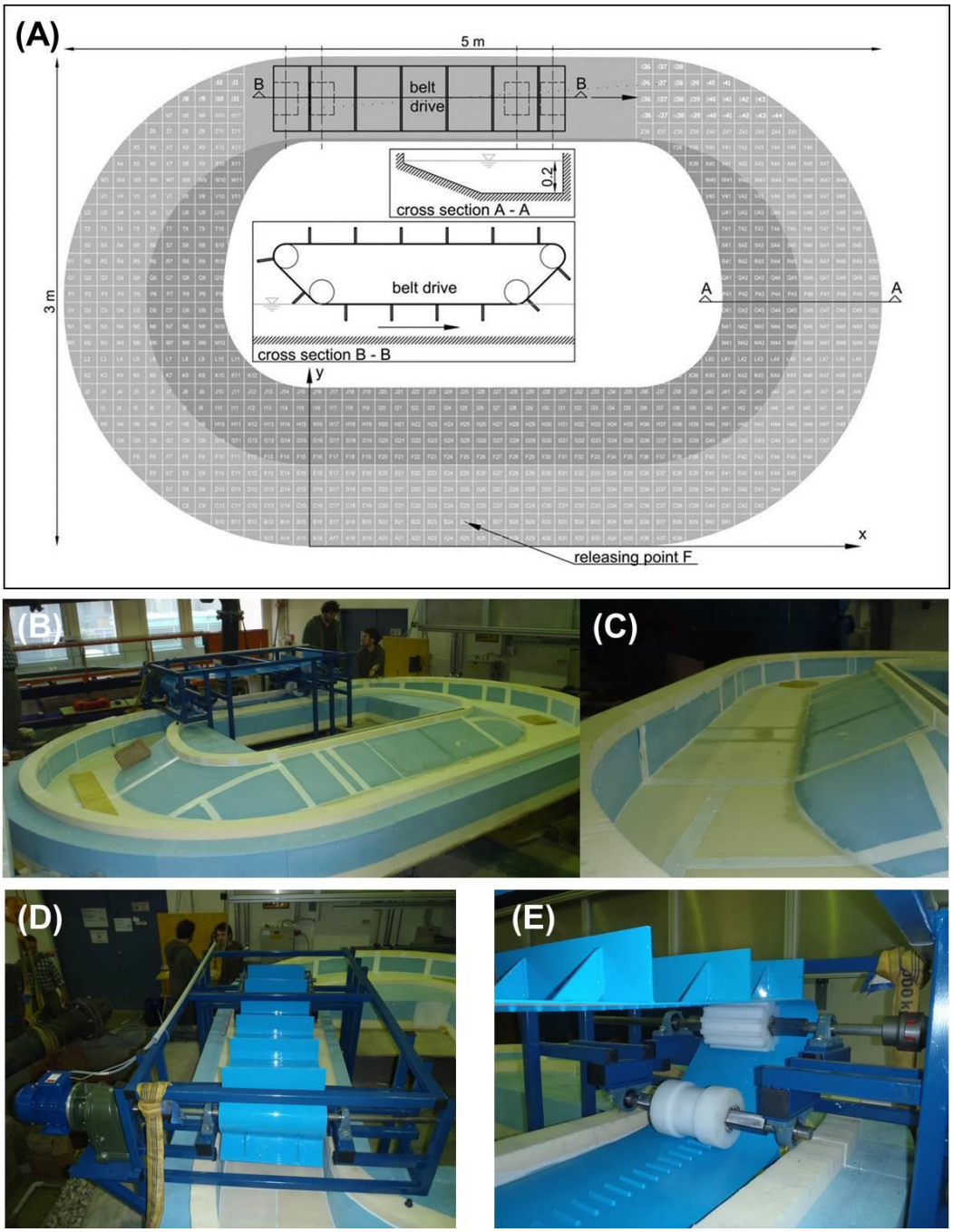


Fig. 1: (A) Schematic diagram of the flume mesocosm illustrating the dimensions, shape and functional principle. The cross section along stretch A-A shows the slope (22° from horizontal) of the inner bank resembling the natural shoreline configuration and inducing the formation of rheogradients. The functional principle of the belt drive is shown along the cross section B-B (arrow indicates direction of movement of belt drive and the induced clockwise water flow). Note the observation grid (10 x 10 cm squares) drawn on the flume floor (Glas et al., 2012). (B) and (C) The entire body of the flume consists of agglutinated Styrodur®-singles. Photographs taken during the final stage of flume installation (prior to drawing of observation grid). (D) and (E) show details of the paddled belt drive. (Photos: H. Keckeis)

Experimental design & conditions

Flume experiments comprised three different velocity scenarios chosen based on the known critical current velocity (V_{crit}) of the larvae. V_{crit} refers to the maximum sustainable swimming speed according to Flore et al. (2001). Experimental flow scenarios represented under-critical, near-critical and over-critical mean flow conditions. In every scenario, a gradient from nearly zero-flow zones to the aforementioned zones with faster flow was present. We conducted our experiments during daylight (i.e. artificial light in the lab) as well as in darkness. Larvae were released at two different release points: one was situated in a shallow (depth = 11 cm) low-flow zone close to the inner bank with an average current velocity of 2.8, 4.2 and 8.4 cm s^{-1}, depending on flow scenario. The other release point was situated in deep water (depth = 20 cm) and faster current velocities of 9.0, 13.1 and 26.3 cm s^{-1}, respectively.

Tab. 1 Numbers of conducted experiments (N) at different flow scenarios during day and night.

Flow-scenario	**Release-point**	**Day**	**Night**	**Total**
under-critical	*low*	8	9	17
	fast	10	6	16
near-critical	*low*	9	3	12
	fast	10	6	16
over-critical	*low*	7	3	10
	fast	10	0	10
Total		54	27	81

Filming & experimental handling

The racetrack flume was filled with fresh water (13.9 ± 1.8°C) at the beginning of each day of experimental work. For each experiment, larvae were captured from the culture-tank and adapted in an aerated container with the same water; before release, each single larva was adapted in a transparent acrylic glass cup with a 400 µm mesh bottom for a few minutes in the flume. This procedure should enable adaptation to the temperature as well as chemical and optical properties of the new surroundings. The inexperienced fish larvae were released individually from this release cup and filmed with a handheld camcorder (Sony®, HDR-CX700VE) over a period of 5 minutes. During daylight, the automatic focus function (AF) was used, whereas in darkness we used the infra-red detectable night-shot function together with an additional infra-red light source (Sony®, HVL-HIRL) and the manual focus (the AF did not perform satisfactorily here). Filming during day and night followed the exactly same procedure: The observer was always positioned in the flume´s eye where it was possible to move freely in order to keep an adequate distance and angle to the studied fish. From release onwards, the fish´s path was continuously followed at zoom settings, resulting in coverage of at least four grid squares simultaneously (to achieve sufficiently detailed information on behavior and the required resolution to determine the position of the larva). When visual contact to the observed fish larvae was lost, the experiment was discarded if the fish could not be redetected immediately (within approx. 30 s). Discarded experiments were repeated with new, inexperienced larvae. After the filming period, the fish larvae were anesthesized and killed using a highly overdosed (approx. 0.2%) solution of MS 222 (tricaine methanesulfonate, Fulka Analytical®). Larvae were individually coded and preserved in 4% formalin for later morphometric measurement and stage identification. Water temperature and oxygen saturation was measured to the nearest 0.1° C and 0.1 mg L^{-1} using an Oximeter (Oxi 330, WTW®) at the beginning and end of each block of experiments.

Video analysis & processing

The recorded videos were processed manually using a fast motion and stop procedure in the software package Adobe® Premiere Pro 5.0/CS5: Via mouse-scrolling, the videos were replayed at fast motion and stopped every time when a larva passed over a grid line with the full length of its body. The position was obtained from the observation grid on the flume floor and noted in a continuous time table with steps of 0.1 seconds. This procedure enabled determining larval speed relative to stationary landmarks for every observation which, according to grid size, approximates every 10 cm of traveled distance. In order to assess the orientation, eight different positions were defined according to angles of -45°, -90°, -135°, 180°, 135°, 90°, 45° and 0° enclosed by the fish´s body axis and the longitudinal gridline in clockwise (negative values) or counterclockwise (positive values) rotation. The angle of the fish`s body axis enclosed with the 2-D current vector (obtained from the hydrological model) was then calculated for any point of observation (Fig. 2). Additionally, the water layer used by the fish larva was noted in terms of *bottom*, *middle* and *surface* third of the water column.

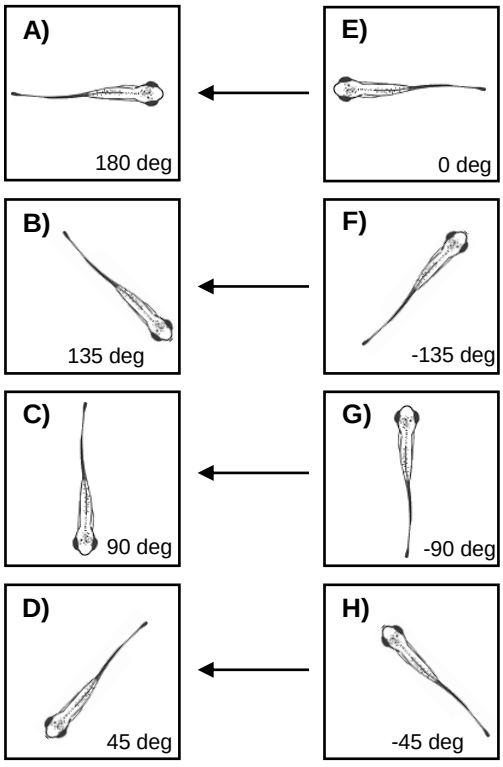


Fig.2: Schematic representation of the eight different orientation categories in relation to the current vector (black arrows). The angle of the body axis enclosed with the current vector is given either in positive or negative values for each category. (A) 180 degree, (B) 135 degree, (C) 90 degree, (D) 45 degree, (E) 0 degree, (F) -135 degree, (G) -90 degree, (H) -45 degree. Note that there are 3 pairs of equivalent categories (in terms of angles) which differ only in the direction: (B) & (F), (C) & (G), (D) & (H).

Determined variables & movement patterns

For every individual larvae used in the experiments, the larval stage according to Peňáz (1974) was identified (see Supplement I: Characteristics of larval stages of *C. nasus* & comments on their identification, Fig. S1). For each larva, the following morphometric characters were measured to the nearest 0.01 mm: (A) Total Length (TL) measured from snout tip to rear margin of caudal fin. (B) Standard Length (SL) from snout tip to end of notochord. (C) Body Height (H), measured approximately one eye-diameter behind rear margin of eye, orthogonally to body axis (Supplement I, Fig. S2). In the larvae used in this study, H represented the greatest dorso-ventral height. Wet weight of each individual was measured to the nearest 0.01 mg (METLER® MT5 analytical microbalance) after turning the fish onto folded, standardized paper (Precision Wipes, Kimberley-Clark Professional®) several times, until adhering formalin-water was removed as completely as possible. All morphometric length measurements (TL, SL and H) were obtained from standardized photographs (compare Supplement I, Fig. S1) showing each larva in plane, lateral view (head left-sided) and at fixed microscope zoom settings (x 7.5), using the imaging software NIS Elements® 3.0 Inc.

Depending on total length, the maximum sustainable water velocity (hereafter termed *critical velocity*; V_{crit}) was calculated for each larva as $V_{crit} = 4.39 + 0.456 * TL$, according to Flore et al. (2001). V_{crit} is defined as the current velocity that does not allow the fish larva to hold its position over ground for longer than 2 minutes.

In order to examine displacement and rheoreaction, the distances covered in upstream direction were extrapolated to 5 minutes observation time (hereafter termed covered upstream distance, CUD) and calculated for each individual larva. The same applies to the distance covered in downstream direction (covered downstream distance, CDD). Displacement was calculated as CUD minus CDD, yielding positive values for larvae that showed net upstream movement or negative values for larvae showing net downstream translocation over the 5-minute observation period, respectively. The type of rheoreaction has been defined as the “preference of fish to move in a certain direction in relation to the

direction of water current" (Pavlov et al., 2011). The positive type represents upstream movement against the current (CUD > CDD). The neutral type demonstrates resistance to move: CUD and CDD compensate each other and the displacement is therefore zero, the individual maintains its position constantly. The negative type of rheoreaction is indicated by downstream movement (CUD < CDD). In order to achieve a standardized value for the rheoreaction type, I introduced the *rheoreactive quotient* (*RQ*) representing relative net displacement with values ranging from -1 to 1, irrespective of the covered distance. This variable consists of a negative and a positive component and was calculated as:

$$RQ = -\frac{CCD}{TCD} + \frac{CUD}{TCD}$$

where TCD is the total covered distance.

Values of -1 indicate that the larva exclusively shows downstream movement, whereas values of 1 show exclusively upstream movement.

For larvae that moved downstream or kept position, the proper movement rate (relative to water flow), *Vf* (in cm s^{-1}) was calculated at any point of observation as the speed of the fish towards stationary landmarks (*Vs*, net speed over ground in cm s^{-1}) minus the current velocity (*Vw*, in cm s^{-1}), according to Pavlov et al. (2011):

$$Vf = Vs - Vw$$

This variable can therefore attain any value, either positive (if the fish moves faster than the surrounding water), negative (fish moves slower than surrounding water) or zero (fish speed equals flow velocity). Hence, *Vf* can be regarded as an expression for a fish´s swimming activity. Both, negative and positive values indicate that propulsive movements are carried out, the intensity of propulsion being reflected in the magnitude of *Vf*. Note, however, that these characteristics of *Vf* and the computation given above apply for downstream movement and position holding. In the case of upstream movement, $Vf_{upstream}$ can be calculated as:

$$Vf_{upstream} = (Vs + Vw) * -1$$

This procedure will yield values of $Vf_{upstream}$ which are negative and smaller than the current velocity but greater in magnitude. Analogously to *Vf*, the magnitude of $Vf_{upstream}$ reflects the propulsion intensity of the swimming fish larva.

