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Key Points:
- We conducted laboratory experiments with live grass carp larvae (Ctenopharyngodon idella), an aquatic invasive species, subject to various levels of turbulence.
- We used quantitative imaging to identify larval response to a range of turbulent features, representing common in-stream obstructions in the field.
- Data showed that larvae might be more sensitive to specific magnitudes and length scales of turbulence flow features.

Supporting Information:
- Supporting Information S1

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Abstract

In this experimental series, we studied the swimming capabilities and response of grass carp (Ctenopharyngodon idella) larvae to flow turbulence in a laboratory flume. We compared three different experimental configurations, representing in-stream obstructions commonly found in natural streams (e.g., a gravel bump, a single vertical cylinder, and patches of submerged rigid vegetation). Grass carp larvae (postgas bladder emergence) were introduced to each experimental configuration and subjected to a variety of hydrodynamic forces of different magnitudes and scales. We varied the flow velocities and water depths and found ranges of turbulent kinetic energy and Reynolds stresses that triggered a response in larval trajectories, identified by measured horizontal and vertical swimming speeds for each flow condition. Larvae apparently actively avoided areas with increased levels of turbulence by swimming away, moving faster in short bursts, and expending more energy. In addition to the magnitude of turbulent kinetic energy, the length scale and time scale of turbulent eddies also influenced the larval response. These findings support the development of new strategies for controlling the spread of grass carp larvae in rivers, as well as the development of numerical tools incorporating active swimming capabilities to predict larval transport in streams.

1. Introduction

The study of dispersal of fish larvae in fluvial freshwater ecosystems is a challenging task, because the small size of larvae prevents a direct observation of this phenomenon in the field. Although many models have used simplified assumptions about larval movement, larvae do not behave simply as inert particles traveling with the flow (Leis, 2007). As larvae develop, their swimming behavior changes and their swimming ability increases. Larvae are able to resist the hydraulic forces in rivers (Lechner et al., 2014; Pavlov et al., 2008), thus requiring an “active” component to drift modeling. All larvae develop swimming capabilities to find safe nursery habitat with desirable water temperature and food availability, minimizing mortality, and maximizing successful dispersal (Keckeis et al., 1997; Pavlov 1994; Schiemer et al., 2003), but behavioral strategies to accomplish those goals are species and developmental-stage-specific (Lechner et al., 2014, 2016). The mechanisms and behaviors by which early life stage larval fish identify and move to those habitats, including fine-scale habitat selection within the river, are particularly poorly understood for riverine fishes (Lechner et al., 2016).

Lacey et al. (2012) introduced a framework to categorize turbulence parameters that affect swimming performance. The IPOS framework, acronym for Intensity, Periodicity, Orientation, and Scale, presents the case for why not only the magnitude of turbulence parameters is relevant for flow-larval interactions, but also the associated time scales, length scales, and orientation of turbulent eddies with respect to the fish. However, at early developmental stages, grass carp larvae have not fully developed other senses, and their response to external mechanical stimuli, as described by the IPOS framework, is not yet well understood. Fish larvae differ from adult fish in their interaction with the physical environment because of their size, as they navigate their environment through coherent flow structures mostly larger than their size (Smith et al., 2014), and are subjected to different hydrodynamic regimes (i.e., viscous versus inertial regime; Voesnek et al., ). Several studies have reported how coherent turbulent structures may decrease fish swimming stability, especially eddies of similar size to the total length of the fish (e.g., Cada & Odeh, 2001; Metaxas, 2001; Pavlov...
et al. 2000). Fish larger than the surrounding eddies are typically unaffected, whereas fish of sizes similar to the eddies can be disturbed and destabilized (Metaxas, 2001; Triticó & Coté, 2010).

If larvae are destabilized by the flow turbulence, they may lose their ability to maintain position and can be swept away by the river currents, affecting their dispersal (Lechner et al., 2018; Reichard & Jurajda, 2007; Schludermann et al., 2012). Understanding the limitations of their swimming capabilities as a function of turbulent flow parameters is thus critical for monitoring, managing, and predicting the dispersal of grass carp during early life stages.

In this study, we are particularly interested in characterizing the swimming response of grass carp larvae to allow better predictions of larval transport in natural streams and movement to nursery areas, because they have become established in the central United States and parts of Europe, where they are considered problematic invaders (Chick & Pegg, 2001; Kočovský et al., 2018; Milardi et al., 2015; Parker et al., 2016). Grass carp have similar early life history to bighead carp (Hypophthalmichthys nobilis), silver carp (Hypophthalmichthys molitrix), and black carp (Mylopharyngodon piceus), and this study may also provide insights to the control of those species, which are also highly problematic invasive species in North America. However, in their native range in China, these four species are revered as food fishes (Kočovský et al., 2018). They are in decline there, in part due to anthropogenic modifications of their environment (Chapman et al., 2016). Understanding the transport, behavior, and habitat selection by the larvae of these species may also be useful in their native range, where physical river conditions might be altered to improve the success of larval movement into nursery habitats.

