



Egg transport and larval behavior of curimba, *Prochilodus lineatus* (Valenciennes, 1836; Characiformes, Prochilodontidae) in a drift simulator channel

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ABSTRACT. Landscape changes and hydrologic alterations associated with dam construction have greatly hindered reproductive success and the recruitment of migratory species. However, little is known about the flow conditions that are necessary for the reproductive success of migrators. We aimed to analyze one of the important migrants of the Paraná River, the curimba, *Prochilodus lineatus* (Valenciennes, 1836), to determine the minimum velocities necessary for egg drift and analyze the behavior of larvae at different flow velocities. Thus, we experimentally evaluated the behavior of eggs and larvae released in a drift simulator channel (DSC) under four flow velocities. Additionally, we investigate the swimming ability of larvae in relation to certain morphometric measures of their bodies. We also performed morphometry and calculations of larval body and fin proportions to determine which structures influence swimming ability. The results indicated that environments with flow velocities greater than 0.081 m s^{-1} were favorable for egg drift and increased the chances of successful transport of eggs to downstream areas. We also observed that soon after hatching, the larvae positioned themselves against the flow, indicating that they had a perception of position and orientation. The larvae were able to maintain and disperse against the flow within the channel 32h after hatching at all velocities, which was mainly due to the development of pectoral and caudal fins that were actively involved in the swimming process, as well as the reduction in the dimensions of the yolk sac, which reduced drag.

Keywords: drifting; fish eggs and larvae; flow velocity; ichthyoplankton.

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Introduction

Studies on the drift of eggs and larvae generate important information, such as the minimum flow required for transport from spawning to development areas, and the effects of hydrological characteristics (speed and direction of flow) on the dynamics of ichthyoplankton assemblages (Pavlov & Mikheev, 2017). Thus, it is important to not only understand how the transport of eggs and larvae is influenced by biological characteristics, such as growth rate, egg diameter and density, and larval swimming potential, but also by hydrodynamics and environmental characteristics of the water body (Garcia, Murphy, Valocchi, & Garcia, 2013).

Knowledge of the behavior and associated movement patterns that determine the trajectory, drift velocity, and destination of fish larvae drift in rivers and their relationship with larval morphology is critical (Pavlov, Mikheev, Lupandin, & Skorobogatov, 2008; Lechner, Keckeis, & Humphries, 2016). The patterns of transport of fish larvae in rivers and other types of moving water must be understood, modeled, or predicted (Lechner et al., 2016), since this knowledge along with a greater understanding of population dynamics are key factors for species conservation, fisheries management, and restoration ecology.

Researchers have been interested in the relationship between fish and water flow for centuries (Lacey, Neary, Liao, Enders, & Tritico, 2012), because movement is a fundamental process in the lives of the majority of organisms (Nathan et al., 2008). The drift of eggs and larvae from spawning sites to initial development

habitats, such as floodplains and lowland areas (Barros & Rosman, 2018), affects the survival and fitness of an individual, and the probability of completing its life cycle (Daewel, Peck, & Schrum, 2011; Hinrichsen, Hüsey, & Huwer, 2012).

The predominance of hydroelectric power in the energy matrix of Brazil and the recent increase in its demand have resulted in the damming of most major hydrographic basins in the country (Sanches et al., 2020). As changes in freshwater ecosystems caused by dams are related to river hydrodynamics, studies on drift can be useful to determine the minimum speeds and distances necessary for eggs and larvae to be transported in the river stretches of reservoirs. Owing to the reduction in flow velocity with the formation of reservoirs (Agostinho, Gomes, & Pelicice, 2007; Pavlov & Mikheev, 2017), eggs of species not adapted to lentic environments, such as migratory species, will likely descend to the deep regions of the reservoir, where there is a minimal chance of survival (Agostinho et al., 2007).

Although studies using drift simulators for flow peak analysis (Bradford, 1997; Halleraker et al., 2003) and flood pulses (Chun et al., 2011; Cocherell et al., 2012) are common in the Northern Hemisphere, no such studies have been conducted in the Neotropical region (particularly Brazil). Therefore, assuming that the life cycle of migratory fish species depends on the characteristics and flow velocities of the rivers, this study aimed to evaluate the transport of eggs and behavior of *P. lineatus* larvae at different flow speeds in a drift simulator channel. In this study, we aimed to answer the following questions: i) what is the minimum flow speed necessary for eggs to be transported, ii) how will *P. lineatus* larvae behave in relation to the different flow velocities, iii) how long after hatching do the larvae actively swim (positive rheotaxis), and iv) which morphological structures most influence larvae swimming?

Materials and methods

Species characteristics, structure, functioning of the drift simulator channel, and experiment

Prochilodus lineatus, commonly known as curimba, is a long migratory species (Baumgartner, Oliveira, Agostinho, & Gomes, 2018; Ota, Deprá, Graça, & Pavanelli, 2018) that needs to annually migrate more than 100 km upstream to complete its gonadal development and spawning processes (Baumgartner et al., 2018). Native to the Paraná River (Ota et al., 2018), this species reaches 78 cm in length and is of great importance to professional fishing (Agostinho, Gomes, Suzuki, & Júlio, 2003). *Prochilodus lineatus* depends on floods for the success of their reproduction and recruitment, and their abundance has been significantly affected by the damming processes (Piana et al., 2017).

Prochilodus lineatus undergoes external fertilization with no parental care for its offspring. Its eggs are translucent and pelagic, with ample perivitelline space (Nakatani et al., 2001), and an accumulation of yolk in their vegetal pole (telolecitic egg) (Botta et al., 2010). The larvae hatch approximately 16 hours after fertilization at 26°C (Nakatani et al., 2001).

The experiment was conducted in a drift simulator channel (DSC) installed at the Environmental Aquaculture Research Institute (InPAA) linked to the *Universidade Estadual do Oeste do Paraná*, Toledo Campus, Paraná, Brazil. The dimensions and structure of the DSC system are shown in Figure 1. The DSC consists of a closed circulation system, whereby the water stored in a reservoir is released through a centrifugal pump into the channel via piping and, after entering the system, it is launched through a 'flow disperser' that reduces the turbulence generated by the water flow. Water then flows through the system and returns to the reservoir, restarting the cycle. The DSC is constructed of steel, with an 8 mm-thick transparent tempered glass front panel for viewing the organisms, and a metric scale to determine the transport distance of the organisms on its rear panel.

The DSC piping consists of a set of polyvinyl chloride (PVC) pipes that connect the reservoir to the pump, pump to the reservoir, and pump to the flow dispenser inside the channel. The pump is a 5 HP centrifugal pump that pushes water into the channel. The return pipe and pipe connecting the pump to the channel are equipped with registers that allow changes to determine and control the flow velocities by adjusting the degrees of opening and closing between them.

The flow velocities were determined by filling a container positioned at the water outlet of the DSC with a known volume and measuring the time taken for filling, and using the following equation: $Q = V \cdot t^{-1}$, where Q is the volumetric flow, V is the volume (L), and t is time (s). This procedure was conducted with ten repetitions for each speed, and the average values were calculated. The units of $L \cdot s^{-1}$ were transformed into $m^3 \cdot s^{-1}$. We then used the equation: $Q = V \cdot A$, where Q is the flow obtained in $m^3 \cdot s^{-1}$, and A is the cross-sectional

area of the DSC in m^2 . The final four speeds obtained were 0.032, 0.044, 0.068, and 0.081 m s^{-1} . These velocities were selected based on the approximate values, which are lower than those observed for the tributaries that forming the arms of the Itaipu Reservoir (Ouchi-Melo et al., 2021).