Concerning different patterns of movement, I refer to the terminology of Pavlov (2008). With regard to the movement direction in relation to the current direction (upstream, downstream and lateral), *Vf*, and the orientation of the fish larva to the current vector, six different patterns of movement were discriminated. The patterns were identified using an IF function in Excel depending on the characteristics enumerated below:

The active pattern of downstream movement is indicated by directional downstream movement and fish larvae moving faster than the flow velocity. Here, fish larvae are orientated with their heads in downstream direction. In this study, movement was therefore termed *active downstream* when:

1) The movement direction was downstream in relation to the water current,
2) the orientation ranged between -45°, 0° and 45°, and
3) $Vf > 1$ cm s^{-1}.

The passive pattern of downstream movement is characterized by downstream movement with no propulsive activity and random orientation of larvae relative to the flow vector. As a result, they move with the same speed as the flow velocity. Movement was thus designated as *passive downstream* when:

1) The movement direction was downstream in relation to the water current,
2) any orientation was applied (at random) and
3) *Vf* ranged between -1 and 1 cm s^{-1} ($Vf \approx 0$ cm s^{-1}).

Another pattern of downstream movement is characterized by orientation of heads in upstream direction and slower movement than the flow-velocity. This is achieved by propulsive movements against the flow. This pattern of downstream movement was termed *active-passive* and was designated in the present study when:

1) The movement direction was downstream in relation to the water current,

2) the orientation ranged between -135°, 180° and 135°

3) $Vf < -1$ cm s^{-1}.

Additionally to those types of downstream movements, one novel type of movement which is not present in the classification scheme of Pavlov (2008) was introduced in the present study. Movement was termed *traversing* when:

1) The movement direction was downstream in relation to the water current,

2) the orientation was -90° or 90° and

3) $Vf > 1$ cm s^{-1}.

Furthermore, the following patterns of movement were assessed:

Active upstream movement was defined as:

1) The movement direction was upstream in relation to the water current,

2) the orientation ranged between -135°, 180° and 135° and

3) $Vf_{upstream}$ < current velocity * -1.

Active lateral movement is characterized by:

1) The movement direction was perpendicular in relation to the water current,

2) the orientation was -135°, 180°, 135° -90° or 90° an

3) Vf ≈ current velocity * -1.

Furthermore, the mean current velocity of the entire swimming trajectory was calculated for every single experiment. This variable represents the average current velocity (in cm s^{-1}) which each individual larva has experienced and is hereafter termed U_{tr}mean. Whenever a larva held position in one grid square for longer than 10 s, this was designated as a *stop*. Any change in orientation in relation to the current vector from one observation grid square to the next was registered as *turn*.

Data analysis

A Kruskal-Wallis-test was used to test for differences of morphological variables (TL, SL, H and W) between different larval stages. Linear regression was used to analyze the relationship between TL and W. The measurements of TL, H, W and TL/SL-ratio of single individuals were used as input variables for a hierarchical cluster analysis using Euclidean distances and the linkage between groups-method. The grouping of larvae achieved by cluster analysis was compared with the developmental stages. This procedure seeks to explore the extent to which larval stages, mainly differentiated by the shape of the notochord and status in fin development, can be separated by morphometric properties. CDD and CUD values were both standardized to 5 minutes observation time. Kruskal-Wallis-tests were used to detect stage- and/or scenario-specific differences in CDD and CUD. To evaluate potential effects of the release-point on dispersal/movement trajectories, CDD and CUD values were compared by pairwise Mann-Whitney-*U*-tests within distinct larval stages and flow scenarios. A similar procedure was performed to compare of CDD and CUD values of day and night experiments when the number of conducted experiments per category was N=3 or higher. Nonlinear regressions were used to describe the relationship between displacement and U_{tr}mean. Frequency distributions of orientation categories were tested against a random distribution using pairwise G-tests. The random frequency distribution of orientation was generated by 500 random selections out of the eight possible orientation categories using the RANDOM function in MS Excel®. To calculate mean *Vf* values at the different orientation categories, outliers derived from burst swimming (i.e. *Vf* values exceeded V_{crit}) were excluded. To quantify the manifestation of different movement patterns, I used mean frequencies (in percent) calculated for each larval stage at the different flow scenarios on the basis of individuals. Consequently, these percentages express average stage-specific frequencies of the use of distinct movement patterns at the given flow-scenario. In the active upstream and the lateral movement pattern, percentages refer to the total number of observed movements (downstream, upstream and lateral movements together) while percentages of the different downstream movement patterns refer to the total number only of downstream movements, if not otherwise stated. Frequencies were arc-sine square root transformed and tested for differences within larval

stages and flow scenarios using Kruskal-Wallis-tests. Differences between day and night in the manifestation of different patterns of downstream movement were tested pairwise using Mann-Whitney-*U*-tests and arc-sine square root transformed frequency data. Concerning differences in the use of different water layers, frequencies of the use of the lower water layer were arc-sine square root transformed and tested pairwise using Mann-Whitney-*U*-tests between day and night. The number of turns was standardized to 1 meter of observed length of the swimming trajectory for each larva. The number of stops was standardized to 1 minute of observation time. The numbers of turns did not differ with respect the release-points, thus data from both release-points were pooled for further analysis. For both frequencies of turns and stops, Mann-Whitney-*U*-tests were performed to detect differences between day and night. In case of multiple pairwise comparisons, obtained significances were Bonferroni corrected by multiplication with the number of performed tests. Statistical analyses were conducted using PASW Statistics 20.0 (SPSS Inc.®, Chicago) or calculated in MS Excel®. Figures and regressions were generated using SigmaPlot 12.5 (Systat Software®, San Jose).

Results

Flow velocity gradients

Flow conditions during experiments ranged from nearly zero-flow zones (< 1 cm s^{-1}) to higher current velocities, depending on the flow scenario (Fig. 3). However, patches with current velocities < 5 cm s^{-1} (clearly under-critical for all tested larval stages) were present in all three flow scenarios (Fig. 4) and accounted for 5.6, 11.4 and 20.6 % of surface area for the over-critical, near-critical and the under-critical flow scenario, respectively. At the under-critical scenario, 98 % of the total area of the flume was characterized by under-critical current-velocities (< 10 cm s^{-1}), when related to the size of the smallest larvae used in this study. Current velocity in this scenario ranged from 0.1 to 11.0 cm s^{-1}, averaging 6.3 (± 2.1) cm s^{-1} (Fig. 3 B). At the near-critical flow scenario the area with under-critical current-

velocities accounted for 39%, whereas the areas with critical and over-critical current velocities accounted for 50% and 11% of the total area, respectively. The mean current velocity in this flow-scenario was 9.4 (± 3.1) cm s^{-1}; current velocities ranged from 0.4 to 15.7 cm s^{-1} (Fig. 3 C). At the over-critical flow scenario (mean = 18.8 ± 6.2 cm s^{-1}, Fig. 3 D), current velocities ranged from 0.9 to 31.4 cm s^{-1}. Approx. 85 % of the surface area was distinctly higher than V_{crit} of the largest larvae (12.3 cm s^{-1}).

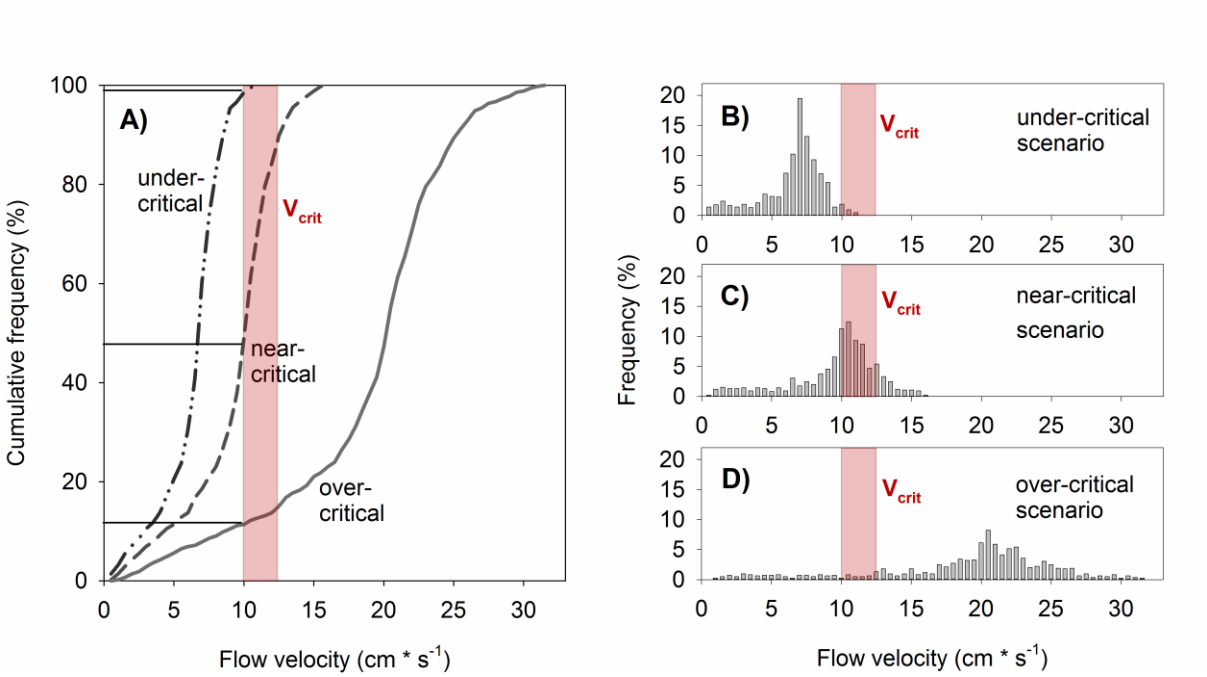


Fig. 3: Cumulative frequencies (A) and frequency distributions (B, C and D) of flow velocities at the three experimental flow scenarios expressed as percentage based on 1996 ADV measurements per scenario. The red-shaded area indicates the size-dependent critical current velocity (V_{crit}) of fish larvae under study.

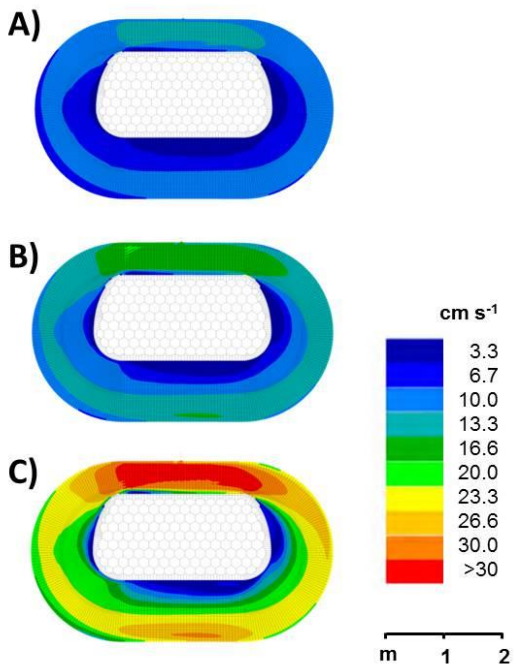


Fig. 4: Spatial distribution of current velocities in the flume in (A) the under-critical, (B) near-critical and (C) over-critical flow-scenario. Colors indicate mean current velocities in the distinct areas.

Morphometrics & development of fish larvae

The age of the nase larvae used in this study ranged from 23 to 43 days post hatch and represented three different larval stages (2nd, 3rd and 4th) according to Peňáz (1974). Hereinafter, these development stages are termed L2, L3 and L4, respectively. Photographs of larvae in these stages and comments on their identification are given in Supplement I, Fig. S1. Stage-specific values of all measured morphometric variables are shown in Tab. 2. Additionally, the calculated critical current velocity is given. Size in terms of total- and standard length turned out to be a very good discrimination variable between these stages. These sizes showed only a marginal overlap, which was more noticeable regarding the variables wet-weight and body height. Significant differences in median values of all morphometric parameters were observed between the different larval stages (all $p<0.001$, Fig. 5).

Tab. 2: Mean values, standard deviation (SD), minimum (Min) and maximum (Max) of morphometric variables and body weight for the three studied larval stages. The number of individuals (N) and the calculated critical current velocity (V_{crit}, cm s^{-1}, according to Flore et al. 2001) is given for each stage (L2, L3, L4).