Grass carp (Ctenopharyngodon idella) larvae go through several developmental stages where marked behavioral and anatomical changes determine their active swimming response (Chapman & George, 2011; George & Chapman 2013; George et al., 2015). After hatching, larvae initially swim vertically, with alternating periods of upward swimming and downward falling resulting in a parabolic shaped track in flowing water (George & Chapman 2015; George et al., 2018; Prada et al., 2018). Like bighead carp and silver carp, grass carp larvae transition to horizontal swimming as the gas bladder develops and inflates, which allows them to move laterally and, under calm water conditions, hold vertical position in the water column without directional swimming (George et al., 2018). This behavioral transition corresponds with the beginning of feeding, and with movement from the drift within large, turbulent, and turbid spawning rivers to low-velocity nursery areas where planktonic prey is likely to be present and feeding is effective (Chick et al., 2020; George et al., 2018). The behaviors and microhabitat selection that result in successful movement from the main channel of a large river to shallow, calm, nursery habitats, needs to be properly understood to be exploited to meet management objectives.

Prada et al. (2020) reported that grass carp larvae responded and swam away from stem-scale eddies created by a submerged vegetation array comprised of rigid elements with stem diameters of the same order of magnitude as the total length of the larvae. Larvae tended to drift above the array where the vorticity generated by the elements was negligible, even though the mean-flow velocity was higher than within the array. Likewise, other laboratory studies (e.g., George et al., 2018; Prada et al. 2018) have documented the swimming response of grass carp larvae to altered flow conditions and various turbulent scales, but did not identify specific ranges of velocity or turbulence for which such response occurred. The estimation of specific magnitudes of turbulence and turbulent spatial and temporal scales that trigger larval response are thus of vital importance to determining how larvae disperse through fluvial systems to their nursery habitat. This knowledge may assist managers in controlling the spread of grass carp and other invasive species with similar early life history.

The objective of this study was to observe the swimming response of grass carp larvae to naturally altered flows recreated in a laboratory flume, and to quantify the physical processes driving such a response. We chose larvae in developmental stage 38 (based on stages described by Yi et al. (1988) and George and Chapman (2015)), a stage with robust ability to swim in any direction and to maintain position in calm water, and the stage at which movement to nursery habitats begins. Three different altered flow configurations were recreated in the flume: (1) a gravel bump, (2) a single vertical cylinder, and (3) rigid-submerged vegetation. These configurations subjected the larvae to a broad range of flow conditions and turbulent scales, driven by changes of flow velocity and water depth. These configurations present a clear difference in larval...
spatial distribution compared with cases with no obstructions in the flume, as reported in Prada et al. (2018, 2020), suggesting that the presence of the structures, rather than potential schooling or other stressors, are responsible for the observed changes in behavior. We report ranges of values of turbulent kinetic energy and Reynolds stresses (i.e., stresses imposed in the flow by turbulent fluctuations) that triggered the response of larvae, based on the measured swimming speeds for each flow condition. The results of this study will contribute to the enhancement of larval sampling and collection in the field, as well as to the development of numerical tools that incorporate active swimming capabilities for the modeling of larval transport in streams.

2. Materials and Methods
2.1. Experimental Setup
2.1.1. Race-Track Flume (RTF)
A series of laboratory experiments with live grass carp larvae was conducted at the Ven Te Chow Hydrosystems Laboratory (VTCHL) of the University of Illinois at Urbana-Champaign. Authorization to use live eggs and larvae for research purposes was obtained from the Illinois Department of Natural Resources (Permit No. 18-050).

The VTCHL is equipped with a unique Odell-Kovasznay type flume (Odell & Kovasznay, 1971), which is a Race-Track Flume (RTF) where the flow is driven by a vertical-axis disk pump (Figure 1a). The flume is specially designed to break down secondary flow structures as water moves in a continuous loop to reach fully developed flows, minimizing effects from the side walls. The disk pump is controlled by a frequency inverter, with a relationship between the inverter frequency \( F \) and the rotation speed \( \Omega \) of the disk pump given by \( \Omega \ [\text{RPM}] = 6.6 \ F \ [\text{Hz}] \). The RTF has a constant width of 0.15 meters (m) and a straight test section of 2.0 m in length (Figure 1a). A 0.1-m thick flat sediment bed was placed for the experimental series along the test section. The sediment bed was composed of a mixture of walnut shells and sand (bulk sediment density \( \rho_p = 1,250 \text{ kilograms per cubic meter (kg/m}^3) \), settling velocity \( w_p = 1.89 \times 10^{-2} \text{ meters per second (m/s)} \), and size \( \{D_{16}, D_{50}, D_{84}\} = \{0.41, 0.54, 0.66\} \text{ millimeters (mm)} \) (Prada et al., 2018).

One of three different in-stream obstructions was placed atop the sediment bed for each set of experiments. The in-stream obstructions included a gravel bump, a single vertical cylinder, and arrays of rigid-submerged vegetation (Figures 1b–1g). Two different water depths were tested, \( H = 0.2 \) and 0.4 m measured over the sediment bed, for four disk-pump frequencies of 10, 15, 20, and 25 Hz that generated mean bulk velocities from 0.04 to 0.2 m/s (Table 1). The width-to-depth ratios in the straight section were 3/4 for \( H = 0.2 \) m and 3/8 for \( H = 0.4 \) m. Secondary flows and boundary layer effects created by the side walls in this type of flume do not affect the mean flow (as described in Odell and Kovasznay (1971), Stephenson and Fernando (1991), Strang and Fernando (2001), Jackson (2006), and Prada et al. (2018)).