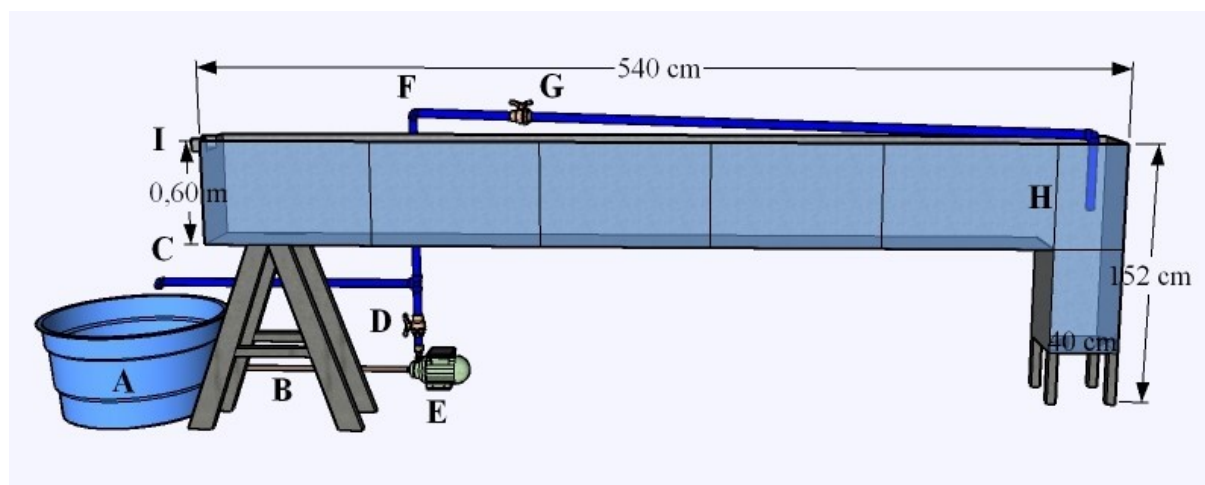


Figure 1. Diagram of the frontal view, dimensions (cm), and structural components of the drift simulator channel (DSC). A) Reservoir; B) connection pipe between the reservoir and centrifugal pump; C) return pipe from the centrifugal pump to the reservoir; D) record of the flow control of the return pipe; E) centrifugal pump; F) water inlet pipe in the DSC; G) flow control record of the inlet pipe; H) flow disperser; I) DSC water outlet.

Development of the experiment

The eggs and larvae used in the experiment were obtained from induced spawning at the InPAA and placed in a 200 L conical-cylindrical fiberglass incubator with constant water circulation and an average temperature of $21.6 \pm 0.3^\circ\text{C}$ until the end of the experiment. For the observations, the organisms (both eggs and larvae) were released into the channel 1 m from the outlet of the flow disperser to avoid turbulence of the water entering the system from influencing the organisms.

For the experiment, ten organisms at each developmental stage (replicates) were used to test each of the four flow velocities. Each egg or larva was individually released into the channel, the horizontal displacement distance and time were recorded, and larval swimming behavior was observed. The eggs were stained with methylene blue to allow visualization inside the channel, and were subjected to tests every 4 hours from fertilization to the phase before hatching. After hatching, the larvae were evaluated every 8 hours until they reached 48 hours of development, followed by 12 hours intervals until the end of the experiment. The sampling times were determined based on previous information on the development of the species (Nakatani et al., 2001; Botta et al., 2010), considering the occurrence of ontogenetic events of embryonic development (initial cleavage stages, early embryo, free tail, and late embryo) and early larval development (hatching, endogenous feeding, and early exogenous feeding). As our objective was to evaluate the swimming behavior of the initial stages of larval development, we only used larvae in the larval vitelline and pre-flexion larval stages, with the classification of these stages according to Nakatani et al. (2001).

For the larvae, only the time spent in the water column was considered, and their behavior was observed until they touched the bottom or side of the channel. For analysis, the time of egg development was grouped into hours post-fertilization, with 0 being freshly fertilized and 24 as pre-hatch. The larvae were grouped based on the number of hours after hatching, with 0 representing newly hatched larvae. The drift velocity of organisms was calculated using the following equation: $V = d \cdot t^{-1}$, where V is the volume (L), d is the horizontal displacement distance (m), and t is displacement time (s).

Before each experiment in the DSC, a sample containing around 20 organisms (both eggs and larvae) from each developmental time were fixed in 4% formaldehyde, and morphometric measurements (expressed in millimeters) were obtained from images captured with a digital camera (OLYMPUS, model DP-25) coupled to a stereoscopic microscope (OLYMPUS, model SZX7) using the DP2-BSW software. Based on the morphometric measurements, the morphometric indices of Gatz Jr.(1979), Watson and Balon (1984), Ferreira (2007), and Andrade et al. (2014) were adapted. These included the relative pectoral fin length (RPFL), pectoral fin length divided by standard length; pectoral fin proportion (PFP), pectoral fin length divided by pectoral fin height; relative caudal fin length (RCFL), caudal fin length divided by standard length; caudal fin proportion (CFP),

caudal fin height divided by square root of caudal fin area; relative body height (RBH), body height divided by standard length; and yolk sac proportion (YSP), yolk sac area divided by body height.

Data analysis

The experiment followed a factorial-type design with two factors (water flow and developmental hours). The results were analyzed using repeated-measures ANOVA tests. To check the assumptions of the analysis, Mauchly's test of sphericity was applied, and if violations were found in the assumed sphericity, Greenhouse-Geisser correction was performed to obtain the valid critical F-value. When a significant difference was observed, the data were subjected to Tukey's test to identify the factor level that differed. When the assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Levene test) were not met, the corresponding nonparametric test (Kruskal-Wallis) was conducted, followed by a median test. A significance level of 5% ($p < 0.05$) was set for all analyses. The effects of the interactions between the development times (h) and flow velocities (m s^{-1}) for eggs and larvae within the DSC were also analyzed. The morphometric indices were analyzed using principal component analysis (PCA) to assess the possible relationships between the speed of larval displacement in the DSC and the development of these structures at the highest flow speed. The analysis was conducted using Statistica® 7.0 (Statsoft, 2004).

Results

During the experiment, a significant difference between the flow velocities was only observed under the highest flow velocity analyzed, 0.081 m s^{-1} (Figure 2).