Stage		L2	L3	L4
N		29	26	26
Total length (mm)	Mean	12.9	14.7	16.4
	± SD	0.4	0.6	0.5
	Min	12.1	13.5	15.7
	Max	13.6	15.7	17.4
Standard length (mm)	Mean	12.4	14.0	15.2
	± SD	0.5	0.5	0.4
	Min	11.5	13.1	14.5
	Max	13.1	15.0	16.2
Body height (mm)	Mean	1.5	1.8	2.1
	± SD	0.1	0.1	0.1
	Min	1.4	1.5	1.9
	Max	1.8	2.2	2.4
Wet weight (mg)	Mean	7.2	13.2	20.2
	± SD	1.1	2.3	2.9
	Min	4.5	8.5	15.0
	Max	10.3	17.5	26.1
V_{crit} *(cm* s^{-1}*)*	Mean	10.3	11.1	11.9
	± SD	0.2	0.3	0.2
	Min	9.9	10.6	11.6
	Max	10.6	11.5	12.3

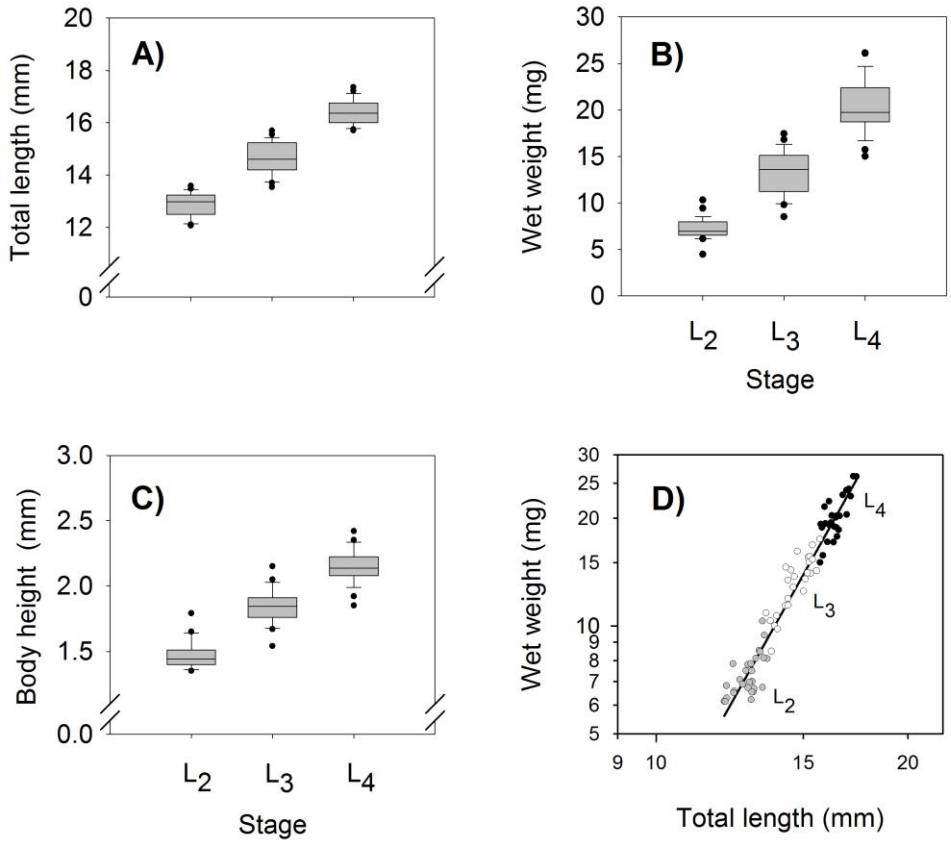


Fig. 5: Relationship between developmental stages and morphometric variables of the studied fish larvae. (A) Total length, (B) wet weight and (C) body height of different larval stages differed significantly between larval stages (all $p<0.001$). (D) Relationship between total length and wet weight for all investigated larvae. Different larval stages according to Peňáz (1974) are indicated by colors. Solid line shows significant linear regression (W = -39.575 + 3.628 * TL, $r^2 = 0.93$, $p<0.001$, N = 81). Note logarithmic scale on both axes.

Grouping of larvae by a Hierarchical Cluster Analysis of these morphometric characteristics coincided well with the estimated larval stages: the overlap was 93% (75 out of 81 larvae, Fig. 6).

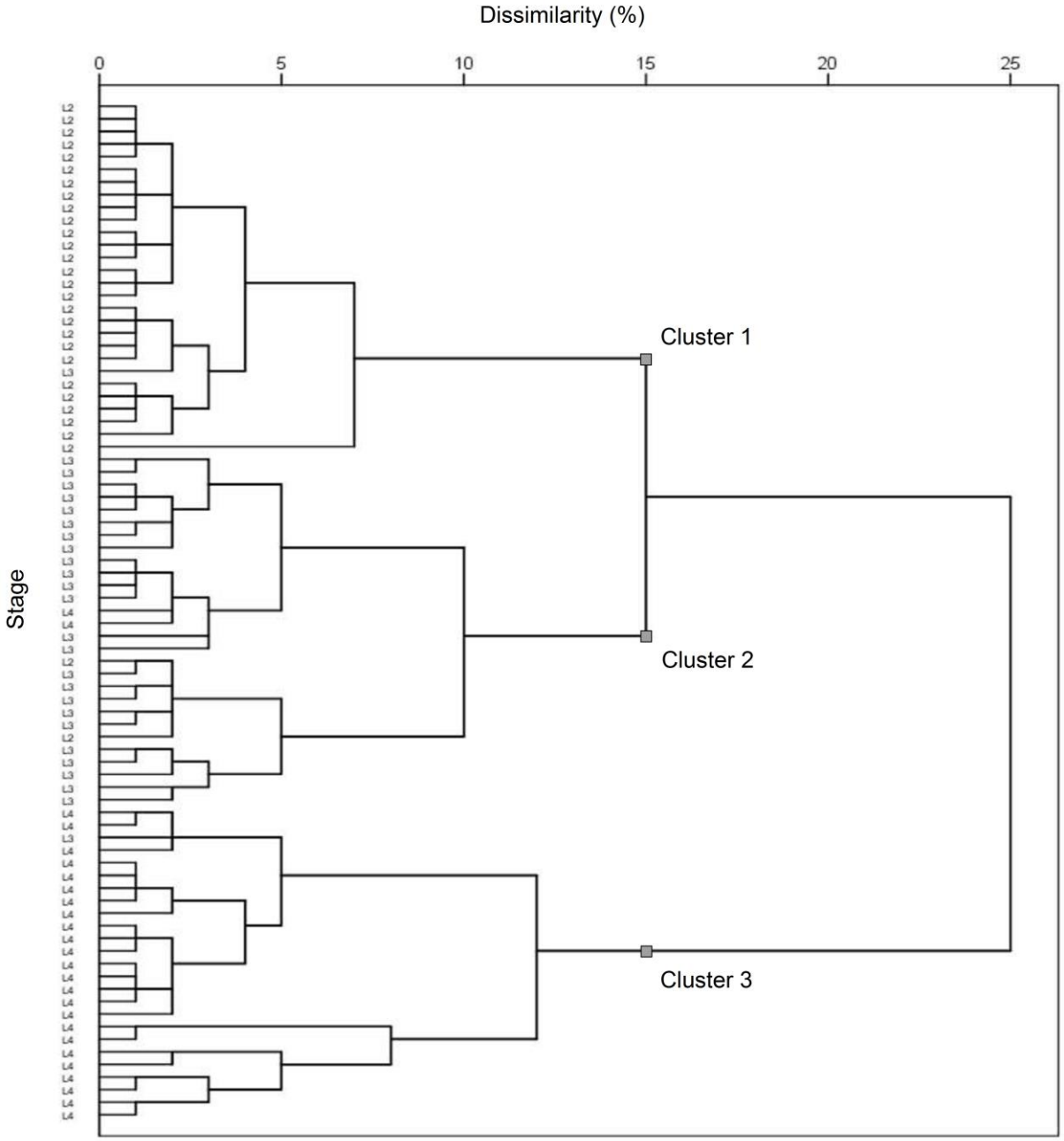


Fig. 6: Dendrogram of the Hierarchical Cluster Analysis based on total length, wet weight, body height and the ratio between total and standard length of all fish larvae used in our experiments (N = 81). Three clusters are separated at 15% dissimilarity and single individuals are labeled according to their larval stage.

Covered distances, displacement & rheoreaction

Individual larvae exhibited great variability in the extent of upstream and downstream movements. As a consequence, displacement and rheoreaction were also subject to considerable individual variation. The CUD values (covered upstream distance in 5 minutes observation time) of single individuals ranged from 0.1 to 10.7 m, whereas CDD (covered downstream distance in 5 minutes observation time) ranged from 0.3 to 56.3 m. The release point showed no significant effect on either CDD or CUD mean values (all $p>0.08$), thus data from both release points were pooled for further analysis. The effect of different flow-scenarios on covered distances was clear: During daytime, mean CDD values were 2 to 12 times higher in the overcritical flow-scenario compared to the under- and the near-critical scenario for all stages ($p<0.001$ in L2-larvae, $p<0.05$ in L3- and L4-larvae). Among larval stages, no significant differences in mean CDD values in the under-critical ($p=0.63$) and in the over-critical flow-scenario ($p=0.173$) were found, although L2-larvae showed CDD values which were on average more than 2 times higher compared to those of L3- and L4-larvae. During daytime, however, L2-larvae showed significant higher mean CDD values at the near-critical flow-scenario ($p<0.05$) compared to L3- and L4-larvae.

CUD values were statistically not significantly different at the under-critical flow scenario compared to the near- and the over-critical scenario in all stages ($p=0.279$ in L2, $p=0.258$ in L3 and $p=0.281$). Also within each flow-scenario, larval stages showed very similar CUD values which did not differ significantly from each other ($p=0.515$ in under-critical, $p=0.53$ in near-critical and $p=0.42$ in over-critical flow-scenario).

During the night, recorded mean CDD values were similar among flow-scenarios and stages and ranged from 0.77 to 3.98 m during 5 minutes. No statistical comparison of both, CDD and CUD values among flow-scenarios and among stages was conducted for the night experiments because in all stages there was at least one flow-scenario in which the number of successfully conducted experiments was zero or only one.

Covered distances did not differ drastically between the day and night experiments: At the under-critical flow scenario, CDD values of L2- and L3-larvae were slightly higher during

the night, whereas L4-larvae showed slightly higher values during day. Additionally, L4-larvae also showed slightly higher CDD values during daylight at the near-critical scenario. These differences, however, were not statistically significant (all $p>0.1$). CUD values at the under-critical and the near-critical flow-scenario were also very similar among night and day experiments and did not show any significant differences (all $p>0.1$).

Net displacement increased with U_{tr}mean, which was shown by highly significant regressions following negative exponential functions (Fig. 7 A). All larval stages under study showed similar displacement patterns. In larvae showing clearly under-critical U_{tr}mean values (< 8 cm s^{-1}), displacement was bi-directional (upstream and downstream) and ranged from -10.50 to 8.26 m 5 min^{-1}; no stage-specific pattern could be discerned. Above the critical velocity, when U_{tr}mean exceeded 12 cm s^{-1}, displacement increased exponentially and was exclusively directed downstream. L2-larvae showed the highest maximum values of downstream displacement compared to the other two older stages. In larvae with U_{tr}mean values > 12 cm s^{-1}, displacement ranged from -5 to -54.3 m 5 min^{-1}.

The rheoreactive quotient (i.e. the “preference” to move upstream or downstream, Fig. 7 B) of larvae at under-critical flow-conditions (U_{tr}mean < 10 cm s^{-1}) appeared to be independent of larval stage. At under-critical U_{tr}mean values, negative, positive and neutral types were observed in all stages and were relatively homogenously distributed with respect to U_{tr}mean. The rheoreactive quotient was clearly negative at highly over-critical U_{tr}mean values > 15 cm s^{-1} in all stages.

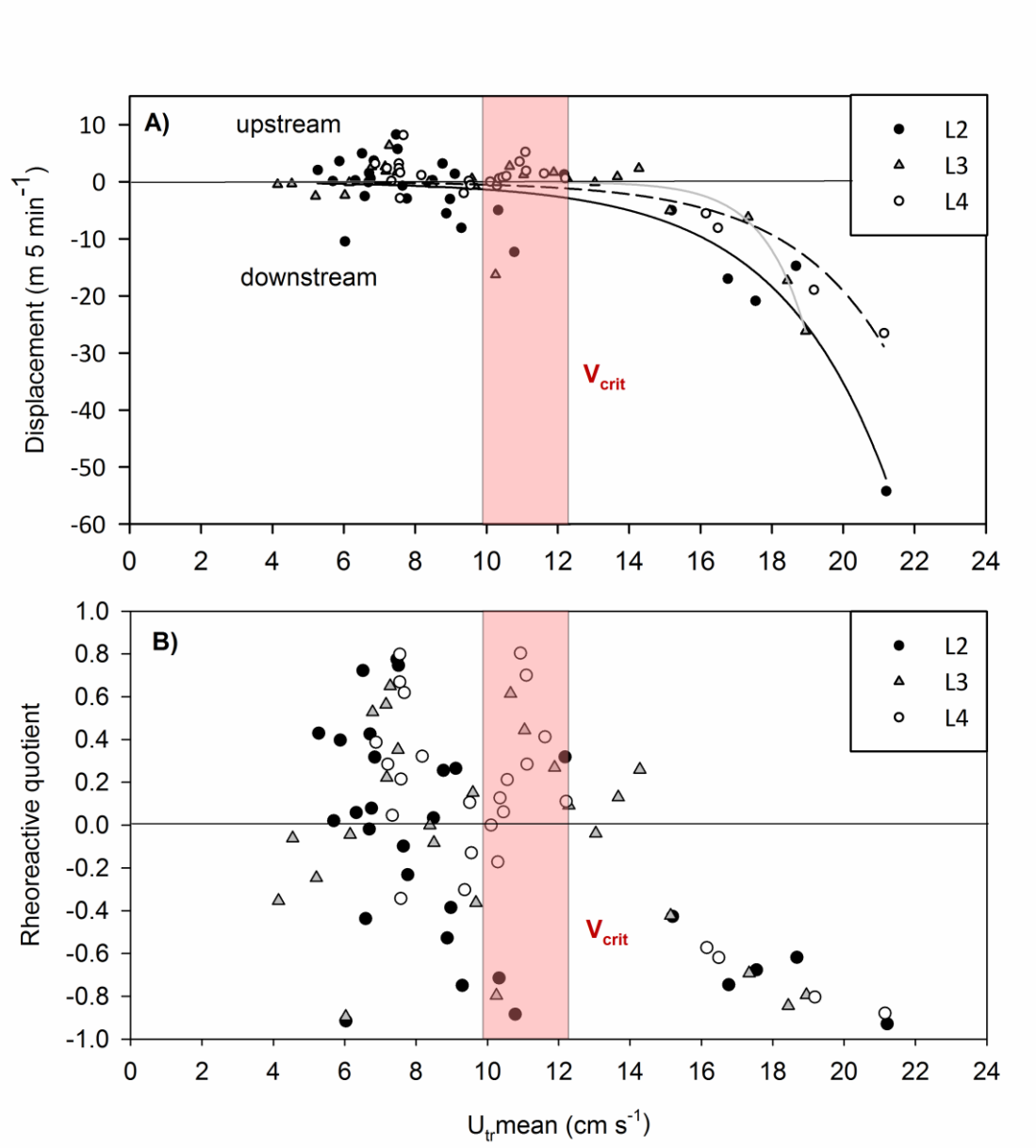


Fig. 7: (A) Displacement (in m 5 min^{-1}) in relation to mean current velocity of swimming trajectories (U_{tr}mean in cm s^{-1}) for all larvae. Data points above the horizontal line indicate upstream displacement, data points below this line downstream displacement. Significant nonlinear regressions were fitted to describe the relationship between these variables for the different larval stages: L2 (black solid line) $y = -0.053 * e^{(0.325 * x)}$, $r^2 = 0.85$, $p < 0.0001$. L3 (gray solid line) y = (4.116 * 10 ^ -6) * $e^{(0.827 * x)}$, r2 = 0.70, $p < 0.0001$. L4 (dashed line) $y = -0.014 * e^{(0.361 * x)}$, $r^2 = 0.81$, $p < 0.001$. B) The rheoreactive quotient in relation to U_{tr}mean. Positive values indicate that larvae showed a tendency for upstream, negative values for downstream movement. The size-dependent critical current velocity (V_{crit}) of fish larvae is highlighted as red-shaded area and different larval stages are indicated by symbols and fills.