The gravel bump had a height of 0.12 m and covered the width of the flume. It was composed of gravel (mean size of 35 mm) contained in a mesh bag (Figures 1b and 1c). The single cylinder embedded in the middle of the test section was a 51 mm (2 in.) diameter PVC pipe that allowed for 49 mm of space between the cylinder and the walls of the flume (Figures 1d and 1e). The rigid-submerged vegetation array extended along the test section (Figures 1f and 1g). The rigid vegetation was arranged in a staggered configuration with average spacing between rods of 0.034 m, porosity of 6.3 \times 10^{-3}, and volumetric frontal area \( a = 1.27 \ [\text{1/m}] \), with acrylic rods of 6.4 mm in diameter and 100 mm in height (i.e., 100 mm protruding above the sand bed). The roughness density \( (ah) \) was 0.127 (intermediate regime), which ensures the onset of a two-layer flow and canopy-scale turbulence (e.g., Nepf, 2012). Larvae tested for these series were 6–7 mm in length, which nearly matches the diameter of the acrylic cylinders, but are much smaller than both the single pile diameter and the gravel bump height.

Instantaneous velocity components in the \( x \), \( y \), and \( z \) directions are denoted by \( u \), \( v \), and \( w \), respectively. The longitudinal dominant mean-flow direction is \( x \), vertical is \( z \), and lateral \( y \). Flow was characterized using 2D Particle Image Velocimetry (PIV), illuminating neutrally buoyant silver-coated hollow glass spheres of 10 micrometers (\( \mu \text{m} \)) in diameter with a laser sheet (thickness < 1 mm) generated by a 5 Watt (W) Continuous-Wave 532 nanometer (nm) laser. Using a cylindrical lens, we create a vertical light sheet, parallel
Figure 1. Schematics of the race-track flume (a, plan view, not to scale) and the 2-m straight test section for each flow scenario (b–g, side views, not to scale). Gravel bump case for $H = 0.4$ m (b) and $H = 0.2$ m (c). Single cylinder case for $H = 0.4$ m (d) and $H = 0.2$ m (e). Rigid-submerged vegetation for $H = 0.4$ m (f) and $H = 0.2$ m (g). Green shadow area indicates the location and orientation of the laser light sheet. Dashed rectangles indicate the field of view for PIV. PIV, Particle Image Velocimetry.

Table 1

|                     | $H = 0.2$ m | $H = 0.4$ m |
|---------------------|-------------|-------------|
|                     | $F$ (Hz)    |            |
|                     | 10          | 15          | 20          | 25          | 10          | 15          | 20          | 25          |
| Case                | $U_c$ (m/s) | 0.044       | 0.064       | 0.084       | 0.104       | 0.080       | 0.113       | 0.150       | 0.187       |
| All                 | $Re_{Rh}$   | 2,400       | 3,491       | 4,582       | 5,673       | 5,053       | 6,947       | 9,474       | 12,000      |
|                     | $Re_{H}$    | 8,800       | 12,800      | 16,800      | 20,800      | 32,000      | 44,000      | 60,000      | 76,000      |
| Gravel              | $Re_{h}$    | 5,280       | 7,680       | 10,080      | 12,480      | 9,600       | 13,200      | 18,000      | 22,800      |
|                     | $Re_{D}$    | 2,244       | 3,264       | 4,284       | 5,304       | 4,080       | 5,610       | 7,650       | 9,690       |
| Cylinder            | $Re_{d}$    | 282         | 410         | 538         | 665         | 512         | 704         | 960         | 1,216       |
| Vegetation          | $Fr$        | 0.031       | 0.046       | 0.060       | 0.074       | 0.040       | 0.056       | 0.076       | 0.096       |

Note: Larvae body length between 6 and 7 mm.
to the longitudinal direction of the flow, located at the centerline of the flume, which enters through the water surface as shown in Figure 1. In the case of vegetation, a small gap of 0.1 m (close to 3 times the mean spacing between cylinders, small enough to avoid gap effects) was set in the middle of the test section to allow optical access to record particle movement (Figures 1f and 1g). All PIV measurements were recorded at 60 frames per second (fps) for 1 min (min) with a 5 megapixel (MP) monochromatic camera after the tests with live fish to avoid: (a) dissolved microspheres affecting larval health, and b) altering larval behavior due to the laser light sheet. The camera resolution, 2,560 × 2,048 pixels, yielded spatial resolution of 0.16 mm/pixel for the deepest, \( H = 0.4 \) m case. Preliminary convergence tests showed that time-averaged velocity, Reynolds stresses, and turbulent kinetic energy converged well below the 60 s duration of the records.

Froude and Reynolds numbers considering the incoming free-stream velocity preceding the obstacle (\( U_c \)) and the corresponding characteristic length scale for each case (e.g., \( Rh = \) hydraulic radius, \( H = \) water depth, \( h = \) gravel bump height, \( D = \) cylinder diameter, \( d = \) rigid element diameter) indicate all flows were within subcritical, turbulent regimes (Table 1). In the case of rigid vegetation, vortex shedding is expected at \( Re_d \approx 50 \), and the wakes become turbulent due to vortex instability for \( Re_d \approx 200 \) (Nepf, 1999), such that even our slowest flow, \( Re_d = 282 \), falls already within a turbulent wake regime. While larger values of velocity and water depth are expected in rivers where grass carp spawn, our study focuses on identifying the features of turbulence and turbulent coherent structures (e.g., intensity, orientation, length scale, and time scales) that most affect the trajectories and spatial distribution of larvae. Identifying which turbulent features drive a change in larval displacement patterns will allow us to use them as hydrodynamic controls to be tested in the field under higher flow conditions.