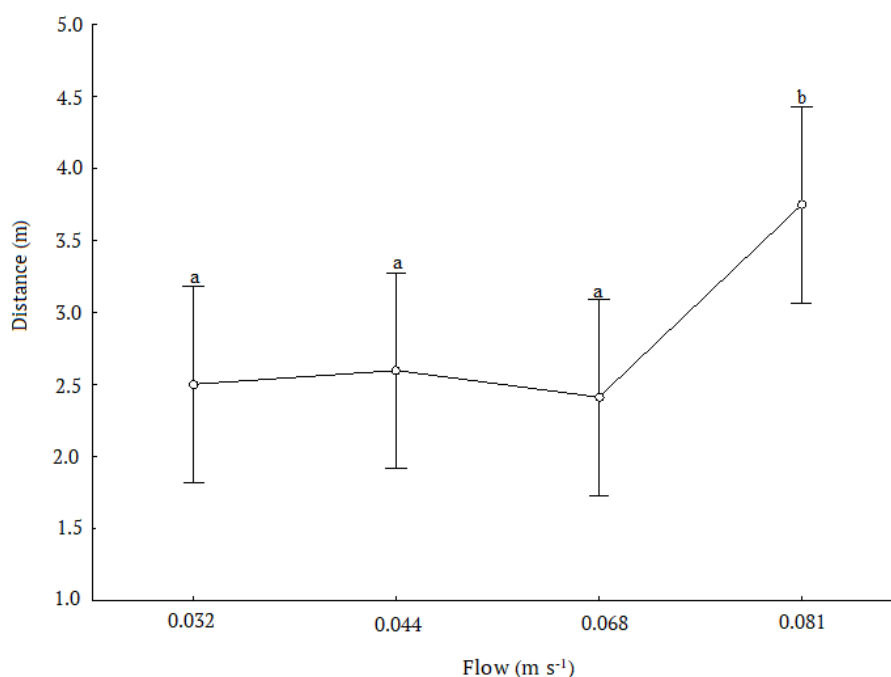


Figure 2. Transport distance of *Prochilodus lineatus* eggs within the drift channel at the four tested flow rates (different letters represent significant differences between the means at 5% according to the Tukey test).

At the lowest speeds evaluated, the percentage of eggs transported along the entire length of the drift channel was below 40%; however, at the highest speed, this percentage exceeded 60%, and at the 4, 16, and 20 hours development times, all released eggs were transported along the entire canal (Figure 3a). Generally, the transport pattern at the lowest speeds (0.032 and 0.044 m s^{-1}) was similar to the reduction in drift speeds throughout development, particularly from the eighth hour after fertilization. At these speeds, a higher oscillation was observed between egg transport speeds throughout the development period (Figure 3b). There was a significant difference between the highest speed of flow and the other velocities, although a reduction in the transport speed under the 0.081 m s^{-1} flow rate was also observed when the eggs were in the final stages of development.

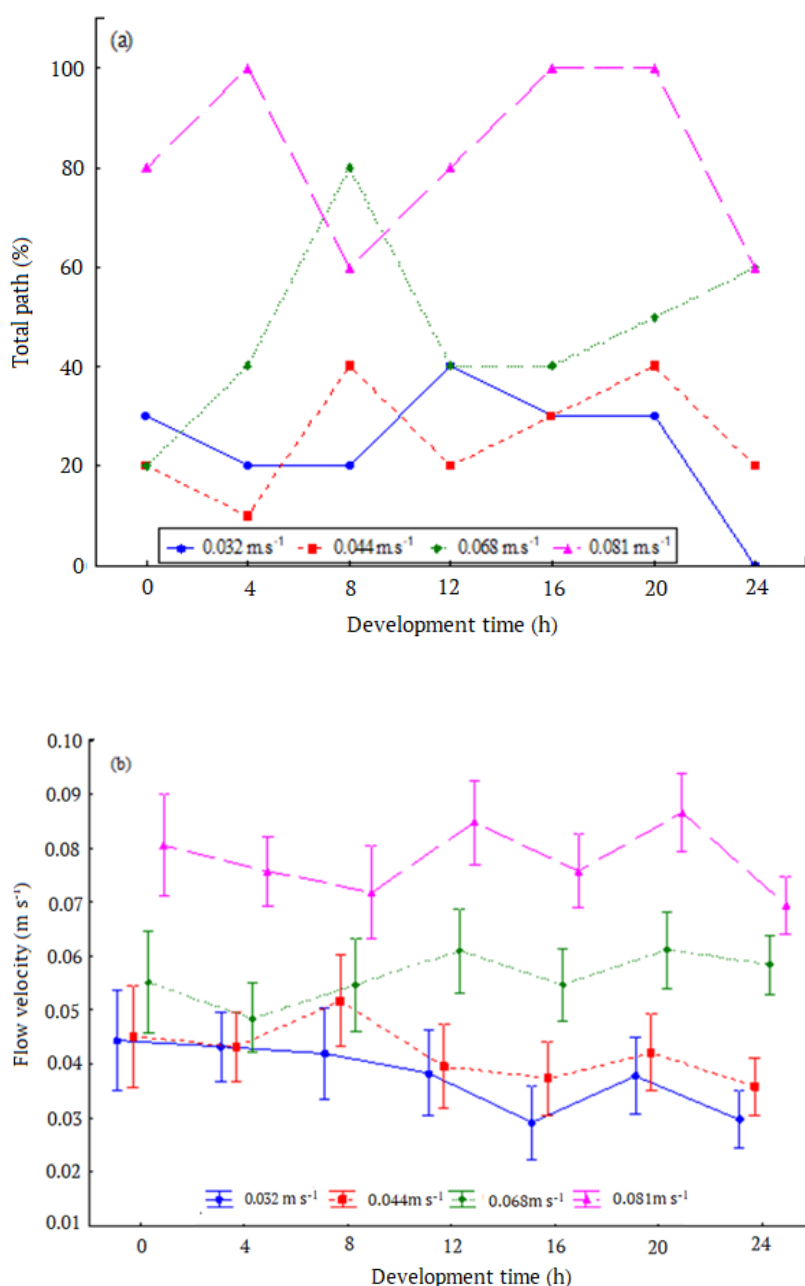


Figure 3. Percentage of transport along the total path (a) and interaction between the flow velocities (m s⁻¹) and egg development times (b) of *Prochilodus lineatus* for the four flow rates tested in the DSC (ANOVA, $p < 0.05$).

In the first stages after hatching (up to 40 hours), the larvae were either completely transported at the highest flow speeds, while there was a significant difference at the highest speed (0.081 m s⁻¹), or they swam in favor of the direction of flow (negative rheotaxis). From 48 to 110 hours after hatching, a drift pattern was observed between the different flow rates and development times. An irregular pattern in the transport speed was then recorded between the flow speeds (Figure 4).

Regarding the behavior of the larvae during the experiment, the newly hatched larvae (larval vitelline stage) were oriented against the flow at all evaluated speeds; however, they searched for the areas of lower flow velocity within the channel (bottom and side), where it was possible to reduce their swimming speed against the direction of flow. Forty-eight hours after development (early pre-flexion stage), active swimming began to occur along with pectoral fin movement, and the larvae remained close to the surface, the canal walls, or even swam against the flow (positive rheotaxis), which became more evident throughout the development time. In the final developmental stages, at 134 and 146 hours (late pre-flexion stage), all larvae exercised active swimming movements against and in favor of the flow direction, traveling up and down in the water column and beyond the observation area of the DS channel.