Tab.3: Average values (Mean ± SD) of CDD and CUD during day and night for the different larval stages and the different flow-scenarios. Number of conducted experiments is given for day and night (NDay, NNight). Minus (-) indicates no data available or SD could not be calculated (N = 0 or 1; these cases were omitted for statistical analysis).

		Day					Night				
Stage	**Scenario**		**CDD**		**CUD**			**CDD**		**CUD**	
		N_{Day}	**Mean**	**SD**	**Mean**	**SD**	**N_{Night}**	**Mean**	**SD**	**Mean**	**SD**
L2	*under-critical*	7	1.95	± 1.46	5.32	±2.89	6	3.98	± 3.48	2.95	± 2.01
	near-critical	8	6.74	± 3.26	3.16	±2.37	3	3.77	± 3.72	2.80	± 0.41
	over-critical	5	25.98	± 18.11	3.58	±1.20	0	-	-	-	-
L3	*under-critical*	6	2.04	± 0.93	4.06	±2.34	5	2.57	± 2.22	2.03	± 1.75
	near-critical	5	5.02	± 7.51	2.18	±1.08	1	0.77	-	2.01	-
	over-critical	7	10.46	± 10.20	3.32	±1.46	2	1.68	± 0.05	1.79	± 0.38
L4	*under-critical*	5	2.51	± 2.04	4.57	±3.50	4	1.87	± 1.03	4.07	± 1.97
	near-critical	6	2.25	± 1.27	2.88	±1.01	5	2.19	± 2.18	3.55	± 2.76
	over-critical	5	14.06	± 10.56	2.34	±0.45	1	2.25	-	2.90	-
	N_{total}	54					27				

Orientation & deviation of fish speed from water velocity

A unimodal distribution pattern of orientation categories (i.e. angles) with a maximum at 180° was observed for all larval stages and flow-scenarios, but differences in frequencies of single categories occurred for different combinations. Key features and vertices of these patterns are first described here based on the pooled data of all studied larvae and experimental conditions in the case of upstream and lateral movement: Unsurprisingly, the frequency distribution of orientation categories was very narrow when larvae were moving upstream: the orientation category 180° (heads in direct upstream direction) accounted for 62% and the categories 135° and -135° together for 32% of all observations during upstream movements. The remaining 6% of observations during upstream movement accounted for orientation categories 90° and -90° together. Likewise, a clear dominance of upstream orientation during lateral movements (strictly perpendicular to the current vector) was recorded: the category 180° made up for 32% and the categories 135° and -135° together accounted for 52% of all observations.

During downstream movement, larvae were also predominantly orientated with their heads in upstream direction, and orientation patterns were relatively uniform among stages, flow-scenarios and among day and night experiments. In all larval stages a distinct peak at the categories 180°, 135° or -135° was noted. At under- and near-critical conditions, category 180° was dominant in all larval stages and ranged from 19.5 to 36.5% of all observations (pooled day and night data). At over-critical conditions, the category -135° was dominant in L2- and L4-larvae, accounting for 24.6 and 29.3% of all observations, respectively. L3-larvae predominantly (30.5% of all observations) used the category 180° during over-critical flows, as had been observed at under- and near-critical conditions. In all stages and flow-scenarios, the categories 0°, 45°, -45°, 90° and -90° were used to a relatively low extent, each of them generally accounting for < 10% of all observations. As a consequence, the frequency distributions of orientation categories during downstream movement of all larval stages differed significantly from a hypothetical random distribution (all $p<0.05$, Fig. 8 A-C) at almost all flow-scenarios. The exceptions were L2-larvae at the under-critical flow-scenario and L3-larvae at the near-critical flow-scenario, in which the

differences were not significant after Bonferroni correction (p=0.27 and 0.081, respectively). In L2-larvae, the frequency distribution of orientation categories during the under-critical scenario was closest to the hypothetical random pattern (which approximated a homogenous distribution) compared to the patterns in L3- and L4-larvae at this flow-scenario. The same applied to lesser extent to L3-larvae at the near-critical flow-scenario. Nonetheless, the characteristic "dome-shaped" pattern was still discernible in both cases.

During downstream movement, means of *Vf* (deviation of fish speed from current-velocity) varied distinctly among orientation categories (Fig. 8 D-F): All larval stages showed negative mean values in case of upstream orientation (categories 180°, 135° and -135°) during all tested flow-scenarios, and positive values when they were orientated with their heads in downstream direction (categories 0°, 45° and -45°). For larvae orientated perpendicularly to the current vector (categories 90° and -90°), the means of *Vf* were also positive but closer to zero compared to the other orientation categories. *Vf* values were relatively similar among flow-scenarios, especially the near- and the over-critical flow scenario. In the under-critical flow-scenario, however, the magnitudes of negative *Vf* values were subtly lower. Means of *Vf* at distinct orientation categories were also very similar between stages. L4-larvae showed slightly higher magnitudes in negative *Vf* values at all flow scenarios and also higher positive *Vf* values at the under-critical flow-scenario compared to L2- and L3-larvae. When larvae were orientated with their heads in upstream direction, means of *Vf* ranged from -2.4 (±2.1) to - 6.5 (±2.7) cm s^{-1}. In case of downstream orientation means of *Vf* ranged from 0.9 (±1.8) to 9.3 (±3.4) cm s^{-1} faster than the current velocity.

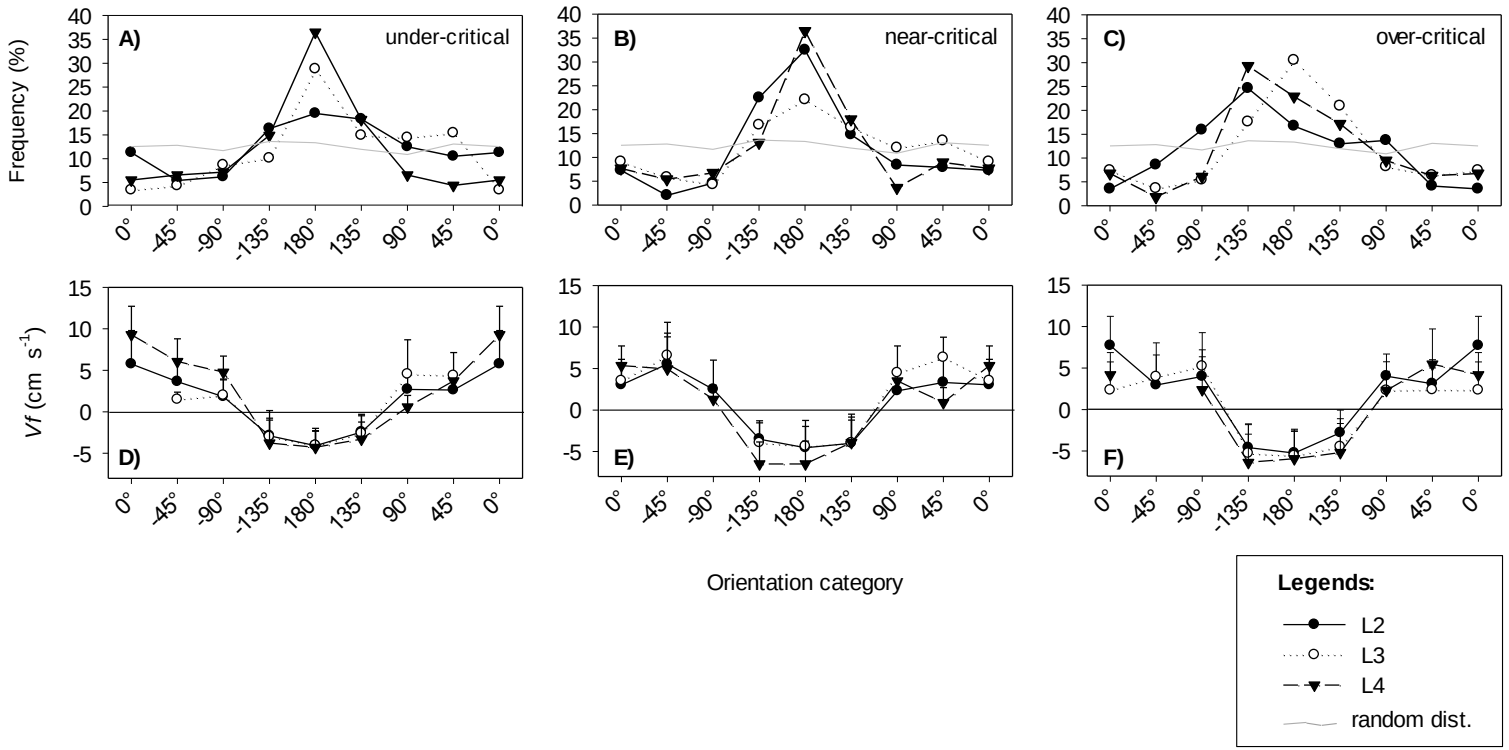


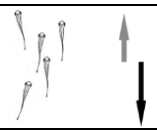
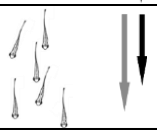
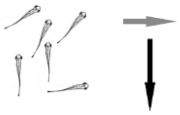
Fig. 8: (A-C) Frequencies of the different orientation categories of larval trajectories at different flow-scenarios during downstream movement for all larval stages. Gray line: hypothetical random distribution. Frequency distributions of almost all larval stages differ significantly from random (all $p<0.05$) except for L2-larvae at the under-critical (A) and L3-larvae at the near-critical flow-scenario (B). (D-F) Means of *Vf* (deviation of fish speed from current velocity) at the different orientation categories during downstream movement. Burst swimming is omitted here because this figure intends to illustrate *Vf* during routine downstream movement.

Movement patterns

Based on the direction of movement (in terms of upstream, downstream and lateral), the orientation (angle towards flow direction) and *Vf*, six different patterns of movement could be distinguished (Tab. 4). Additionally to those patterns of downstream movement proposed by Pavlov (2011), a new pattern was found in which larvae showed orientations roughly perpendicular to the current vector (orientation categories 90° and -90°) and *Vf* values > 1 cm s^{-1}. This pattern is hereafter termed *traversing* and is characterized by a strong lateral component.

Mean frequencies of distinct movement patterns calculated for all larvae and flow-scenarios together were very similar between day and night and differed by less than 7.0% in all movement patterns; in three out of six movement patterns the differences in frequencies between day and night were even smaller (< 2.5 %). For this reason, data from day and night experiments were pooled for further analysis of movement patterns of distinct larval stages.

Tab. 4: Observed movement patterns and their characteristics. Schematic orientation (i.e. posture) of fish larvae during each type of movement. Arrows indicate the direction of fish movement (gray arrows) relative to current vector (black arrows). Arrow lengths represent the resulting fish speed relative to flow-velocity. Orientation towards the current vector is given in angles (categories of orientation) enclosed by the fish´s body axis and the current vector (see Materials & Methods, Fig. 2). *Vf* was calculated as fish speed towards stationary landmarks minus current velocity. *Vf* was estimated with an accuracy of ± 1 cm s^{-1}. During active upstream movement $Vf_{upstream}$ can be calculated as fish speed towards stationary landmarks plus current velocity times -1.

Movement pattern	**Scheme**	**Direction of movement**	**Orientation in relation to current vector**	***Vf* (deviation of fish speed from current velocity)**
Active upstream		upstream	-135°, 135°, 180°	$Vf_{upstream} < Vw * -1$
Active downstream		downstream	-45°, 0°, 45°	$Vf > 1$ cm s^{-1}
Passive downstream		downstream	any orientation possible, at random	$Vf \approx 0$ cm s^{-1}
Active-passive downstream		downstream	-135°, 135°, 180°	$Vf < -1$ cm s^{-1}
Traversing		downstream with lateral component	-90°, 90°	$Vf > 1$ cm s^{-1}
Lateral		strictly lateral	-90°, -135°, 180°, 135°, 90°	$Vf \approx Vw * -1$

In general, *active upstream* movement accounted for a considerable part of all observations. Means (±SD) of larval stages ranged from 14.5% (±9.9) in L2-larvae during the over-critical scenario to 60.3% (±21.5) in L4-larvae at the under-critical scenario. Less surprisingly, a decreasing trend in frequencies of the *active upstream* movement pattern with increasing mean current velocities of flow-scenarios was evident in all stages: Highest values of the *active upstream* movement pattern were found at the under-critical scenario

and lowest values at the over-critical scenario. Intermediate frequencies of *active upstream* movement occurred at the near-critical scenario.

In *lateral* movement, no clear trends were discernible, neither among flow scenarios within larval stages, nor between stages in the same flow scenarios. However, L2-larvae tendentially showed slightly lower values in *lateral* movements than the two older stages: in L2-larvae, mean frequencies of *lateral* movement ranged from 10.1% (±8.5) to 18.1% (±15.3), whereas mean frequencies in L3- and L4-larvae ranged from 17.8% (±15.1) to 27.9% (±16.7) of all movements.