In the case of rigid vegetation, the velocity decelerates within the canopy due to the canopy drag, and a mixing layer is formed just above the canopy (Chen et al., 2013). The initial deceleration extends from the leading edge of the canopy until a distance \( X_D \) downstream that scales with the canopy drag length scale \( (L_c) \) and the canopy drag coefficient \( (C_D) \) as \( X_D = 1.5L_c (1 + 2.3C_D ah) \) (Chen et al., 2013). \( L_c \) is a function of the canopy density \( (a) \), and can be approximated as \( L_c = 2 / C_D a \) (Belcher et al., 2003). We estimated \( C_D \) from the rigid element Reynolds number \( (Re_d) \) using an empirical relation presented by White (1991), \( C_D = 1 + 10Re_d^{-0.23} \). Within the range of \( Re_d \) in our study \( (Re_d = 282\text{--}1,216, \text{Table 1}) \), we can estimate a drag coefficient of \( C_D = 1.1 \). The canopy drag length scale yields \( L_c = 1.43 \) m, with an initial deceleration zone \( X_D = 2.83 \) m. The straight test section of the RTF is 2.0 m long \((<X_D)\), and the PIV measurements for rigid vegetation were made 1.3 m downstream from the leading edge of the canopy. Our results are thus within the initial adjustment region over the canopy, representative of finite vegetated patches in streams, rather than continuous fully developed vegetated flows. Flow at the top of the canopy, however, does generate the expected mixing layer given our canopy density, \( ah > 0.1 \), high enough to create an inflection point in the velocity profile and shift the maximum turbulence and Reynolds stress levels to the top of the canopy, allowing us to explore the effect of turbulent eddies at the stem-scale and at the canopy-scale.

The gravel bump is intended to represent gravel-bed topographic features that may arise from various processes and generates a recirculation zone downstream of the obstruction. A shear layer develops between the low velocity behind and high velocity above the obstruction, resulting in larger-than-larvae eddies oriented mainly on a vertical plane. The single cylinder is representative of bridge piles, lunkers, logs, tree branches, or similar emergent structures that can be modeled with a simplified geometry. In contrast with the other two scenarios, the single cylinder does not produce a two-layer flow, but rather three-dimensional, larger-than-larvae eddies due to the wake past the cylinder, with time scales and length scales as a function of its diameter and \( Re_d \).

### 2.1.2. Live Larvae

As described in Prada et al. (2020), hormone injections were used to induce spawning in adult grass carp at the U.S. Geological Survey Columbia Environmental Research Center (CERC) in Columbia MO. At CERC, spawned eggs were fertilized and then transported to the VTCHL in Urbana, IL (a 4.5-h transport time). In long-term (4 days) experiments covering a wide range of developmental stages, Prada et al. (2020) used the fertilized eggs to investigate the dispersal of eggs and response of larvae to the three flume configurations described in section 2.1.1. (Figures 1a, 1b, 1d, and 1f). However, Prada et al. (2020) maintained a constant water depth of 0.4 m and a constant flow velocity for all experiments. On day 5 of each experiment, at which
point the larvae were at developmental stage 38 according to Yi et al. (1988), George and Chapman (2015), and Prada et al. (2020) carefully removed the larvae from the flume hatching jars (control), assessed morality, and concluded each experiment. The surviving stage 38 larvae from Prada et al. (2020) were carefully returned to the straight section of the flume and immediately used for the experiments presented in this manuscript.

To investigate the response of stage 38 grass carp larvae to a range of velocities, flow depths, and turbulence features, a shorter series of experiments was conducted in the same flume as Prada et al. (2020), but with various velocities and water depths (Table 1). The total number of stage 38 larvae used for the experiments with the gravel bump, single cylinder, and vegetation array, were 2,058, 2,482, and 2,482, respectively (the single cylinder and rigid-submerged vegetation cases used the same larvae). Despite having a large number of larvae in the flume at a single time, the larvae were spread around the flume and not in sufficient density to cause substantial alterations to the flow. It is known that densely laden flows can experience turbulence modulation due to the particles traveling within the flow (Bennett et al., 2013; Best et al., 1997; Szupiany et al., 2012; Zhong et al., 2015), but in the case of grass carp larvae, recent studies have shown that their presence does not measurably change the averaged turbulent kinetic energy in the flow (Li et al., 2020).

Developmental stage 38 grass carp larvae are 6–7 mm in length, after more than 50 h beyond egg hatching at a temperature of 23–24 °C, and have an inflated gas bladder allowing the larvae to swim horizontally and hold position within the water column (George & Chapman, 2015). Based on their developmental similarity with bighead carp (H. nobilis), silver carp (H. molitrix), and black carp (M. piceus), larvae from these species at this stage move into off-channel low-velocity habitats considered nursery areas, where recruitment to juvenile stages likely occurs (Chick et al., 2020; Nico et al., 2005). Although the same batch of larvae was used for all velocities with a given structure, larvae adaptation by remembering the flow they previously experienced was not a concern, because previous studies (e.g., Roberts et al., 2013) suggest that larval response can be highly context-specific, such that memory may not be the primary driver of larval behavior at this developmental stage.