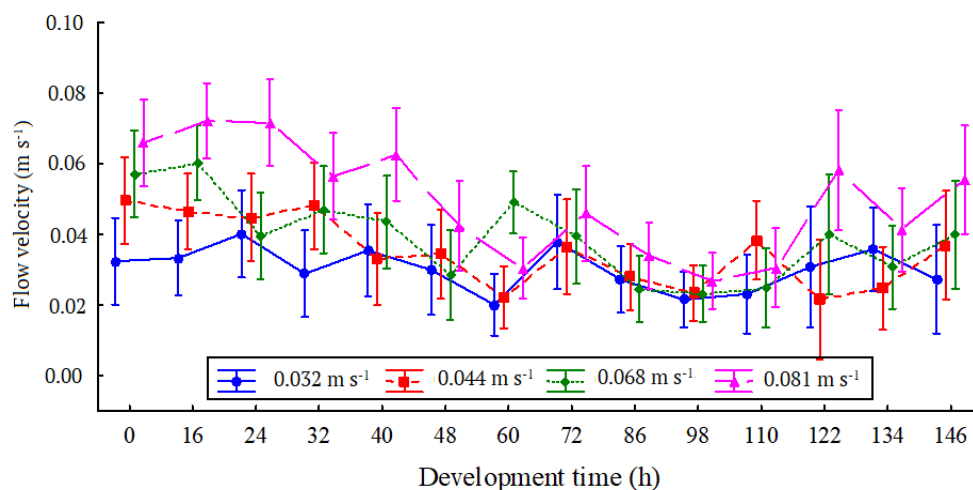


Figure 4. Interactions between the flow velocities (m s^{-1}) and development times of *Prochilodus lineatus* larvae for the four flow rates tested in the drift simulator channel.

The morphometric indices were analyzed using PCA, which summarized 69.43% of the data variability, 41.15% of which was contained in the first axis (PCA 1) and 28.28% in the second axis (PCA 2). The results of PCA applied to the morphological data and larval displacement velocity indicated the formation of two groups (Figure 5a). The first group consisted of organisms with 0, 16, and 24 hours of development time, which were positively associated with the PCA 1 axis. The second group included animals belonging to other development times (32, 48, 86, 98, 134, and 146 hours), which were negatively associated with the PCA 1 axis. By analyzing the first axis of PCA, it was observed that the yolk sac proportion (YSP) and larval drift velocity (LDV) were more strongly positively associated with PCA 1, while the pectoral fin proportion (PFP) and caudal fin portion (CFP) were more strongly negatively associated with PCA 1 (Figure 5b).

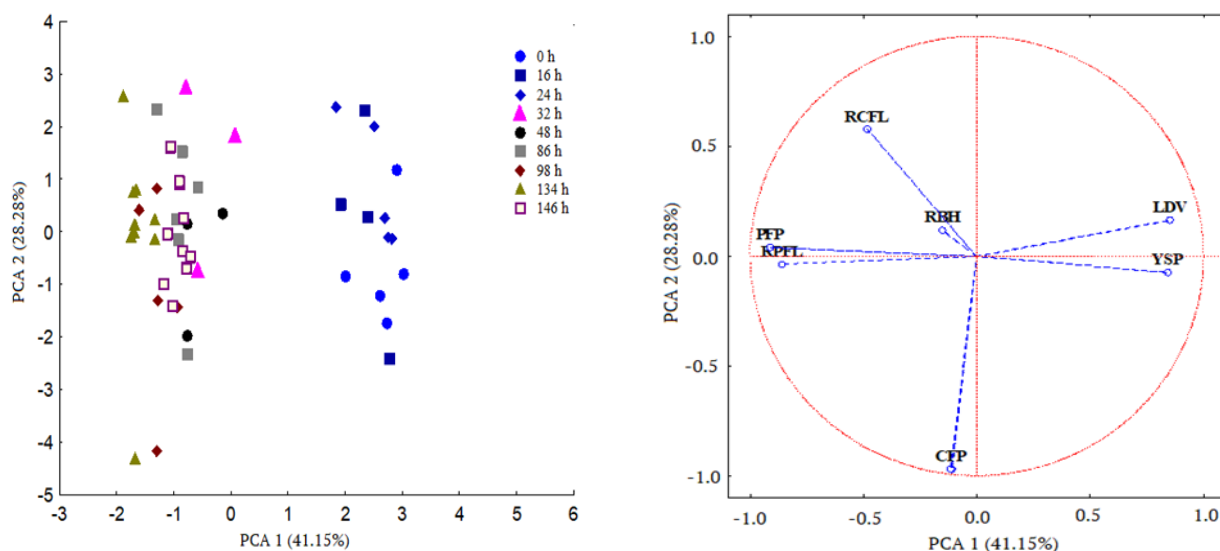


Figure 5. Spatial projection of the larvae sample scores for each development time (a) and vectors of the morphometric indexes and displacement speed (b) in the two axes of the PCA under a flow speed 0.081 m s^{-1} in the DSC. RPFL - relative pectoral fin length; PFP - pectoral fin proportion; RCFL - relative caudal fin length; CFP - caudal fin proportion; RBH - relative body height; YSP - yolk sac proportion and LDV - larval drift velocity.

Therefore, from the analysis, we observed that PFP and CFP were inversely associated with LDV, indicating that after 32 hours of development, these fins began to influence the larvae by providing greater autonomy and swimming capacity. The yolk sac showed a positive association with larval drift, indicating that larvae with larger yolk sacs presented greater buoyancy and consequently higher drift velocities.

Discussion

The transport of eggs during the experiment was proportional to the increase in flow, with only small vertical oscillations possibly caused by turbulence within the channel. At the highest speed, the eggs mainly floated close to the surface, whereas at the lowest speed, although the eggs that immediately entered the flow showed fluctuations, the flow was insufficient to transport them along the entire course of the channel. This relates to the pelagic characteristics of the eggs of this species, which in addition apart to influencing buoyancy and shape, cause them to drift passively (Widmer, Fluder, Kehmeier, Medley, & Valdez, 2012). Eggs of migratory species do not have adaptations that guarantee their buoyancy, such as oil drops in species that spawn in lentic environments (sedentary species), and therefore need adequate flow speeds for transportation (Nakatani et al., 2001; Agostinho et al., 2007; Sanches et al., 2020). Thus, if the eggs of migratory species drift to areas where there is no minimum speed to guarantee their buoyancy and transport, they generally drift to the bottom, where no adequate conditions would exist for hatching (Agostinho et al., 2007). The reduction in egg transport during the most advanced development times can be attributed to the increase in the density of eggs in the final stages of development, which decreases their buoyancy (Petereit, Hinrichsen, Franke, & Köster, 2014) and transport by the flow. These characteristics were confirmed mainly in the lower flow rates, when lower percentages of eggs were transported along the total DSC (total path), indicating that egg transport would be minimal at these speeds. Therefore, if an environment had a slow flow rate, the eggs would not drift to the development areas (Worthington, Brewer, Farless, Grabowski, & Gregory, 2014). At the highest flow speed, the longest drift distances and percentage of eggs transported across the entire channel length were observed, demonstrating that speeds above 0.081 m s^{-1} were efficient in transporting *P. lineatus* eggs at all stages of embryonic development to downstream areas. These results corroborate the study by Murphy and Jackson (2013), who determined that eggs of grass carp would settle at mean current velocities of flow lower than 0.081 m s^{-1} . Thus, at flow velocities below 0.081 m s^{-1} , such as those recorded in the arms of the Itaipu HPP reservoir, which served as the basis for determining the flow velocities used in this study (0.0 to 0.050 m s^{-1}), eggs would not be transported to the lower reaches and would tend to settle to the bottom where the conditions for their development are not favorable (Agostinho et al., 2007).