Concerning patterns of downstream movement (*active*, *active-passive*, *passive* and *traversing*), significant differences (all $p<0.01$) among their frequencies were detected within all flow-scenarios and larval stages (Fig. 9 D-F). The *active-passive* pattern was clearly dominant in all stages during all tested flow-scenarios. Furthermore, the *active-passive* pattern was the most frequently observed pattern of all movement patterns (including *active upstream* and *lateral* movement) at the over-critical flow-scenario in all stages. No strong stage-specific differences in the manifestation of the *active-passive* pattern were found. However, the mean frequencies of the *active-passive* pattern in L2-larvae at the under- and the over-critical flow-scenario (49.2% (±24.0)) and 48.3% (±5.0) of all downstream movements, respectively) were slightly lower than in L3- and L4-larvae, whose mean values ranged from 60.1% (±33.1) to 79.5% (±19.1) of all downstream movements.

In all stages, the *passive* pattern of downstream movement was manifested in low to moderate mean frequencies and ranged from 6.9% (±6.0) in L3-larvae at the near-critical flow-scenario to 19.4% (±15.3) in L2-larvae at the under-critical flow-scenario. In general, L2-larvae showed slightly higher values in the *passive* downstream movement pattern compared to later stages. Concerning flow-scenarios, no trends were evident in the *passive* downstream movement pattern.

The newly found pattern *traversing* was highest in L2-larvae at the over-critical flow-scenario, where this pattern accounted for 22.6% (±5.0) of all downstream movements. In other larval stages and flow-scenarios, *traversing* was rather rare with means ranging from

3.1% (±5.7) to 6.8% (±11.9) of all downstream movements. No significant trends were discernible among flow-scenarios.

Frequencies of the *active downstream* movement pattern were low to moderate and ranged from 6.6% (±7.3) to 20.4% (±16.2) of downstream movements. As in other movement patterns, stage- and/or scenario-specific differences could not be observed.

Day and night differences in the manifestation of patterns of downstream movement were minor (based on pooled data of all larval stages, Fig. 9 G-H). In general, the dominance of the *active-passive* pattern was even stronger at night, especially at the near- and over-critical flow-scenario. Accordingly, mean frequencies of the patterns *active downstream*, *passive downstream* and *traversing* were slightly lower at night. For most patterns of downstream movement these differences were not significant ($p>0.1$). However, the differences were significant for the *active downstream* pattern at the near-critical flow-scenario with 19.0% (±13.8) during day versus 6.2% (±10.4) during night and for the *active-passive* pattern at the near-critical flow-scenario with 64.9% (±18.3) during day versus 82.1% (±25.7) during night (both $p<0.05$). Furthermore, no *traversing* occurred at the over-critical flow-scenario during night, whereas this pattern accounted for 11.3% (±8.8) during day at the same flow scenario, a significant difference ($p<0.05$).

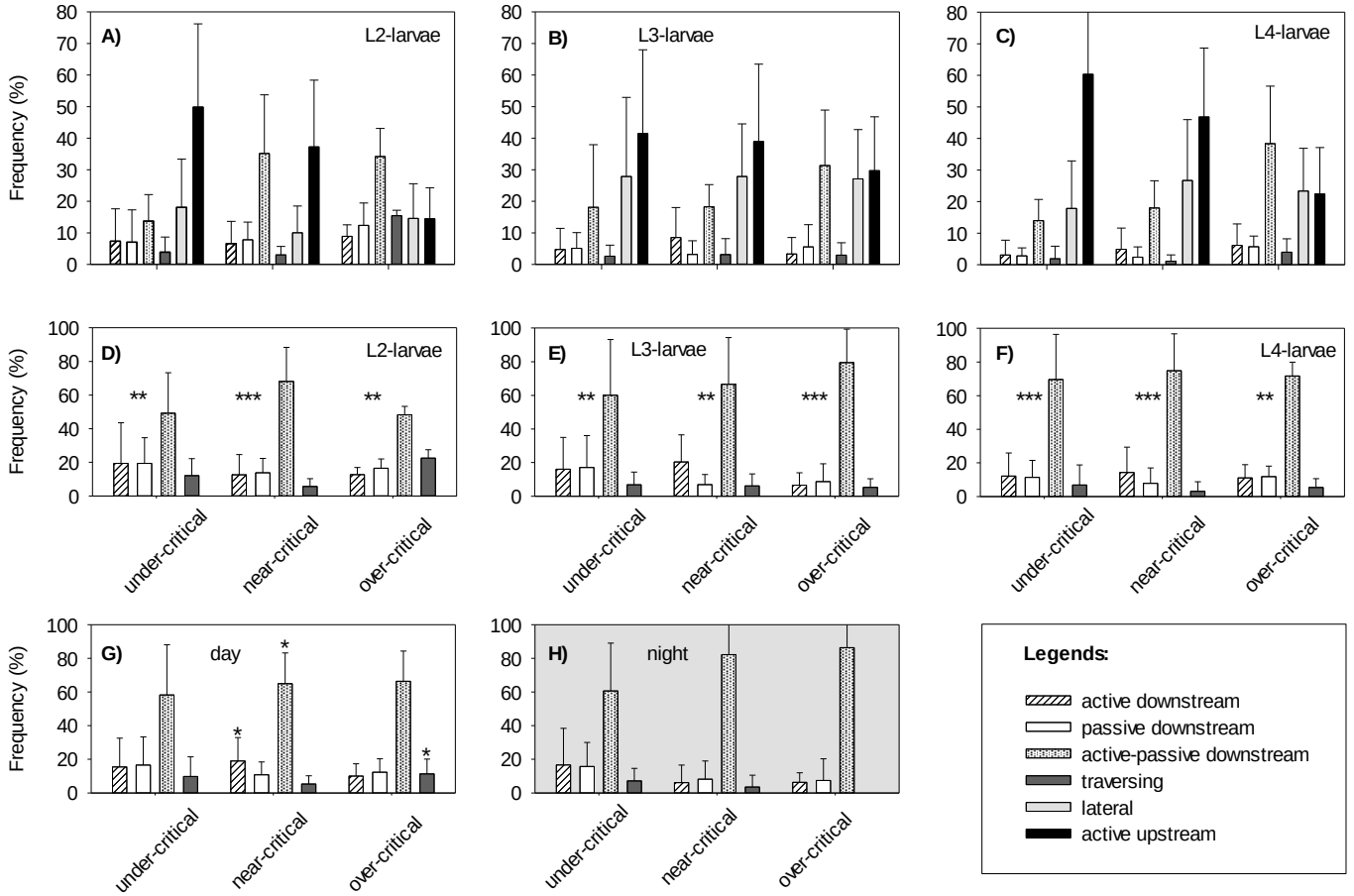


Fig. 9: Mean frequencies of all movement patterns (A-C) and of patterns of downstream movement only (D-F) of different larval stages at three different flow-scenarios. Different movement patterns are indicated by fill colors/patterns. Error bars show standard deviation. Asterisks in D-F indicate significance among patterns of downstream movement within flow-scenarios: * $p<0.05$, ** $p<0.01$, *** $p<0.001$. G) and H) show mean frequencies of patterns of downstream movement of all larvae for day and night, respectively. Asterisks in G) indicate significance between day and night: * p<0.05, ** p<0.01, *** p<0.001.

Used water layer

In general, the lower water layer was used more frequently than the middle or upper water layer (Fig. 10). This applied to almost all larval stages and flow-scenarios. The exception was the over-critical flow-scenario during day, where all layers were used to a similar extent by all larval stages. Furthermore, L2-larvae at the near-critical flow-scenario during day also used all three water layers to a similar extent. At night, however, L3- and L4-larvae almost exclusively used the lower layer at the over-critical flow-scenario (no data for L2-larvae). Likewise, at the near-critical flow-scenario, all larval stages almost exclusively used the lower water layer at night. During day, the mean use frequencies of the lower layer accounted for only 19.5% (±20.7) in L2-larvae, 54.5% (±31.1) in L3-larvae and 38.6% (±32.8) in L4-larvae, at the over-critical flow-scenario. At night, the mean use frequencies of the lower layer in L3- and L4-larvae were, 98.7% (±1.9) and 92.0% (no SD, N=1) at the same flow-scenario, respectively (no data for L2-larvae). At the near-critical flow scenario, the values of the lower layer were 96.0% (±3.6), 96.3% (no SD, N=1) and 97.5% (±1.9) for L2-, L3- and L4-larvae, respectively. The day/night differences in this layer were significant for L2- and L4-larvae at the near-critical flow-scenario ($p<0.05$ and $p<0.01$, respectively; no test for L3-larvae). At the over-critical flow-scenario the differences were close to significance in L3-larvae ($p=0.056$, no data for L2-larvae, no test for L4-larvae). At the under-critical flow-scenario, values of the lower water-layer ranged from 65.4% (±40.1) in L2-larvae to 77.0% (±20.7) in L4-larave and were similar between night and day (all $p>0.1$).

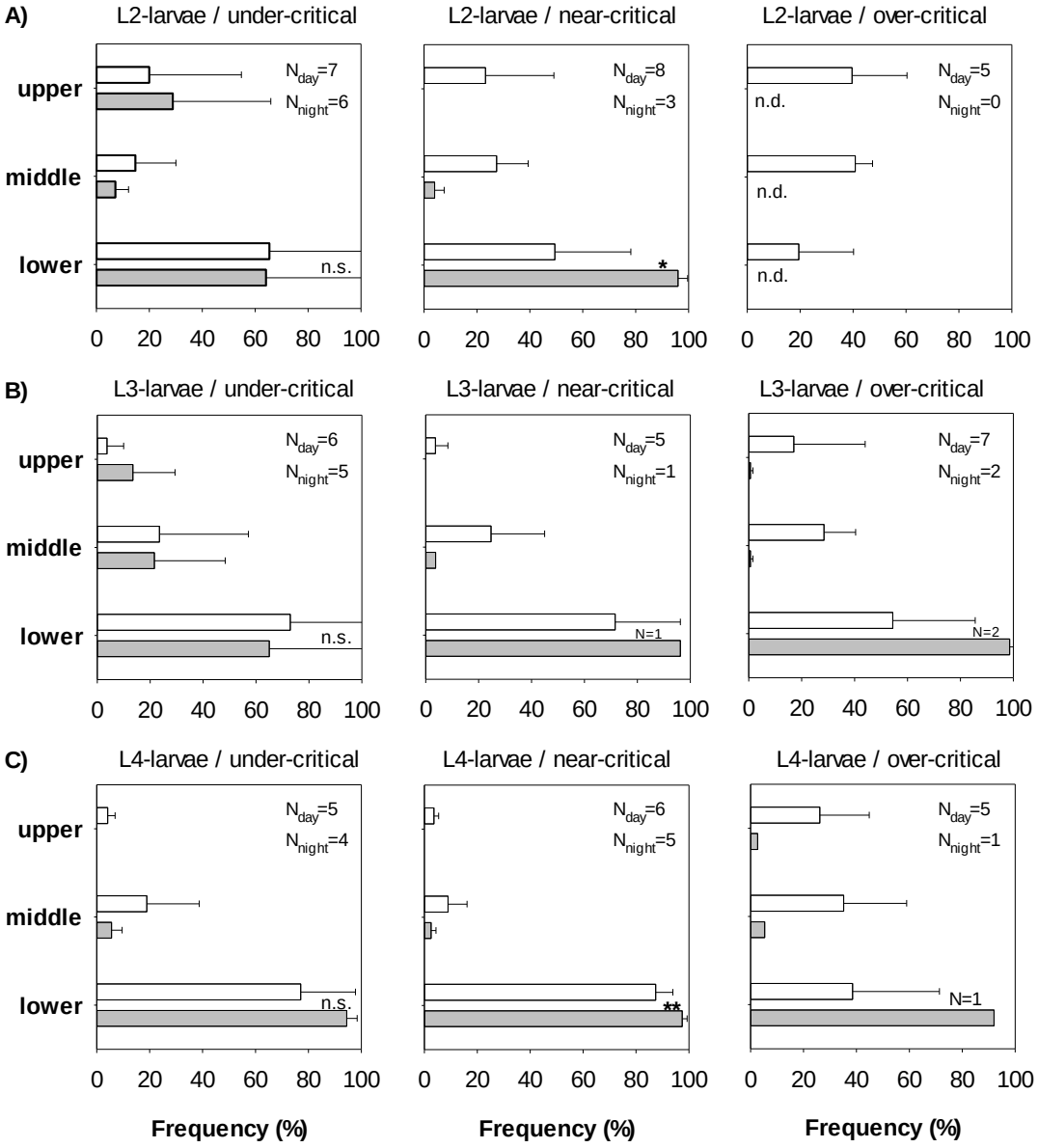


Fig. 10: (A-C) Mean frequencies of the use of upper, middle and lower water layer for L2-, L3- and L4-larvae for all flow-scenarios for day and night. Error bars are standard deviation. Asterisks indicate significance between day and night in the use of the lower water layer: * $p<0.05$, ** $p<0.01$. No data (n.d.) available for L2-larvae at the over-critical flow scenario at night.

Turns & stops

The number of turns was strongly dependent on the total length of the observed swimming trajectory (which is approx. equivalent to the number of observations per individual). A highly significant linear regression described this relationship: $T_n = 0.735 + 3.923 * L_{TR}$, where T_n is the number of turns and L_{TR} the length of the observed swimming trajectory in meters (N=80, r^2=0.78, p<0.001). For this reason, the number of turns was standardized for 1 m of observed swimming trajectory to evaluate potential effects of other variables. Turns m^{-1} ranged from 1.2 to 7.5 among individuals; approx. 60% of all individuals showed values of 3 to 5 turns m^{-1}. The means (±SD) for the different larval stages calculated for all flow-scenarios together were similar: 3.9 (±1.2) in L2-, 4.2 (±1.5) in L3- and 3.7 (±1.2) in L4-larvae. The number of turns m^{-1} within larval stages and flow scenarios were very similar with respect to the different release-points and did not differ significantly (all p>0.1). The flow-scenario also showed no discernable effect on the number of turns m^{-1} (Fig. 11). The mean values of L2- and L3-larvae showed no recognizable tendencies regarding the different flow-scenarios. In L4-larvae, however, a slightly increasing trend among flow-scenarios was noted, but this was not significant. In most cases, the number of turns m^{-1} were slightly higher at night. This difference applied to L4-larvae at the under- and the near-critical flow-scenario, to L3-larvae at the under- and the over-critical flow-scenario and to L2-larvae at the under-critical scenario. In contrast, the mean values were marginally higher during the day for L2- and L3-larvae at the near-critical scenario. In all tested cases, day/night differences within larval stages and flow-scenarios were not significant in pairwise comparison (all p>0.05).