Larval swimming performance around the in-stream obstructions for each velocity and water depth was recorded in 5-min videos with a Nikon D5300 camera (1,920 x 1,080 video resolution) at 30 fps. On a field of view of 0.40 m, this yields a resolution of 0.37 mm/pixel. If the PTV algorithm misses the centroid of a larva by one pixel between frames, it yields a potential error in the measured velocity of 0.01 mm/s. The camera was placed 0.5 m in front of the RTF’s test section. LED Edge Lit panels (Knema, LLC) were used to illuminate the test section from behind, creating a uniform illumination field. At the end of each test case, all larvae were euthanized using MS-222 (tricaine methanesulfonate) according to protocol, to prevent the escape of eggs or larvae from the facility. Remaining organic material was filtered and incinerated.

2.2. Data Processing

2.2.1. Flow Data

Series of images of the illuminated tracer particles in the flow were processed using the open-source tool PIVlab in MATLAB (Thielicke & Stamhuis, 2014). Postprocessing yields instantaneous velocity fields $u(x,z)$ and $w(x,z)$ across the images field of view, where $x$ is the horizontal coordinate increasing downstream from each obstruction and $z$ is the vertical coordinate increasing upwards from the sediment bed.

Instantaneous velocity fields were time-averaged over 1 min to obtain the mean velocities $\bar{U}(x,z) = \frac{1}{T} \int_{0}^{T} u(x,z) dt$ and $\bar{W}(x,z) = \frac{1}{T} \int_{0}^{T} w(x,z) dt$ (with overbar denoting time average). Reynolds decomposition is used to calculate instantaneous turbulent fluctuations as $u'(x,z) = u(x,z) - \bar{U}(x,z)$ and $w'(x,z) = w(x,z) - \bar{W}(x,z)$. Turbulent kinetic energy ($k$) and Reynolds stresses ($\tau_{xz}$) were estimated as:

$$k(x,z) = 0.5 \left( 2u'^2(x,z) + w'^2(x,z) \right)$$

$$\tau_{xz}(x,z) = \rho \left( u'(x,z) \cdot w'(x,z) \right)$$
where \( \rho \) is the water density. Given our 2D PIV data, and considering the three-dimensional nature of turbulence, we estimated the spanwise turbulence intensity as \( \sqrt{\hat{u}'^2} = \hat{\nu}'^2 \), as corroborated by previous studies (e.g., Tanino & Nepf, for vegetated flows). Profiles of \( k \) and \( \tau_{xz} \) for the gravel bump and rigid vegetation cases were scaled using the characteristic velocity difference \( (U_s) \) for plane mixing layers (Pope, 2000) given as:

\[
U_s = U_h - U_l
\]

where \( U_h \) and \( U_l \) are the mean uniform velocities of each layer \( (U_h > U_l) \). For the single vertical cylinder, \( U_l \) for a plane wake is given as:

\[
U_s = U_c - U(x,z)
\]

where brackets \( \langle \rangle \) represents spatial averaging over \( x \) within the field of view (Pope, 2000).

### 2.2.2. Larval Tracking

Videos of larval swimming past the in-stream obstructions were processed with Particle Tracking Velocimetry (PTV) scripts in MATLAB® (based on Brevis et al., 2011). For every flow condition in each flume setup, 1,800 consecutive frames (i.e., 1-min data) were extracted from the videos to track the position of individual larvae over time. The PTV routine produced binary images, which were processed using Gaussian particle detection routines to obtain the centroid of each larva \( (x,z) \) coordinates. In stage 38, although larvae are still very small, pigmentation in the eyes and back allowed reliable detection of each individual larva, by setting threshold levels of pixel intensities that contrasted with the white background.

Algorithms of cross correlation (Brevis et al., 2011; Hassan et al., 1992; Uemura et al., 1989) were implemented in the PTV codes to match identified larvae between consecutive frames. The algorithm identifies individual particles \( (i.e., \text{larvae}) \), and finds the highest cross-correlation coefficient in consecutive images to identify the displacement of each larva. It uses square interrogation windows, which size is selected based on the maximum expected displacement of the particles (similar to the interrogation subwindows for PIV). The velocity associated with a matched particle is then estimated between pairs of consecutive frames, which we call in this study “larvae traveling speeds,” \( u_l \) and \( w_l \) for each component in the \( x \) and \( z \) directions, respectively. The code keeps track of initial and final position of each particle between image pairs to identify trajectories of the same particle.

Because we measured the distribution of mean-flow velocities \( (U \text{ and } W) \) with two-dimensional-PIV and larvae traveling speeds with two-dimensional-PTV, we can estimate a two-dimensional field of larval swimming speeds \( u_{sw} \) and \( w_{sw} \) as:

\[
\begin{align*}
    u_{sw} &= U - u_l \\
    w_{sw} &= W - w_l
\end{align*}
\]

Two-dimensional fields of traveling speeds \( (u_l \text{ and } w_l) \) were interpolated to match the size of the two-dimensional fields of mean velocity \( (U \text{ and } W) \) to allow for direct calculation of two-dimensional fields of swimming speeds \( (u_{sw} \text{ and } w_{sw}) \). We notice that some of the recorded swimming speeds account for rapid bursts, and represent instantaneous escape responses, which do not always represent a sustained mean swimming speed, i.e., a larva does not maintain the same speed for several tail beats (George et al., 2018; Müller & van Leeuwen, 2004). According to our definition in equations \( (5) \) and \( (6) \), longitudinal swimming speeds are positive if the larva swims against the flow, and negative if it follows flow direction. Positive vertical swimming speeds indicate larvae trying to swim downwards, while negative vertical swimming speeds indicates upwards swimming.