Soon after hatching (approximately 4 hours after hatching or the larval vitelline stage), the larvae had attempted to position themselves against the flow within the channel, indicating that they could perceive their position and orientation, despite their low level of development. This orientation occurs because the larvae create a flow field when swimming that generates an impulse that reaches the lateral line receptors on the body surface (Feitl, Ngo, & McHenry, 2010). Even fish larvae without completely developed fins can generate powerful edge vortices during undulatory swimming that enhance thrust only with oscillating appendages (paired fins and tail fins), fin folds, and body undulations (Li, Müller, Leeuwen, & Liu, 2016).

It is clear that the swimming performance and morphology of larvae are related, as the performance depends on the drag and propulsion force that the organism will develop (Videler, 1993); however, there are other factors related to the development and swimming ability of the larvae, such as eye development, (and other sensory organs) because both swimming ability and visual acuity increase with size and are more limited in the early stages (Flore, Reckendorfer, & Keckeis, 2000). For the *P. lineatus* larvae used in this study, eye pigmentation started soon after hatching and was completed at the beginning of the pre-flexion stage.

The ability of newly hatched larvae to orient themselves is a major factor for their survival, as they need to develop the ability to swim almost immediately after hatching to find food before they have completely exhausted their vitelline reserves (Voesenek, Muijres, & Leeuwen, 2018). Newly hatched larvae have limited yolk supplies and need to feed before the reserve runs out (Hunter, 1981). Starvation is the result of the inability of the larvae to find sufficient food and was originally suggested to be the main cause of mortality during this 'critical period' of mass mortality (China, Levy, Liberzon, Elmaliach, & Holzman, 2017). In addition, the relationship between swimming ability and vision development is important for fleeing predators. Compared to sound or touch, visual responses result in more escape trajectories directed away from the stimulus (Voesenek et al., 2018). Swimming also supports breathing, renews the diffusive limit layer of gases and ions (Green, Ho, & Hale, 2011), and aids in maintaining and controlling the body's orientation (Ehrlich & Schoppik, 2017).

In the early stages of development, *P. lineatus* larvae were transported throughout the channel or followed the flow direction (negative rheotaxis). In the first few hours after hatching (approximately 4 hours), the caudal beats were accelerated, but were insufficient to achieve active swimming. Many characteristics are important for defining the swimming capacity of the larvae, such as the proportion and aspect of the fins

(Sambilay Jr., 1990; Fisher, Bellwood, & Job, 2000), length of the caudal fin (Nanami, 2007), and development of the dorsal and anal fins (Drucker & Lauder, 2005; Standen & Lauder, 2005; 2007). However, fins and supporting elements of the caudal fin (ural bones) are not present during the early stages of development, as observed in this study (pre-flexion stage).

Caudal beats decrease with larval growth due to muscle development (Voosenek et al., 2018) and changes in shape. From 48h after hatching, the larvae engaged in active swimming concomitantly with the movements of the pectoral fins, which typically appear soon after hatching in teleosts (Blaxter, 1988; Osse & Boogaart, 1999) and play important roles in controlling body position and propelling them during steady and unsteady swimming behaviors (Drucker & Lauder 2002). It should be noted that variations in pectoral fin shape in damselfishes are highly related to their behavior (Aguilar-Medrano, Frédérich, Balart, & de Luna, 2013).

Fin morphology strongly affects the swimming and kinematic capacity of the body (Muller & Leeuwen, 2004; Muller, Boogaart, & Leeuwen, 2008; Hale, 2014). Typically, even fins (pectoral and pelvic) are more related to stability and swimming direction (Casatti & Castro, 2006). However, Aguilar-Medrano et al. (2013) reported that, in addition to aiding in controlling body position, fish swimming at high speeds using the pectoral fin in a flapping motion tend to have elongated fins for propulsion during steady and unsteady swimming behaviors. This was evidenced by the ordering of the components obtained by PCA, where the PFP, RPFL, and YSP indicated that the pectoral fins and yolk sac dimensions were the major factors influencing the larval dispersal process.

The results of the analysis and visual observation of the larvae inside the DSC indicated a decrease in drift and an increase in swimming power associated with the development of pectoral fins. The opposite was true for the yolk sac, as a larger yolk sac (in the first hours of development) corresponded to a lower swimming power and a greater area of contact with the water, causing greater drag with the flow. This also related to an increase in buoyancy of the larvae, despite the reduction in swimming power. Thus, with a larger yolk sac, the likelihood of passive drift increases, and these individuals are redistributed according to their body shape and buoyancy (Copp, Faulkner, Doherty, Watkins, & Majecki, 2002).

During the experiment, the larvae searched for the regions with the lowest flow close to the bottom or walls of the simulator channel from the first hours after hatching. This behavior may be related to the need to save energy, since without a properly formed caudal fin, most larvae use high amplitude body undulations to swim, which can be energetically expensive (Downie, Illing, Faria, & Rummer, 2020), and can reach 80% of a larval fish's energy budget in the Atlantic cod (*Gadus morhua*) (Ruzicka & Gallagher, 2006).

This behavior has also been observed in natural environments, where larvae generally seek to float on the margins and not in the center of the stream (Reichard & Jurajda, 2004; Kynard, Parker, Kynard, & Parker, 2007), allowing them to maintain their position without being swept by the flow. Some species of larvae are able to determine their position in the water column (Schludermann, Tritthart, Humphries, & Keckeis, 2012), even at low levels of light and currents by responding to hydraulic gradients. This was observed in studies with zebrafish larvae that were guided by neuromasts of the lateral line, allowing them to sense water flows and orient themselves in currents (Olszewski, Haehnel, Taguchi, & Liao, 2012; Stewart, Cardenas, & McHenry, 2013). This behavior indicates that the larvae are rheotactic and can manage this perception by locating the lowest speeds at which they can remain at a lower energy cost and avoid stress (Pavlov et al., 2008).

Other studies have also found that larvae exert active movement in flow situations and do not behave as passive particles (Robinson, Clarkson, & Forrest, 1998; Schludermann et al., 2012; Lechner et al., 2017; Zens, Glas, Tritthart, Habersack, & Keckeis, 2017). Owing to the verification of the existence of active movements in the dispersion of the larvae, Lechner et al. (2016) proposed a new concept of drift for larvae, referred to as "active-passive", as there would not be adequate separation between active and passive drift because of the variability in larval behavior and movement patterns, and correlations between drift densities and discharge and flow velocity are common (Lechner et al., 2013), as observed in this study.

Marine species have been more commonly studied than freshwater species in terms of flow. Staaterman, Paris, and Helgers (2012), and Mouritsen, Atema, Kingsford, and Gerlach (2013), stated that fish larvae do not undergo passive transport and can detect signals from the environment and exhibit sophisticated behavior, such as directional swimming, which is important for effective dispersion. Observations of larvae moving in hydraulic gradients (Stoll & Beeck, 2012), regulating swimming speeds and drifting to the predominant flow regime (Hogan & Mora, 2005), and adapting their depth distribution according to the currents (Kunze, Morgan, & Lwiza, 2013) also indicate active responses to the hydrodynamic environment.