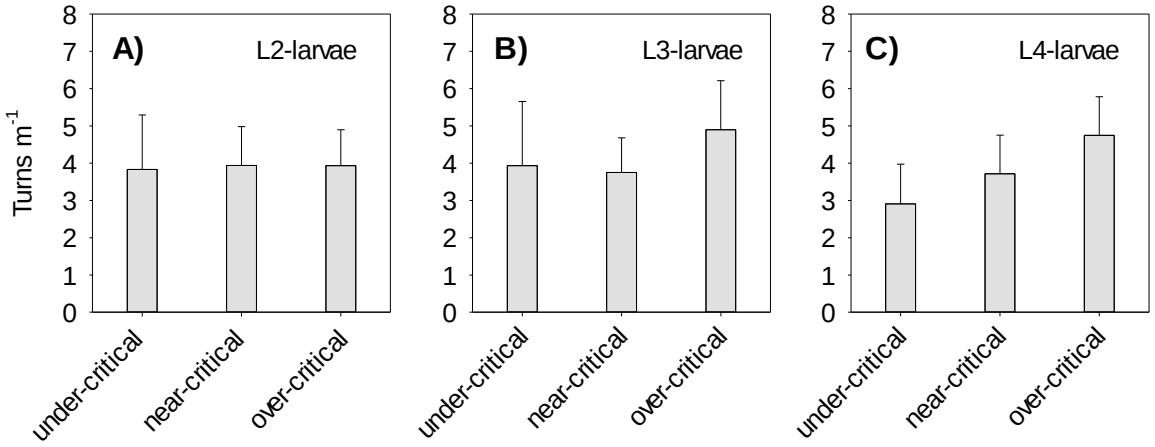


Fig. 11: (A-C) Mean number of turns per meter of swimming trajectory at the different flow-scenarios for L2-, L3- and L4-larvae. Error bars show standard deviation.

The number of stops was standardized to one minute of observation time (Fig. 12). Stops min^{-1} ranged from 0 to approx. 2 in individuals. No stops were recorded in 12 out of 81 individuals (14.8% of total), i.e. they moved continuously. Movement without stops occurred in all larval stages, at all flow-scenarios and by day and night. Mean values of stops for different stages were 0.72 (±0.57), 0.93 (±0.64) and 0.87 (±0.60) for L2, L3 and L4, respectively. Numbers of stops were similar irrespective of release-point (all p>0.1). In almost all stages and flow-scenarios, means of stops min^{-1} tended to be higher during the night. These differences, however, were not significant within stages and flow scenarios (all p>0.1).

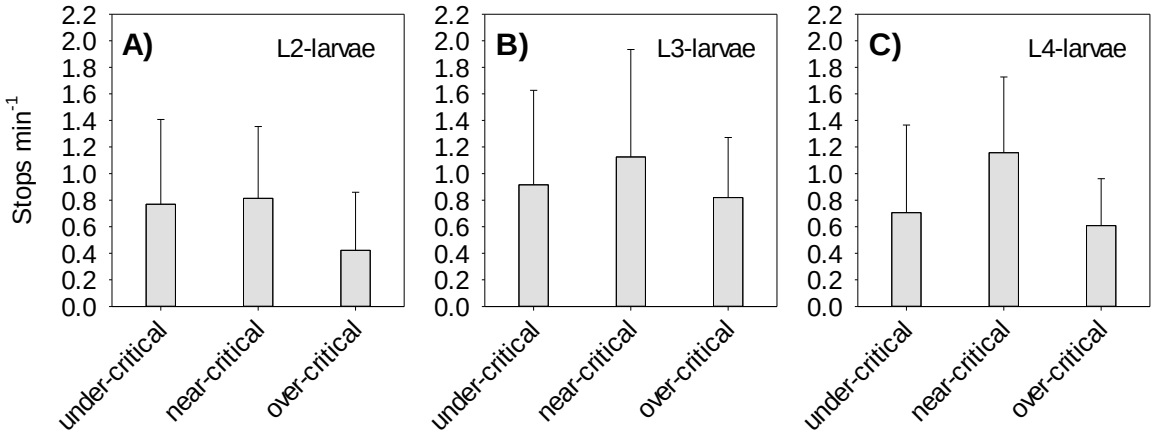


Fig. 12: (A-C) Means of stops per one minute of observation time at the different flow-scenarios for L2-, L3- and L4-larvae. Error bars show standard deviation.

Discussion

Flow velocity gradients

The three different flow-scenarios used in this study, were representative for flow conditions in natural habitats of the nase. The entire area of the flume at the under-critical and approx. 50% of the flume area at the near-critical flow-scenario provided current velocities equal to those found in appropriate nursery habitats in the wild (i.e. current velocities < 12 cm s^{-1}, Keckeis et al., 1997), and the current conditions at the over-critical flow-scenario were similar to those found at sites where larval nase were sampled via drift-nets in the Danube River (Lechner, pers. communication).

Morphometrics & development

The morphometric measurements, length-weight relationships and ontogenesis of the larvae used in the experiments were typical and in accordance to published literature on the species early development (compare e.g. Peňáz 1974, Flore and Keckeis 1998, Flore et al. 2001, Lechner et. al 2014). These findings indicate that the rearing process was successful in terms of producing vital and healthy larvae. Concerning fin development in L4-larvae, we noticed that anlagen of ventral fins were present in only 5 out of 26 individuals. According to the literature used for stage identification (Peňáz 1974), the origin of ventral fins is a characteristic feature of the fourth larval stage. Thus, we assume that most L4-larvae used in our experiments represented early individuals of this stage (valid identification of the L4-stage was based on completed differentiation of caudal fin with typical dorsal tubercle). The grouping of larvae by Hierarchical Cluster analysis coincided strongly with the classification based on developmental stages (i.e. similarity approx. 93%). This finding demonstrates that larval stages presented convenient operational units with respect to morphometrics (i.e. size, shape, proportions).

Covered distances, displacement & rheoreaction

Upstream movement accounted for more than 50% of total longitudinal movement in approx. 70% of tested larvae at under-critical flow conditions. So far, very few studies have detected upstream movement of larvae in rivers. Schludermann et al. (2012) documented upstream movement of larval nase in a mark-recapture study along a shoreline nursery habitat of the Danube River. Lechner et al. (2014) recaptured marked L4-larvae up to 150 m upstream of a release-point on the 5th day after release. In the study of Schludermann et al. (2012), a very small proportion of released larvae remained in the study reach; these were classified as "retained". Out of those, however, the vast majority (22 out of 24 larvae) were found upstream of the release-point. They were significantly larger than larvae of the same release event which were captured in drift nets. The results of the present laboratory study do not support this finding: larvae with positive rheoreactive quotient (i.e. net upstream displacement) were almost identical in size to those with negative rheoreactive quotient (i.e. net downstream displacement). The present study suggests that fish larvae use zero and low-flow zones along the shoreline as corridors for upstream migration to a hitherto unknown large extent. Larval riverine fish thereby possibly compensate for accidental drift and/or exploit profitable nursery habitats upstream. From this perspective, the continuity of corridors along river shorelines which enable upstream movement is very important and potentially strongly promotes recruitment.

Downstream displacement increased drastically at over-critical flow conditions. This finding was expected because such flow conditions prohibit fish larvae from holding their position for longer than 2 minutes per definition (Kaufmann, 1990; Flore et al., 2001). However, compared to the displacement of a passive particle, the displacement of fish larvae was markedly lower: The highest downstream displacement in 5 minutes observation time was approx. 54 m at an average current velocity (U_{tr}mean) of 21.2 cm s^{-1}: a passive particle would have reached a distance of approx. 64 m in 5 minutes at this flow-velocity. In other individuals with similar U_{tr}mean values, downstream displacement was even lower (i.e. did not exceed 30 m in 5 minutes). These findings are in accordance to published literature: Schludermann et al. (2012) estimated that released larvae would have passed

through the study reach of 200 m length after approx. 6 minutes if they would have drifted totally passive. Instead, larvae were captured for up to 120 minutes after release and the authors therefore concluded that larval movement was not entirely passive.

The present study was unable to detect significant differences in covered downstream distances (CDD) between day and night. These results were not expected, considering the known nocturnal peak of drift in most studied species (D'Amours et al., 2001; Johnson & McKenna, 2007) including nase (e.g. Keckeis et al. 1997; Reichard et al., 2001). Potentially, other environmental factors in rivers act beyond to the level of illumination to promote the extent of nocturnal downstream movement. These were absent in the experimental flume and might include predator-prey interactions, schooling effects or higher turbulences in natural systems. Note, however, that the number of successfully conducted experiments was markedly higher during the day, especially at the over-critical flow-scenario.

Pavlov et al. (2011) distinguished between the positive, the neutral and the negative type of rheoreaction with respect to the preferred direction of movement in relation to the water current direction. This classification scheme can be extended because phases of upstream movement and downstream movement can alternate within seconds to minutes. This pattern is reflected in the presented rheoreactive quotient. At under-critical flow conditions, the rheoreactive quotient was relatively homogeneously distributed between -0.92 and 0.80. This finding shows that the preference to move either upstream or downstream is subject to large individual variation. Nonetheless, the mean rheoreactive quotients of L4-larvae were slightly higher than those of L2- and L3-larvae (0.33 in L4 versus 0.20 and 0.07 in L2 and L3, respectively), implying a higher tendency for upstream movement of older stages in under-critical flow conditions. These differences potentially reflect increased swimming abilities of larger larvae and lower physiological costs. The findings are partly supported by published literature. Lechner et al. (2014) released marked L2- and L4-larvae simultaneously. On the 5th day after release, only L4-larvae were detected upstream of the release-point while L2-larvae were exclusively recaptured downstream.

Orientation & deviation of fish speed from water velocity

The observed dominance of larval orientation in upstream direction is clearly related to a high proportion of upstream movement, the high frequency of using the active-passive pattern during downstream movement, and the association of lateral movement with orientation in upstream direction. Very few studies have focused on the orientation of riverine larvae. In this study, larvae were clearly not randomly orientated during downstream movement. Pavlov et al. (2011) reported that in a laboratory study using juvenile roach (*Rutilus rutilus*), carp (*Cyprinus carpio*), bream (*Abramis brama*) and perch (*Perca fluviatilis*), all investigated fish showed orientation patterns that differed significantly from random distribution during downstream movement. That study was, however, performed with juvenile fish, and the authors reported that prior studies conducted with larvae revealed that those stages are randomly orientated during downstream movement in the dark.

Larvae showed *Vf* values that clearly deviated from zero in almost all cases. This finding demonstrates that active propulsive movements were executed in most observations and, consequently, passive translocation by the current was rare. Similar results were reported by Pavlov et al. (2011), who detected significant differences between fish speed and water current velocity in juveniles of the species mentioned above. For juveniles orientated with their heads downstream, Pavlov et al. (2011) measured *Vf* values up to 4.8 cm s^{-1} (up to 12.6 cm s^{-1} in starved roach), which is similar to the values detected in the present study (means: 0.9 (±1.8) to 9.3 (±3.4) cm s^{-1}).

Movement patterns

Concerning types of downstream movements, the *active-passive* type was overwhelmingly dominant in all tested flow scenarios. This pattern was evident during under-, near- and over-critical flow conditions as well as in all larval stages, both during day and night. Previously, it was assumed that the active-passive pattern of downstream movement mainly reflects inhibition of swimming abilities due to starvation or lower water

temperature (Pavlov et al. 2008). In our experiments, flume water temperatures equaled those in the rearing tank and were near the physiological optimal temperature of the species (Kamler and Keckeis, 2000). Additionally, larvae in the rearing tanks were fed ad libitum throughout the study period. I therefore conclude that starvation and/or a decrease in water temperature do not underlie the observed dominance of the active-passive pattern. Pavlov et al. argued later (2011) that active-passive movement might be a manifestation of rheoreaction and not merely reflect inhibited locomotive activity in juveniles of different eurytopic cyprinid species. The authors analyzed patterns of downstream movement in juvenile carp (*Cyprinus carpio*), bream (*Abramis brama*) and roach (*Rutilus rutilus*) and found that the proportion of specimens using the active-passive type was highest in bream (50.0 %) and roach (36.8 %) and relatively low in carp (18.6%). Evidence for the occurrence of the active-passive type of downstream movement at over-critical flows has also been reported in larvae of an oyster reef gobiid, *Gobiosoma bosc* (Breitenburg et al. 1994). Moving downstream active-passively possibly provides several benefits to fish larvae. These benefits could be associated with greater skills in sensing, orientating and maneuvering, thereby favoring successful settlement in appropriate habitats, prey capture and predator avoidance. For example, Pavlov et al. (2011) discovered that juveniles had a greater chance to enter micro eddy-zones when they were moving downstream with head in upstream direction. Moreover, reducing downstream displacement in over-critical currents (e.g. during accidental drift in case of floods) seems to provide a reasonable strategy and would reflect evolutionary adaption to life in fast-running waters. Pavlov et al. (2008) suggested the passive form of downstream movement to be typical for early larvae and to be in general higher at night and/or highly turbid conditions. In the present study, the use of the passive pattern was slightly higher in L2-larvae than in L3- and L4-larvae. This finding partly supports both the thesis of Pavlov et al. (2008) and the hypothesis posed in the present study. The swimming style in many cyprinids (including *C. nasus*) is known to shift from anguilliform (flexion of the whole body) in early larvae to subcarangiform (single tail flicks alternating with gliding phases) in later larval stages (Kaufmann 1990, Flore et al. 2001). Speculatively, these differences would reasonably explain shifts in the preference for different movement patterns. Nevertheless, the differences between L2-larvae and older stages detected in this study were relatively low. Concerning diurnal effects, day/night differences in the

manifestation of the passive movement pattern were marginal and statistically not significant. Instead, the dominance of the active-passive pattern was even more pronounced during night. The newly described movement pattern (traversing) occurred in almost all larval stages and flow-scenarios, but in a low percentage of all observed movement patterns. The only exception were L2-larvae at the over-critical flow-scenario where traversing accounted for 22.6% (±5.0) of all downstream movements. Traversing was indicated by strong propulsive activity (clearly positive values of Vf) and orientation perpendicular to the current. Based on these characteristics, traversing could be an effect of spontaneous burst swimming and/or represents a brief attempt to scan the surroundings in lateral direction, e.g. in order to approach favorable or escape unfavorable zones. Pavlov et al. (2011) described that juvenile carp, bream and roach were sometimes orientated such that "the axis of the fish body was perpendicular to the vector of current and the head was always directed to the same stationary landmark". Proportions of this type of orientation accounted for 13.6, 0.0 and 15.8% of individuals of the species mentioned above, respectively. However, the combination of active swimming and perpendicular orientation towards the current vector has not yet been designated as a specific movement pattern in itself. The manifestation of the active downstream movement was low to moderate in the present study. This finding suggests differences between larvae and juveniles: Pavlov et al. (2011) discovered relatively high proportions (47.4 to 64.4%) of juvenile cyprinids moving downstream in the active form and assumed that the manifestation of active downstream movement depends on the motivational state of the individuals. The dissimilarity in findings of the two studies implies that active downstream movement is more typical for juveniles than for larvae, possibly related to lower swimming capabilities.