### 3. Results and Discussion

We have chosen to present the results with interpretation and discussion immediately following each part of the study because each section of the results builds upon the previous section and the interpretations therein. We first present the PIV results and characterize the flow fields and turbulence parameters for each
experimental configuration. Next, we present the larval swimming speeds in relation to the mean-flow and turbulence parameters. Finally, we present the spatial distributions of larvae in relation to the mean-flow and turbulence parameters as well as the swimming capabilities of the larvae.

### 3.1. Flow Field and Turbulence Characterization

In the cases of the gravel bump and rigid vegetation, we observe a two-layer flow, high velocity above the obstacle, and low velocity within the obstructed region (Figures 2a and 2d—gravel, Figures 2c and 2f—vegetation). At the interface of the two layers, there is a mixing zone with increased levels of shear and turbulence. Figures 3a, 3c, 3d and 4a, 4c, 4d show how $k$ (from equation (1)) and $\tau_{zz}$ (from equation (2)) increase in the mixing zone for all flow conditions compared to the two bounding layers. Further detail of the turbulence in all cases is presented as supplementary material, where we show scaled profiles of turbulent intensities $u_{rms}/U_i$ (Figure S1) and $w_{rms}/U_i$ (Figure S2). In contrast, data from the single cylinder (Figures 2b and 2e) shows a nearly uniform velocity field over the vertical, with no significant shear, velocity gradients or areas of high turbulence (Figures 3b, 3e and 4b, 4c). Data for the fastest case ($F = 25$ Hz, $U_i = 0.104$ m/s for $H = 0.2$ m or $U_i = 0.187$ m/s for $H = 0.4$ m) in the vertical cylinder are not shown due to sediment movement that biased the PIV and PTV results for both water depths.

The difference in definitions of $U_i$ for each case (equation (3) for gravel and vegetation, equation (4) for single cylinder) produces a significant difference in the nondimensional values in Figures 3 and 4. However, we can conduct a direct comparison between the two 2-layer flows (gravel and vegetation), as they
show similar patterns. While both cases have similar profiles of Reynolds stresses (Figures 3a and 3d versus Figures 3c and 3f) and turbulent kinetic energy (Figures 4a and 4d versus Figures 4c and 4f), we notice a difference of almost an order of magnitude between turbulent kinetic energy magnitudes with the gravel and vegetation scenarios. If turbulence intensity were the dominant driver of larval responses to flow, we would expect the higher turbulence levels of the gravel case to yield the most evident response. However, as we will discuss in the next sections, the type of turbulence features (i.e., orientation and length scale of turbulent eddies), also plays a role on driving such responses.

### 3.2. Larvae Swimming Speeds

Individual trajectories of larvae (as shown in Figure 5 for case with $U_c = 0.113$ m/s, 15 Hz) shows us: (i) the main paths followed by larvae, ii) whether larvae accumulate in specific areas around the tested obstructions, (iii) if there is clustering independently of the structure, (iv) whether larvae are still able to move vertically or they just travel with the flow, and (v) whether an active behavior can be inferred from the trajectories or if larvae act as passive particles. Larvae passing above the gravel (Figure 5a) show pat-
terns following flow streamlines traveling mostly horizontally, whereas larvae behind the gravel show more vertical and recirculating motions. Larvae swimming past the cylinder (Figure 5b) shows a more three-dimensional pattern, with more irregular trajectories and accumulation within one diameter downstream of the cylinder hinting at wake effects. Larvae passing through and above the vegetation array (Figure 5c) shows similarities with the gravel case, with streamlined trajectories far above the plants, and more vertical displacements within the array.

To better understand the spatial distribution of larvae and how it may be affected by swimming behaviors, we needed to quantify their swimming capabilities. For all the flow conditions investigated, we used PTV and PIV data to estimate horizontal and vertical burst swimming speeds (Figure 6, from equations (5) and (6)). Time-averaged traveling speeds (Figure 6c) are subtracted from time-averaged water velocities (Figure 6b) to obtain time-averaged swimming speeds (Figure 6a). Given our reference frame (flow moving right to left in Figure 6), positive flow speed and traveling speeds indicate movement in the direction of the flow (right to left in Figure 6). Positive swimming speeds (Figure 6a), as defined in equation (5), indicate

Figure 4. Profiles of nondimensional turbulent kinetic energy spatially averaged within the field of view for all flow conditions for the gravel bump (left panels; a and d), the vertical cylinder (middle panels; b and e), and the rigid vegetation (right panels; c and f) at \( H = 0.2 \) m (top panels) and \( H = 0.4 \) m (bottom panels). Data for the fastest free-stream velocities for the vertical cylinder are not shown due to sediment movement that biased the results for both water depths.
larvae are swimming against the current, trying to move upstream even if they do not swim fast enough to overcome the flow (hence positive traveling speeds in Figure 6c). Zero swimming speeds indicate passive drifting with the flow. Negative swimming speeds indicate that they are actively swimming downstream, propelling them to move faster than the mean flow.