The results of this study revealed that *P. lineatus* larvae exhibited active behavior during the first hours of development (approximately 4 hours after hatching in the larval vitelline stage), which was indicated by the decrease in larval drift in the final stages of larval development and swimming behavior. They also searched for areas with low flow within the simulator channel. Therefore, larval movement should be considered when attempting to understand and model patterns of fish larval dispersion in rivers, as the assumption of only passive transport is generally inaccurate.

Conclusion

Based on the data obtained in this study, we can conclude that environments with flow velocities greater than 0.081 m s^{-1} are favorable to the drift of *P. lineatus* eggs, which may extend to other species of migratory fish because these speeds provide greater probabilities of success in transporting the eggs to downstream areas. We can also conclude that the larvae exhibited active behavior during the first few hours of development, similar to that presented in a natural environment. The larvae were able to maintain themselves and disperse against the flow within the channel 48 hours after hatching (early pre-flexion stage), mainly because of the development of pectoral and caudal fins that are actively involved in the swimming process, as well as a reduction in the dimensions of the yolk sac, allowing for the reduction of drag through the flow.

Studies on the influence of flow velocity on the transport and behavior of ichthyoplankton are scarce, and further research should be conducted in this area to gain a greater understanding of the processes involved in the transport and survival of eggs and larvae, which can contribute to future conservation measures.

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References

- Aguilar-Medrano, R., Frédérich, B., Balart, E. F., & Luna, E. (2013) Diversification of the pectoral fin shape in damselfishes (Perciformes, Pomacentridae) of the Eastern Pacific. *Zoomorphology*, 132(2), 197-213.
- Agostinho, A. A., Gomes, L. C., Suzuki, H. I., & Júlio, H. F. (2003) Migratory fish from the upper Paraná River basin, Brazil. In J. Carolsfeld, B. Harvey, C. Ross, A. Baer, & C. Ross (Eds.), *Migratory fishes of South America: biology, social importance and conservation status* (p. 19-99). Victoria, CA: World Fisheries Trust, the World Bank and the International Development Research Centre.
- Agostinho, A. A., Gomes, L. C., & Pelicice, F. M. (2007). *Ecologia e manejo de recursos pesqueiros em reservatórios do Brasil*. Maringá, PR: Eduem.
- Andrade, F. F., Makrakis, M. C., Lima, A. F. D., Assumpção, L. D., Makrakis, S., & Pini, S. F. (2014). Desenvolvimento embrionário, larval e juvenil de *Hemisorubim platyrhynchos* (Siluriformes, Pimelodidae) da bacia do rio Paraná. *Iheringia. Série Zoologia*, 104(1), 70-80. DOI: <http://dx.doi.org/10.1590/1678-4766201410417080>
- Barros, M. D. L. C., & Rosman, P. C. C. (2018). A study on fish eggs and larvae drifting in the Jirau reservoir, Brazilian Amazon. *Journal of the Brazilian Society of Mechanical Sciences and Engineering*, 40(2), 1-16. DOI: <http://dx.doi.org/10.1007/s40430-017-0951-1>
- Baumgartner, M. T., Oliveira, A. G., Agostinho, A. A., & Gomes, L. C. (2018). Fish functional diversity responses following flood pulses in the upper Paraná River floodplain. *Ecology of Freshwater Fish*, 27(4), 910-919. DOI: <https://doi.org/10.1111/eff.12402>
- Blaxter, J. H. S. (1988). Pattern and variety in development. In W. S. Hoar, & D. J. Randall (Eds.), *Fish physiology* (Vol. XIA, p. 1-58). New York, NY: Academic Press.
- Botta, P., Sciara, A., Arranz, S., Murgas, L. D. S., Pereira, G. J. M., & Oberlender, G. (2010). Estudio del desarrollo embrionario del sábalo (*Prochilodus lineatus*). *Archivos de Medicina Veterinaria*, 42(2), 109-114.
- Bradford, M. J. (1997). An experimental study of stranding of juvenile salmonids on gravel bars and in side channels during rapid flow decreases. *Regulated Rivers: Research & Management*, 13(5), 395-401. DOI: [https://doi.org/10.1002/\(SICI\)1099-1646\(199709/10\)13:5<395::AID-RRR464>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1099-1646(199709/10)13:5<395::AID-RRR464>3.0.CO;2-L)

- Casatti, L., & Castro, R. M. C. (2006). Testing the ecomorphological hypothesis in a headwater riffles fish assemblage of the rio São Francisco, southeastern Brazil. *Neotropical Ichthyology*, 4(2), 203-214. DOI: <https://doi.org/10.1590/S1679-62252006000200006>
- China, V., Levy, L., Liberzon, A., Elmaliach, T., & Holzman, R. (2017). Hydrodynamic regime determines the feeding success of larval fish through the modulation of strike kinematics. *Proceedings of the Royal Society B: Biological Sciences*, 284(1853), 20170235. DOI: <http://dx.doi.org/10.1098/rspb.2017.0235>
- Chun, S. N., Cocherell, S. A., Cocherell, D. E., Miranda, J. B., Jones, G. J., Graham, J., & Cech, J. J. (2011). Displacement, velocity preference, and substrate use of three native California stream fishes in simulated pulsed flows. *Environmental Biology of Fishes*, 90(1), 43-52. DOI: <https://doi.org/10.1007/s10641-010-9716-8>
- Cocherell, S. A., Chun, S. N., Cocherell, D. E., Thompson, L. C., Klimley, A. P., & Cech, J. J. (2012). A lateral-displacement flume for fish behavior and stranding studies during simulated pulsed flows. *Environmental Biology of Fishes*, 93(1), 143-150.
- Copp, G. H., Faulkner, H., Doherty, S., Watkins, M. S., & Majecki, J. (2002). Diel drift behaviour of fish eggs and larvae, in particular barbel, *Barbus barbus* (L.), in an English chalk stream. *Fisheries Management and Ecology*, 9(2), 95-103. DOI: <https://doi.org/10.1046/j.1365-2400.2002.00286.x>
- Daewel, U., Peck, M. A., & Schrum, C. (2011). Life history strategy and impacts of environmental variability on early life stages of two marine fishes in the North Sea: an individual-based modelling approach. *Canadian Journal of Fisheries and Aquatic Sciences*, 68(3), 426-443. DOI: <https://doi.org/10.1139/F10-164>
- Downie, A. T., Illing, B., Faria, A. M., & Rummer, J. L. (2020). Swimming performance of marine fish larvae: review of a universal trait under ecological and environmental pressure. *Reviews in Fish Biology and Fisheries*, 30(1), 93-108. DOI: <https://doi.org/10.1007/s11160-019-09592-w>
- Drucker E. G., Lauder G. V. (2002) Wake dynamics and locomotor function in fishes: interpreting evolutionary patterns in pectoral fin design. *Integrative and Comparative Biology*. 42(5), 997-1008. DOI: <https://doi.org/10.1093/icb/42.5.997>
- Drucker, E. G., & Lauder, G. V. (2005). Locomotor function of the dorsal fin in rainbow trout: kinematic patterns and hydrodynamic forces. *Journal of Experimental Biology*, 208(23), 4479-4494. DOI: <https://doi.org/10.1242/jeb.01922>
- Ehrlich, D. E., & Schoppik, D. (2017). Control of movement initiation underlies the development of balance. *Current Biology*, 27(3), 334-344. DOI: <https://doi.org/10.1016/j.cub.2016.12.003>
- Feitl, K. E., Ngo, V., & McHenry, M. J. (2010). Are fish less responsive to a flow stimulus when swimming? *Journal of Experimental Biology*, 213(18), 3131-3137. DOI: <https://doi.org/10.1242/jeb.045518>
- Ferreira, K. M. (2007). Biology and ecomorphology of stream fishes from the rio Mogi-Guaçu basin, Southeastern Brazil. *Neotropical Ichthyology*, 5(3), 311-326.
- Fisher, R., Bellwood, D. R., & Job, S. D. (2000). Development of swimming abilities in reef fish larvae. *Marine Ecology Progress Series*, 202(1), 163-173. DOI: <https://doi.org/10.3354/meps202163>
- Flore, L., Reckendorfer, W., & Keckeis, H. (2000). Reaction field, capture field, and search volume of 0+ nase (*Chondrostoma nasus*): effects of body size and water velocity. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(2), 342-350.
- Garcia, T., Jackson, P. R., Murphy, E. A., Valocchi, A. J., & Garcia, M. H. (2013). Development of a fluvial egg drift simulator to evaluate the transport and dispersion of Asian carp eggs in rivers. *Ecological Modelling*, 263(1), 211-222. DOI: <https://doi.org/10.1016/j.ecolmodel.2013.05.005>
- Gatz Jr, A. J. (1979). Community organization in fishes as indicated by morphological features. *Ecology*, 60(4), 711-718.
- Green, M. H., Ho, R. K., & Hale, M. E. (2011). Movement and function of the pectoral fins of the larval zebrafish (*Danio rerio*) during slow swimming. *Journal of Experimental Biology*, 214(18), 3111-3123. DOI: <https://doi.org/10.1242/jeb.057497>
- Hale, M. E. (2014). Developmental change in the function of movement systems: transition of the pectoral fins between respiratory and locomotor roles in zebrafish. *American Zoologist*, 54(2), 238-249. DOI: <https://doi.org/10.1093/icb/icu014>