Used water layer

The vertical position of fish larvae in the water column and associated effects on dispersal have been extensively studied in marine and estuarine environments (e.g. by Frotier & Legett, 1983; Leis, 1986; Leis, 1991) but have received comparatively little attention in freshwater lakes and rivers (e.g. Oesmann, 2003; Pavlov et al., 1995). These

studies have, however, focused on vertical distribution of larvae at large scale i.e. the entire water column of large rivers. The present study detected significant diurnal differences in the use of different water layers at a much smaller scale i.e. over a maximum depth of 20 cm. Significant differences in the use of the three water layers (upper, middle and lower) were detected between day and night at the near-critical flow-scenario. In general, at night, larvae preferred the lower water layer at the over-critical flow-scenario, whereas the distribution was more homogeneously during the day. Pavlov et al. (2000) studied the vertical distribution of juvenile cyprinids (*Leuciscus leuciscus* and *L. idus*) under different illumination regimes and found that fish were more evenly distributed in the water column in the dark, while they mainly (51.6% of individuals) used the upper layer (0-1 m in a 6 m deep aquarium) during uniform illumination. Note, however, that these experiments were conducted with a much greater maximum depth and in lentic conditions. Nevertheless, differences in the vertical position of larval and juvenile cyprinids seem possible.

Turns & stops

The number of turns appeared to be independent of larval stage or day and night. Concerning the different flow-scenarios, a slightly increasing (non-significant) trend with mean current velocities was observed only in L4-larvae. This increase could be related to higher turbulence and the need for larvae to re-orientate themselves in order to maintain their preferred swimming trajectories or movement patterns.

Stops were slightly reduced at the over-critical flow-scenario. This result was expected because position holding would mean high energetic costs under such conditions. Fish would be unable to hold their position for more than 2 minutes when experiencing over-critical flows, by definition (Flore et al. 2001, Kaufmann 1990). Nonetheless, the decreasing trend in the number of stops was not significant, potentially reated to the presence of low-flowing zones even at the over-critical flow-scenario.

Conclusions

Knowledge about different patterns of downstream movement, and the relevant behavioral features associated with them, has significant implications for modelling the dispersion of fish larvae in running waters. The relevance involves different dispersal velocities of fish moving downstream in relation to current velocity. This study is the first attempt to investigate different patterns of movement in larvae of a rheophilic cyprinid species. The results underline that nase larvae must not be regarded as passive particles when attempting to understand, model or predict their dispersal patterns. Instead, early stages apparently possess active behavioral components governing downstream transport and actively move upstream at low-flow conditions, i.e. in velocity gradients. By applying the active-passive type of downstream movement, they actively decrease downstream displacement and probably obtain further benefits. These advantages could include a greater chance to detect and access suitable nursery habitats and/or enhanced skills in maneuvering, which might facilitate prey capture and predator avoidance. Future research should examine the ecological reasons and consequences of this behavior. This would involve exploring dispersal-relevant behavioral features and subsequent movement patterns in larvae of other freshwater species and the ecological advantages associated with these patterns, thereby further contributing to our understanding of the dynamics and recruitment of riverine fish populations.

Acknowledgements

First, I am very grateful to Hubert Keckeis for his great supervision. He always had an open ear for my questions and never missed an opportunity to give most helpful advice throughout my Master`s study, no matter how busy he was. Michael Stachowitsch kindly improved the English. Furthermore, I would like to thank my colleagues Aaron Lechner, Franz Lumesberger-Loisl, Reinhard Krusch, Florian Keil, David Ramler, Maximilian Sehr, Astrid Toth and Holger Villwock for their support, advice and friendship. All of them are great enthusiasts in freshwater fish and I am thankful for the numerous discussions on fish ecology and identification.

Special thanks are dedicated to Martin Glas and Michael Tritthart for very fruitful collaboration during this study. I also acknowledge the FWF for funding the wider project (MODI P22631-B17 “Modelling dispersal patterns of fish larvae in a large river”) of which this thesis is a part.

Last but not least, I would like to thank my family, especially my parents and my brother for their great support.

Danksagung

An erster Stelle möchte ich mich bei Hubert Keckeis für seine hervorragende Betreuung bedanken. Während meines gesamten Master Studiums hatte er stets ein offenes Ohr für meine Fragen und hat es nie verabsäumt mit höchst hilfreichen Ratschlägen zur Seite zu stehen, ganz gleich wie beschäftigt er sonst war. Michael Stachowitsch verbesserte dankenswerterweise das Englisch. Bedanken will ich mich auch ganz besonders bei meinen Kollegen Aaron Lechner, Franz Lumesberger-Loisl, Reinhard Krusch, Florian Keil, David Ramler, Maximilian Sehr, Astrid Toth und Holger Villwock für ihre Unterstützung, Ratschläge und nicht zuletzt für ihre Freundschaft. Sie alle sind Enthusiasten in Sachen Süßwasserfische, wie man sie nur selten findet. Ich werde mich immer gerne an die zahlreichen Diskussionen zum Thema Fische, deren Ökologie und Bestimmung erinnern.

Besonderer Dank gebührt Martin Glas und Michael Tritthart für die sehr erfolgreiche Zusammenarbeit während dieser Studie. Dank geht auch an den FWF für die Finanzierung des übergeordneten Projektes (MODI P22631-B17 “Modelling dispersal patterns of fish larvae in a large river”) von dem meine Arbeit Teil ist.

Zu guter Letzt ein großes „Danke“ meiner lieben Familie, ganz besonders meinen Eltern und meinem Bruder die mich immer unterstützt haben so gut es ging.

Literature

Bischoff, A., Wolter, C. (2001) The flood of the century on the River Oder: Effects on the 0+ fish community and implications for floodplain restoration. *Regulated Rivers-Research & Management* **17,** 171-190.

Breitburg, D. L. (1994). Behavioral response of fish larvae to low dissolved oxygen concentrations in a stratified water column. *Marine Biology* **120 (4)**, 615-625.

Brown, A.V., Armstrong, M.L. (1985) Propensity to Drift Downstream among Various Species of Fish. *Journal of Freshwater Ecology* **3**, 3-17.

Copp, G.H., Cellot, B. (1988) Structure and Function of Ecosystems of the French Upper-Rhone River: Drift of Embryonic and Larval Fishes, Especially *Lepomis gibbosus* (L), in the Upper-Rhone River. *Journal of Freshwater Ecology* **4**, 419-424.

D'Amours, J., Thibodeau, S., Fortin, R. (2001) Comparison of lake sturgeon (*Acipenser fulvescens*), *Stizostedion* spp., *Catostomus* spp., *Moxostoma* spp., quillback (*Carpiodes cyprinus*), and mooneye (*Hiodon tergisus*) larval drift in Des Prairies River, Quebec. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **79**, 1472-1489.

Fiksen, Ø., Jørgensen, C., Kristiansen, T., Vikebø, F., Huse, G. (2007) Linking behavioural ecology and oceanography: larval behaviour determines growth, mortality and dispersal. *Marine Ecology Progress Series* **347**, 195–205.

Flore, L., & Keckeis, H. (1998) The effect of water current on foraging behaviour of the rheophilic cyprinid Chondrostoma nasus (L.) during ontogeny: evidence of a trade-off between energetic gain and swimming costs. *Regulated Rivers: Research & Management* **14 (1)**, 141-154.

Flore, L., Keckeis, H., & Schiemer, F. (2001) Feeding, energetic benefit and swimming capabilities of 0+ nase (Chondrostoma nasus L.) in flowing water: an integrative laboratory approach. *Archiv für Hydrobiologie, Supplementband. Large rivers* **12 (2-4)**, 409-424.

Fortier, L., Leggett, W. C. (1983) Vertical migrations and transport of larval fish in a partially mixed estuary. *Canadian Journal of Fisheries and Aquatic Sciences* **40(10)**, 1543-1555.

Fuiman, L. A., & Werner, R. G. (Eds.). (2009) *Fishery science: the unique contributions of early life stages*. John Wiley & Sons.

Gallego, A., North, E., & Petitgas, P. (2007) Introduction: status and future of modelling physical-biological interactions during the early life of fishes. *Marine Ecology Progress Series* **347**, 122-126.

Garner, P. (1999) Swimming ability and differential use of velocity-patches by 0+ cyprinids. *Ecology of Freshwater Fish* **8,** 55-58.

Glas, M., Tritthart, M., Zens, B., Keckeis, H., Hauer, C., Habersack, H. (2012) Larval dispersal patterns: observations and particle tracing simulations in a racetrack flume. *In Institute of Water Management, Hydrology and Hydraulic Engineering, University of Natural Resources and Life Sciences, Vienna, 9th International Symposium on Ecohydraulics.*

Humphries, P., King, A. J. (2004) Drifting fish larvae in Murray-Darling Basin rivers: composition, spatial and temporal patterns and distance drifted. *Canberra Workshop* June 2003.

Johnson, J.H., McKenna, J.E. (2007) Diel periodicity of drift of larval fishes in tributaries of Lake Ontario. *Journal of Freshwater Ecology* **22,** 347-350.

Johnston, T.A., Gaboury, M.N., Janusz, R.A., Janusz, L.R. (1995) Larval fish drift in the Valley River, Manitoba: Influence of abiotic and biotic factors, and relationships with future year class strengths. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 2423-2431.

Kamler, E., Keckei, H., & Bauer-Nemeschkal, E. (1998) Temperature-induced changes of survival, development and yolk partitioning in Chondrostoma nasus. *Journal of Fish Biology* **53 (3)**, 658-682.

Kamler, E., Keckeis, H. (2000) Reproduction and early life history of *Chondrostoma nasus*: implications for recruitment (a review). Polskie Archiwum Hydrobiologii **47(1)**, 73-86.

Kaufmann, R. (1990) Respiratory cost of swimming in larval and juvenile cyprinids. *Journal of Experimental Biology* **150 (1)**, 343-366.

Keckeis, H., Frankiewicz, P., Schiemer, F. (1996) The importance of inshore areas for spawning nase *Chondrostoma nasus* (Cyprinidae) in a free-flowing section of a large river (Danube, Austria). Large Rivers **10**, 51-64.

Keckeis, H., Winkler, G., Flore, L., Reckendorfer, W., & Schiemer, F. (1997) Spatial and seasonal characteristics of 0+ fish nursery habitats of nase, *Chondrostoma nasus* in the River Danube, Austria. *Folia Zoologica* **46**, 133-150.

Kottelat, M., & Freyhof, J. (2007) Handbook of European freshwater fishes. *Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany.* p. 186.

Lechner, A., Keckeis, H., Schludermann, E., Loisl, F., Humphries, P., Glas, M., Tritthart, M., Habersack, H. (2014) Shoreline configurations affect dispersal patterns of fish larvae in a large river. *ICES Journal of Marine Science: Journal du Conseil*, **71(4)**, 930-942.

Leis, J.M. (1986) Vertical and horizontal distribution of fish larvae near coral reefs at Lizard Island, Great Barrier Reef. *Marine Biology* **90 (4)**, 505-516.

Leis, J.M. (2007) Behaviour as input for modelling dispersal of fish larvae: behaviour, biogeography, hydrodynamics, ontogeny, physiology and phylogeny meet hydrography. *Marine Ecology Progress Series* **347**, 185-193.

Makeyeva, A. P., Pavlov, D. S., Pavlov, D. A. (2011) Atlas of larvae and juvenile of freshwater fishes of Russia. *Moscow, KMK Scientific Press,* 200-201

Metaxas, A. (2011) Behaviour in flow: perspectives on the distribution and dispersion of meroplanktonic larvae in the water column. *Canadian Journal of Fisheries and Aquatic Sciences* **58 (1)**, 86-98.

Mion, J.B., Stein, R.A., Marschall, E.A. (1998) River discharge drives survival of larval walleye. *Ecological Applications* **8**, 88-103.

Oesmann, S. (2003) Vertical, lateral and diurnal drift patterns of fish larvae in a large lowland river, the Elbe. *Journal of Applied Ichthyology* **19**, 284-293.

Pavlov DS, P.A., Kuragina GN, Nezdoliy VK, Nekrasova NP, Brodskiy DA, Ersler AL. (1978) Some Features of the Downstream Migrations of Juvenile Fishes in the Volga and Kuban Rivers. *Journal of Ichthyology* **17**, 63-374.

Pavlov, D.S. (1994) The Downstream Migration of Young Fishes in Rivers - Mechanisms and Distribution. *Folia Zoologica* **43**, 193-208.