Two-dimensional maps of horizontal and vertical swimming speeds (as in Figure 6) were spatially averaged over the longitudinal ($x$) direction to obtain vertical profiles of longitudinal (Figure 7) and vertical (Figure 8) swimming speeds for all three cases (gravel bump, vertical cylinder, and rigid vegetation), for both water depths and all free-stream velocities shown in Table 1. Notice that the spatial averaging is calculated over the area downstream of the gravel and cylinder, and within the observation gap for vegetation, to reduce the longitudinal variability in the profiles.

Larvae consistently oriented upstream, but swimming speeds never exceeded the flow velocity, meaning that all larvae traveled downstream in all cases. For the gravel bump case (Figures 7a and 7d) the horizontal swimming speeds increased within the mixing layer (shaded area in Figure 7) for all flow conditions up to $F = 15$ Hz ($U_c = 0.064$ m/s) with $H = 0.2$ m shown. Positive PIV and PTV velocities indicate movement from right to left (in the direction of the flow). Positive swimming speeds indicate larvae are trying to swim against the current. PIV, Particle Image Velocimetry.

**Figure 5.** Sample trajectories of larvae detected over a 1-min interval using particle tracking velocimetry (PTV) for $H = 0.4$ m at an intermediate speed (15 Hz, $U_c = 0.113$ m/s) and three flow configurations: (a) gravel bump, (b) vertical cylinder, and (c) rigid vegetation (each colored line represents the trajectory of a single larva).

**Figure 6.** Larval swimming speeds ($a$, from equation (5)) are obtained by subtracting the traveling speeds ($c$, from PTV) from the time-averaged velocities $U$ ($b$, from PIV), i.e., $a = b - c$. Gravel bump at $F = 15$ Hz ($U_c = 0.064$ m/s) with $H = 0.2$ m shown. Positive PIV and PTV velocities indicate movement from right to left (in the direction of the flow). Positive swimming speeds indicate larvae are trying to swim against the current. PIV, Particle Image Velocimetry.
the flow velocity (i.e., $u_{sw}/U \sim 1$). While the flow velocity may be higher elsewhere in the water column, the increased level of turbulence in the mixing layer seemingly forced them to swim faster. While instantaneous events could be responsible for transporting larvae away from these regions of high turbulence (e.g., sweeps and ejections within the mixing layer), we evaluate the changes in the velocity of larvae in a given region based on the time-averaged turbulence statistics in that region, rather than looking at intermittent instantaneous events. For the cylinder (Figures 7b and 7e), most of the swimming speeds for both water depths were negative and some of them greater than the mean flow ($u_{sw}/U < -1$), hinting that they swam in the direction of the flow to escape from the turbulent wake downstream of the cylinder. For the rigid vegetation case (Figures 7c and 7f), rather than an increase of swimming speed within the mixing layer as in the gravel bump, faster swimming speeds were measured above the canopy in response to higher velocities. The contrast in larval response between the two 2-layer flow scenarios (gravel and vegetation, Figures 7a and 7d versus Figures 7c and 7f), added to their difference in turbulent kinetic energy (Figure 4) confirms that other factors are driving the differences in swimming behaviors.

Figure 7. Horizontal swimming speeds scaled with time-averaged longitudinal flow speed as a function of the water depth for the gravel bump (left panels; a and d), the vertical cylinder (middle panels; b and e), and the rigid vegetation (right panels; c and f) at $H = 0.2$ m (top panels) and $H = 0.4$ m (bottom panels). Data for the fastest free-stream velocities for the vertical cylinder are not shown due to sediment movement that biased the results for both water depths. Shaded areas represent an approximate width of the mixing layer in each case.
According to our definition (equation (6)), positive vertical swimming speeds indicate that larvae are actively trying to move downwards, whereas negative vertical swimming speeds indicate that they try to move upwards. Vertical profiles of vertical swimming speeds (Figure 8) for all three cases, at both water depths and all disk-pump frequencies show that, for the gravel bump, there is an apparent increase of the vertical swimming speeds within the mixing layer, and larvae are able to overcome the vertical flow velocities \( w_{sw}/W > 1 \) (Figures 8a and 8d) even up to 10 times faster, moving upwards above the mixing layer and downwards below it (Figures 8a and 8d). However, time-averaged vertical velocities are near negligible, thus the ratio \( w_{sw}/W \) does not provide sufficient information to assess larval response. To improve the assessment of vertical response, we scaled the vertical swimming speeds with the vertical turbulent intensities (\( w_{rms}' \))—Figure 9. The magnitude of the ratio \( w_{sw}/w_{rms}' \) is lower within the mixing layer (grayed areas in Figure 9) than outside of it. This shows that instantaneous turbulent fluctuations in vertical velocity at the interface are larger than the observed vertical swimming speeds. When compared with the ratio \( w_{sw}/W \) in Figure 8, our data suggest that evaluating mean vertical velocities at the interface is not enough to assess how larvae will behave within and across these type of mixing layers. The use of turbulent parameters, such as the

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**Figure 8.** Vertical swimming speeds scaled with the time-averaged vertical flow speed as a function of the water depth for the gravel bump (left panels; a and d), the vertical cylinder (middle panels; b and e), and the rigid vegetation (right panels; c and f) at \( H = 0.2 \) m (top panels) and \( H = 0.4 \) m (bottom panels). Data for the fastest free-stream velocities for the vertical cylinder are not shown due to sediment movement that biased the results for both water depths. Shaded areas represent an approximate width of the mixing layer in each case.
increased turbulent fluctuations at the interface, can then improve predictions of the expected swimming response from larvae. For instance, larvae can swim away from areas with low mean vertical velocities (Figure 8), but assessment of whether larvae will choose to stay there or be able to go across such interfaces (as we discuss in section 3.3) requires knowledge about turbulent fluctuations at the interface (Figure 9).