- Halleraker, J. H., Saltveit, S. J., Harby, A., Arnekleiv, J. V., Fjeldstad, H. P., & Kohler, B. (2003). Factors influencing stranding of wild juvenile brown trout (*Salmo trutta*) during rapid and frequent flow decreases in an artificial stream. *River Research and Applications*, 19(5-6), 589-603. DOI: <https://doi.org/10.1002/rra.752>
- Hinrichsen, H. H., Hüsey, K., & Huwer, B. (2012). Spatio-temporal variability in western Baltic cod early life stage survival mediated by egg buoyancy, hydrography and hydrodynamics. *ICES Journal of Marine Science*, 69(10), 1744-1752. DOI: <https://doi.org/10.1093/icesjms/fss137>
- Hogan, J. D., & Mora, C. (2005). Experimental analysis of the contribution of swimming and drifting to the displacement of reef fish larvae. *Marine Biology*, 147(1), 1213-1220. DOI: <https://doi.org/10.1007/s00227-005-0006-5>
- Hunter, J. R. (1981). Feeding ecology and predation of marine fish larvae. In R. Lasker (Ed.), *Marine fish larvae* (p. 34-77). Washington, DC: Washington Sea Grant Program.
- Kunze, H. B., Morgan, S. G., & Lwiza, K. M. (2013). Field test of the behavioral regulation of larval transport. *Marine Ecology Progress Series*, 487(1), 71-87. DOI: <https://doi.org/10.3354/meps10283>
- Kynard, B., Parker, E., Kynard, B., & Parker, T. (2007). *Dispersal characteristics, drift distance, and wintering behaviour of young Kootenai River White Sturgeon: a laboratory study* (Final Report to Idaho Department of Fish and Game). Boise, ID: Idaho Department of Fish and Game.
- Lacey, R. J., Neary, V. S., Liao, J. C., Enders, E. C., & Tritico, H. M. (2012). The IPOS framework: linking fish swimming performance in altered flows from laboratory experiments to rivers. *River Research and Applications*, 28(4), 429-443. DOI: <https://doi.org/10.1002/rra.1584>
- Lechner, A., Keckeis, H., Schludermann, E., Humphries, P., McCasker, N., & Tritthart, M. (2013). Hydraulic forces impact larval fish drift in the free flowing section of a large European river. *Ecohydrology*, 7(2), 648-658. DOI: <https://doi.org/10.1002/eco.1386>
- Lechner, A., Keckeis, H., & Humphries, P. (2016). Patterns and processes in the drift of early developmental stages of fish in rivers: a review. *Reviews in Fish Biology and Fisheries*, 26(1), 471-489. DOI: <https://doi.org/10.1007/s11160-016-9437-y>
- Lechner, A., Keckeis, H., Glas, M., Tritthart, M., Habersack, H., Andorfer, L., & Humphries, P. (2017). The influence of discharge, current speed, and development on the downstream dispersal of larval nase (*Chondrostoma nasus*) in the River Danube. *Canadian Journal of Fisheries and Aquatic Sciences*, 75(2), 247-259. DOI: <https://doi.org/10.1139/cjfas-2016-0340>
- Li, G., Müller, U. K., Leeuwen, J. L. van, & Liu, H. (2016). Fish larvae exploit edge vortices along their dorsal and ventral fin folds to propel themselves. *Journal of The Royal Society Interface*, 13(116), 20160068.
- Mouritsen, H., Atema, J., Kingsford, M. J., & Gerlach, G. (2013). Sun compass orientation helps coral reef fish larvae return to their natal reef. *PLoS One*, 8(6), e66039. DOI: <https://doi.org/10.1371/journal.pone.0066039>
- Muller, U. K., & Leeuwen, J. L. van (2004). Swimming of larval zebrafish: ontogeny of body waves and implications for locomotory development. *Journal of Experimental Biology*, 207(Pt 5), 853-868. DOI: <http://dx.doi.org/10.1242/jeb.00821>
- Muller, U. K., Boogaart, J. G. van den, & Leeuwen, J. L. van (2008). Flow patterns of larval fish: undulatory swimming in the intermediate flow regime. *Journal of Experimental Biology*, 211(2), 196-205. DOI: <http://dx.doi.org/10.1242/jeb.005629>
- Murphy, E. A., & Jackson, P. R. (2013). *Hydraulic and water-quality data collection for the investigation of Great Lakes tributaries for Asian carp spawning and egg-transport suitability*. Reston, VA: US Geological Survey.
- Nakatani, K., Agostinho, A. A., Baumgartner, G., Bialezki, A., Sanches, P. V., Makrakis, M. C., & Pavanelli, C. S. (2001). *Ovos e larvas de peixes de água doce: desenvolvimento e manual de identificação*. Maringá, PR: Eduem; UEM-Nupélia; Eletrobrás.
- Nanami, A. (2007). Juvenile swimming performance of three fish species on an exposed sandy beach in Japan. *Journal of Experimental Marine Biology and Ecology*, 348(1-2), 1-10. DOI: <https://doi.org/10.1016/j.jembe.2007.02.016>
- Nathan, R., Getz, W. M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., & Smouse, P. E. (2008). A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences*, 105(49), 19052-19059. DOI: <https://doi.org/10.1073/pnas.08003751>