Pavlov, S., Nezdoliy, V. K., Urteaga, A. K., Sanches, O. R. (1995). Downstream migration of juvenile fishes in the rivers of Amazonian Peru. *Journal of Ichthyology* **35**, 227–248.

Pavlov, D. S., Sadkovskii, R. V., Kostin, V. V., & Lupandin, A. I. (2000) Experimental study of young fish distribution and behaviour under combined influence of baro-, photo-and thermo-gradients. *Journal of fish biology* **57 (1)**, 69-81.

Pavlov, D.S., Mikheev, V.N., Lupandin, A.I., Skorobogatov, M.A. (2008) Ecological and behavioural influences on juvenile fish migrations in regulated rivers: a review of experimental and field studies. *Hydrobiologia* **609**, 125-138.

Pavlov, D.S., Kostin, V. V., Zvezdin, A. O. (2011) Patterns of movements of fish with negative type of rheoreaction. *Journal of Ichthyology* **51**, 536-542.

Peňáz M. Early development of the nase carp, *Chondrostoma nasus* (Linnaeus, 1758). (1974) *Zoologické Listy* **23 (3)**, 275-288.

Peňáz M. Chondrostoma nasus - its reproduction strategy and possible reasons for a widely observed population decline - a review. (1996) *Conservation of Endangered Freshwater Fish in Europe ALS Advances in Life Sciences* 279-285.

PiNeDa, J. (2007) Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanography* **20 (3)**, 22.

Reckendorfer, W., Keckeis, H., Tiitu, V., Winkler, G., Zornig, H., & Schiemer, F. (2001) Diet shifts in 0+ nase, Chondrostoma nasus: Size-specific differences and the effect of food availability. *Archiv für Hydrobiologie. Supplementband. Large rivers*, **12 (2-4)**, 425-440.

Reichard, M., Jurajda, P. and Vaclavik, R. (2001) Drift of larval and juvenile fishes: a comparison between small and large lowland rivers. *Archiv für Hydrobiologie - Supplement* **135 (2-4)**, 373-389.

Reichard, M., Jurajda, P., Ondrackova, M. (2002) The effect of light intensity on the drift of young-of-the-year cyprinid fishes. *Journal of Fish Biology* **61**, 1063-1066.

Reichard, M., Jurajda, P. (2007) Seasonal dynamics and age structure of drifting cyprinid fishes: an interspecific comparison. *Ecology of Freshwater Fish* **16**, 482-492.

Schiemer, F., Keckeis, H., & Kamler, E. (2002) The early life history stages of riverine fish: ecophysiological and environmental bottlenecks. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **133 (3)**, 439-449.

Schludermann, E., Tritthart, M., Humphries, P., Keckeis, H. (2012) Dispersal and retention of larval fish in a potential nursery habitat of a large temperate river: an experimental study. *Canadian Journal of Fisheries and Aquatic Sciences* **69**, 1302-1315.

Spindler, T., (1988) Ökologie der Brutfische in der Donau bei Wien. *Dissertation Universität Wien*, p. 119.

Stoll, S., Beeck, P. (2012) Larval fish in troubled waters – is the behavioural response of larval fish to hydrodynamic impacts active or passive? *Canadian Journal of Fisheries and Aquatic Sciences* **69**, 1576-1584.

Szabó, T., Medgyasszay, C., & Horváth, L. (2002) Ovulation induction in nase (Chondrostoma nasus, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone. *Aquaculture* **203 (3)**, 389-395.

White, J.L., Harvey, B.C. (2003) Basin-scale patterns in the drift of embryonic and larval fishes and lamprey ammocoetes in two coastal rivers. *Environmental Biology of Fishes* **67,** 369-378.

Wolter, C., & Sukhodolov, A. (2008) Random displacement versus habitat choice of fish larvae in rivers. *River research and applications* **24 (5)**, 661-672.

Supplement I

Characteristics of larval stages in C. nasus & comments on their identification

L2-larvae are characterized by a fully depleted yolk sac (start of exclusively exogenous feeding) and a filled anterior chamber of the swim bladder. Beside large remnants of the embryonic fin fold, these larvae possess differentiated pectoral fins. In L4-larvae, fin development is characterized by a successive reduction of the embryonic fin fold and formation of fin rays (lepidotrichia) in the dorsal and anal fin. Additionally, the posterior chamber of the swim bladder is filled. Anlagen of ventral fins appear during the L4-stage. According to Peňáz (2001) these features can also be applied to any other species of Cyprinidae. Further important characteristics, most applicable for stage identification, are provided in the explanatory text to Fig. S1.

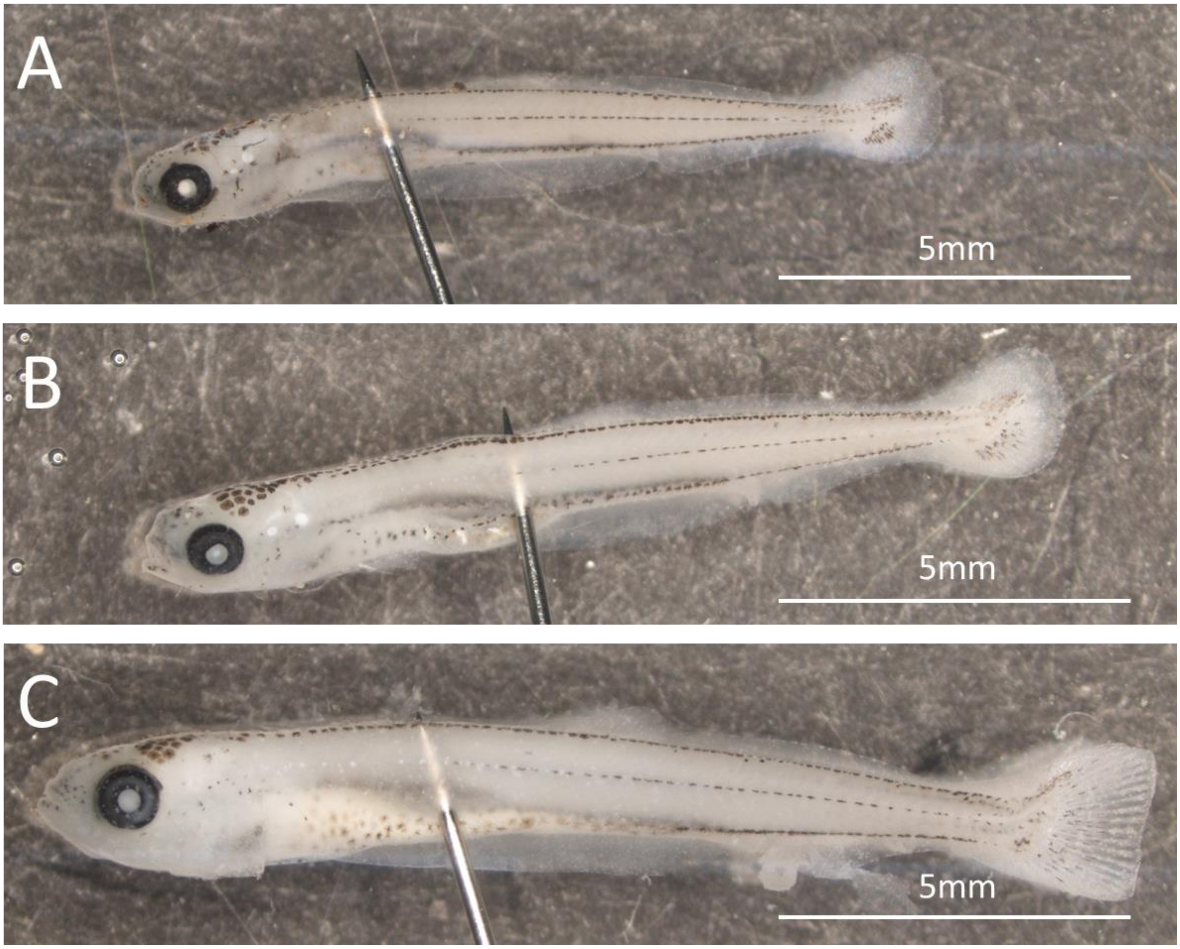


Fig. S1: Different larval stages (according to Peňáz 1974) of *Chondrostoma nasus* used in this study and hints on the most important features for their identification: (A) L2-larva (TL = 11.6 mm). Note especially the asymmetrical caudal fin and slightly up-curved notochord. (B) Typical L3-larva (TL = 13.6 mm) showing advanced growth of lepidotrichia in caudal fin nearly reaching the margin of the fin and more up-curved notochord compared to L2-larvae. (C) L4-larva (TL = 16.2 mm) characterized by completed differentiation of the (nearly homocercal) caudal fin and growth of lepidotrichia in dorsal and anal fin which begin to separate in the embryonic fin fold.

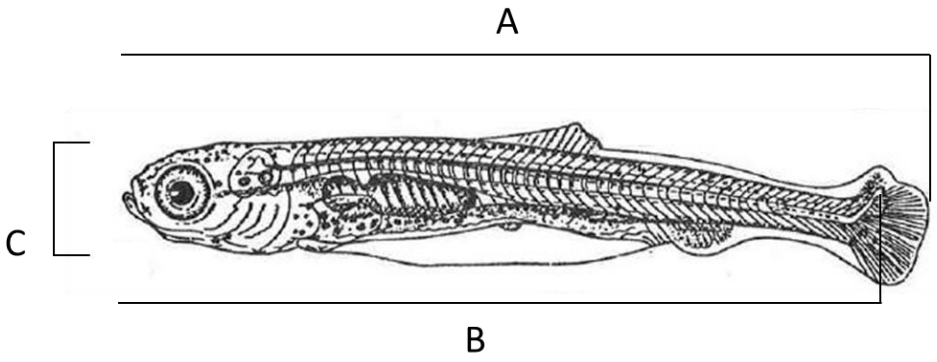


Fig. S2: Schematic representation of the morphometric measurements. (A) Total length, (B) standard length and (C) body height measured approx. one eye-diameter behind the eye. (Scheme modified from Makeyeva et al. 2011)

Supplement II

Curriculum vitae

CONTACT INFORMATION

Bernhard Zens, BSc

Esterhazygasse 20/12

1060 Vienna, Austria

Email: berni.zens@gmail.com

Mobile: +43 676 6209504

PERSONAL INFORMATION

Date of birth: 20 October 1987 (Wiener Neustadt)

Nationality: Austria

EDUCATION

WS 2011-SS2015	Master´s study of ecology at the University of Vienna, Austria.
WS2007-SS2011	Bachelor´s study of biology (major: ecology) at University of Vienna, Austria. Bachelor´s thesis: *Depths used by fish in sublittoral zones of the river Danube (Austria) during high flow conditions*. Supervisor: ao. Univ.-Prof. Dr. Hubert KECKEIS
2006-2007	compulsory community service (Austrian Red Cross)
1997-2006	Bundesrealgymnasium Gröhrmühlgasse
1993-1997	Volksschule Waldegg

JOURNAL ARTICLES

LECHNER A., KECKEIS H., LUMESBERGER-LOISL F., ZENS B., KRUSCH R., TRITTHART M., GLAS M., SCHLUDERMANN E. 2014: The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environmental Pollution* **188**: 177-181.

ZENS B., GLAS M., TRITTHART M., HUMPHRIES P., KECKEIS H., 2013: Linking behavior and hydraulics: Rheoreaction and movement patterns in larvae of a fluvial specialist. S*TAGES, Newsletter of the Early Life History Section of the American Fisheries Society* **34/2**.

PRESENTATIONS & CONGRESS CONTRIBUTIONS

2015	Poster: ZENS B., GLAS M., KECKEIS H.: Linking behavior and hydraulics: Rheoreaction and movement patterns in a fluvial specialist. Presented at 39th Annual Larval Fish Conference 2015, Vienna, Austria.
2013	Talk: ZENS B., GLAS M., TRITTHART M., HUMPHRIES P., KECKEIS H.: Linking fish behavior and hydraulics: Rheoreaction and movement patterns in fish larvae. Presented at *Science Day of the Faculty Center of Ecology 2013* University of Vienna, Austria.

WORK EXPERIENCE & SPECIFICATIONS

2012-2013	Field work at pilot study "*Witzelsdorf*" (various fishing techniques, see below)
2013	Field and laboratory work at different stages of the FWF-funded research project "P22631-B17: *Modelling dispersal patterns of fish larvae in a large river*" (including rearing of fish larvae, staining of otoliths, drift sampling, sample processing, preparation and microscopy of otoliths)
2012-2014	Tutorial at University of Vienna, Austria. Lecture: „300365 SE+UE - *Übungen zur funktionellen Ökologie - Biodiversität und Funktionalität von verbauten und unverbauten Flußabschnitten am Beispiel der Wien*" (including electrofishing and data analysis)
2006	Employment at the Austrian Red Cross (rescue and ambulance service, driver)

SKILLS AND QUALIFICATIONS

Languages

German (first language)

English (proficient in speech and writing)

Computer literacy

MS Office, statistics (R, SPSS, sigma plot), graphics (sigma plot), video editing (Adobe Premiere Pro 5), programming and modelling (NetLogo)

Additional qualifications

Driving license (classes A & B, motorboat up to 10 m on lakes, rivers and waterways)

Knowledge and extensive field experience in various fishing techniques applied for rivers and lakes including boat- and shore-based electrofishing, wading electrofishing, long-line fishing, drift sampling and gill netting

Detailed knowledge on and experience in identification of native and non-native fish species in Central Europe

Workshop on the assessment of fish communities in running waters according to quality standards of EU Water Framework Directive: “Methodikkurs 2013 – Qualitätselement Fische. Beprobung sowie ökologische Beurteilung von Fließgewässern mittels Fischbeständen gemäß Arbeitsanweisung des BMLFUW im Rahmen der Gewässerzustandsüberwachung in Österreich 2013-2015”

Mapping of breeding birds according to quality standards of “Methodenstandards zur Erfassung der Brutvögel Deutschlands, P. Südbeck, Max-Planck-Inst. für Ornithologie, Vogelwarte Radolfzell, 2005”