- Olszewski, J., Haehnel, M., Taguchi, M., & Liao, J. C. (2012). Zebrafish larvae exhibit rheotaxis and can escape a continuous suction source using their lateral line. *PloS One*, 7(5), e36661. DOI: <https://doi.org/10.1371/journal.pone.0036661>
- Osse, J. W. M., & Boogaart, J. G. M. van den (1999). Dynamic morphology of fish larvae, structural implications of friction forces in swimming, feeding and ventilation. *Journal of Fish Biology*, 55(sA), 156-174. DOI: <https://doi.org/10.1111/j.1095-8649.1999.tb01053.x>
- Ota, R. R., Deprá, G. D. C., Graça, W. J. D., & Pavanelli, C. S. (2018). Peixes da planície de inundação do alto rio Paraná e áreas adjacentes: revised, annotated and updated. *Neotropical Ichthyology*, 16(2), e170094. DOI: <https://doi.org/10.1590/1982-0224-20170094>
- Ouchi-Melo, L. S., Amaral, B., Tavares, D. A., Bartozek, E. C., de Souza, J. E., Pereira, L. H., ... & Peres, C. K. (2021). Brazilian vs. Paraguayan streams: Differences in water quality in a cross-border subtropical region. *Limnologica*, 90, 125904. DOI: <https://doi.org/10.1016/j.limno.2021.125904>
- 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(1), 125-138. DOI: <https://doi.org/10.1007/s10750-008-9396-y>
- Pavlov, D. S., & Mikheev, V. N. (2017). Downstream migration and mechanisms of dispersal of young fish in rivers. *Canadian Journal of Fisheries and Aquatic Sciences*, 74(8), 1312-1323.
- Petereit, C., Hinrichsen, H. H., Franke, A., & Köster, F. W. (2014). Floating along buoyancy levels: Dispersal and survival of western Baltic fish eggs. *Progress in Oceanography*, 122(1), 131-152. DOI: <https://doi.org/10.1016/j.pocean.2014.01.001>
- Piana, P. A., Cardoso, B. F., Dias, J., Gomes, L. C., Agostinho, A. A., & Miranda, L. E. (2017). Using long-term data to predict fish abundance: the case of *Prochilodus lineatus* (Characiformes, Prochilodontidae) in the intensely regulated upper Paraná River. *Neotropical Ichthyology*, 15(3), e160029. DOI: 10.1590/1982-0224-20160029
- Reichard, M., & Jurajda, P. (2004). The effects of elevated river discharge on the downstream drift of young-of-the-year cyprinid fishes. *Journal of Freshwater Ecology*, 19(3), 465-471.
- Robinson, A. T., Clarkson, R. W., & Forrest, R. E. (1998). Dispersal of larval fishes in a regulated river tributary. *Transactions of the American Fisheries Society*, 127(5), 772-786.
- Ruzicka, J. J., & Gallagher, S. M. (2006). The importance of the cost of swimming to the foraging behavior and ecology of larval cod (*Gadus morhua*) on Georges Bank. *Deep Sea Research Part II: Topical Studies in Oceanography*, 53(23-24), 2708-2734. DOI: <https://doi.org/10.1016/j.dsr2.2006.08.014>
- Sambily Jr, V. C. (1990). Interrelationships between swimming speed, caudal fin aspect ratio and body length of fishes. *Fishbyte*, 8(3), 16-20.
- Sanches, P. V., Gogola, T. M., Silva, R. O., Topan, D. A., Santos Picapedra, P. H., & Piana, P. A. (2020). Arms as areas for larval development of migratory fish species in a Neotropical reservoir and the influence of rainfall over abundances. *Journal of Fish Biology*, 97(5), 1306-1316. DOI: <https://doi.org/10.1111/jfb.14474>
- 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(8), 1302-1315. DOI: <https://doi.org/10.1139/f2012-061>
- Staaterman, E., Paris, C. B., & Helgers, J. (2012). Orientation behavior in fish larvae: a missing piece to Hjort's critical period hypothesis. *Journal of Theoretical Biology*, 304(1), 188-196. DOI: <https://doi.org/10.1016/j.jtbi.2012.03.016>
- Standen, E. M., & Lauder, G. V. (2005). Dorsal and anal fin function in bluegill sunfish *Lepomis macrochirus*: three-dimensional kinematics during propulsion and maneuvering. *Journal of Experimental Biology*, 208(14), 2753-2763. DOI: <https://doi.org/10.1242/jeb.01706>
- Standen, E. M., & Lauder, G. V. (2007). Hydrodynamic function of dorsal and anal fins in brook trout (*Salvelinus fontinalis*). *Journal of Experimental Biology*, 210(Pt 2), 325-339. DOI: <https://doi.org/10.1242/jeb.02661>
- Statsoft, I. (2004). *Statistica (Data Analysis Software System), Version 7*.
- Stewart, W. J., Cardenas, G. S., & McHenry, M. J. (2013). Zebrafish larvae evade predators by sensing water flow. *Journal of Experimental Biology*, 216(3), 388-398. DOI: <https://doi.org/10.1242/jeb.072751>

- 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(1), 1576-1584. DOI: <https://doi.org/10.1139/F2012-086>
- Videler, J. J. (1993). *Fish swimming*. London, UK: Chapman & Hall.
- Voesenek, C. J., Muijres, F. T., & Leeuwen, J. L. van (2018). Biomechanics of swimming in developing larval fish. *Journal of Experimental Biology*, 221(1), jeb149583. DOI: <https://doi.org/10.1242/jeb.149583>
- Watson, D. J., & Balon, E. K. (1984). Ecomorphological analysis of fish taxocenes in rainforest streams of northern Borneo. *Journal of Fish Biology*, 25(3), 371-384.
- Widmer A. M., Fluder J. J., Kehmeier J. W., Medley C. N., & Valdez R. A. (2012). Drift and retention of pelagic spawning minnow eggs in a regulated river. *River Research and Applications*, 28(2), 192-203. DOI: <https://doi.org/10.1002/rra.1454>
- Worthington, T. A., Brewer, S. K., Farless, N., Grabowski, T. B., & Gregory, M. S. (2014). Interacting effects of discharge and channel morphology on transport of semibuoyant fish eggs in large, altered river systems. *PLoS One*, 9(5), e96599. DOI: <https://doi.org/10.1371/journal.pone.0096599>
- Zens, B., Glas, M., Tritthart, M., Habersack, H., & Keckeis, H. (2017). Movement patterns and rheoreaction of larvae of a fluvial specialist (nase, *Chondrostoma nasus*): the role of active versus passive components of behaviour in dispersal. *Canadian Journal of Fisheries and Aquatic Sciences*, 75(2), 193-200. DOI: <https://doi.org/10.1139/cjfas-2016-0276>