In contrast to the patterns seen in the two-layer flows, the single cylinder case shows no specific trend in the distributions of the vertical swimming speeds (Figures 8b and 8e), and larvae could move easily in any direction without being concentrated in any specific zone (Figures 9b and 9e).

The observed larval behavior described here highlights the importance of rheotactic stimuli in the dispersal of grass carp. Because other senses (i.e., chemosense, vision, hearing) are not yet well developed at the stages when larvae begin to leave the drift, the inferred response to mechanical stimuli such as flow and turbulence would indicate that these may be some of the main factors driving dispersal at early life stages, which could allow for the implementation of turbulence-based control mechanisms in streams that would

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**Figure 9.** Vertical larval swimming speeds scaled with the vertical turbulent intensity ($w'_v$) for the gravel bump (left panels; a and d), the vertical cylinder (middle panels; b and e), and the rigid vegetation (right panels; c and f) at $H = 0.2$ m (top panels) and $H = 0.4$ m (bottom panels). Positive values indicate upward swimming and negative values indicate downward swimming. Data for the fastest free-stream velocities for the vertical cylinder are not shown due to sediment movement that biased the results for both water depths. Shaded areas represent an approximate width of the mixing layer in each case.
work at early life stages when other senses have not yet developed. The difference in swimming response with turbulence generated by the gravel obstacle (Figures 7a and 7d) and turbulence generated by the vegetation array (Figures 7c and 7f), even when the magnitude of turbulent kinetic energy is close to an order of magnitude higher for gravel (Figures 4a and 4d), suggests that at this stage, larvae can already sense this disruptions in the flow.

Numerical models are also needed to forecast the dispersion of grass carp larvae in natural streams. By computing the swimming speeds, we provide evidence that larvae do not simply drift with the flow and behave as passive particles, but actually exhibit some resistance to the flow and respond to hydrodynamic conditions. Larvae oriented upstream in their swimming motion, increasing their swimming speeds (potentially equaling the horizontal flow velocity), when they encountered zones of increased turbulence. This previously unquantified behavior allows for a wider range of dispersal possibilities than would be predicted by passive drift models. Using mean velocity as a threshold is not enough to establish passage of larvae. Using turbulence intensity is also not enough to determine larval response. The type of turbulent coherent structure in the flow needs to be determined to get more accurate predictions of larval response, which can be determined through the assessment of vortex shedding frequency, turbulent integral time scale, turbulent integral length scale, autocorrelation functions, or frequency spectra analysis, depending on the type of data available (e.g., Tinoco et al., 2020). This study provides much-needed observational data and insight into larval behavior at early life stages which can improve larvae drift models, inform larval sampling strategies and techniques, and guide management decisions and control efforts.

### 3.3. Larvae Spatial Distribution

Centroids of larvae captured with PTV codes (as shown in the trajectories in Figure 5) were used to generate maps of their spatial distribution within the field of view of each obstruction. Figure 10 shows examples of these spatial distributions for each case with $H = 0.2$ m (Figures 10a–10c) and $H = 0.4$ m (Figures 10d–10f) with free-stream velocity $U_c$ of 0.064 m/s and 0.113 m/s, respectively ($F = 15$ Hz). Each pixel in these plots represents an area of $1 \times 1$ cm and the color indicates the percentage of larval centroids detected in each pixel compared to the total number of larval centroids detected in the field of view during 1 min. Because a larva can be captured in the same pixel in consecutive frames, this ratio indicates how frequently larvae are observed at a specific location, rather than the total number of different larvae that pass through a pixel.

We interpret the high concentrations of larvae in the low-velocity zone behind the gravel bump as indication that the larvae actively swim toward this zone (Figures 10a and 10d). In this zone, larvae were subjected to lower drag forces than in the high-velocity region above it, allowing larvae to freely swim in any direction, and yet they opted for areas close to the obstruction. In high-velocity regions, however, larvae show a clear preference for swimming against the current (Prada et al., 2020), a behavior known as rheotaxis, which is common but not universal among drifting fish larvae (Lechner et al., 2016). However, in the high-velocity regions of the present experiments and those of Prada et al. (2020), larval swim speeds ($u_{sw}$) were insufficient to overcome the mean longitudinal flow velocity ($U_c$) resulting in all larvae traveling downstream even for the slowest case of $U_c = 0.044$ m/s and $H = 0.2$ m.

The mean and turbulent flow field for the gravel bump and rigid vegetation cases differ significantly from the flow field generated by the single cylinder (Figures 3 and 4). This is reflected in the observed swimming speeds (Figures 7 and 8) and the spatial distribution of larvae (Figure 10). Such differences indicate that a single flow parameter (whether mean velocity, or turbulence intensity) cannot be used to accurately predict larval response, but rather the combined effects of velocity, velocity gradients, turbulent kinetic energy, and type of turbulence (e.g., turbulent length scales and time scales) must be considered for more accurate predictions.

The mean velocity fields for the gravel bump and the rigid vegetation (Figures 2a, 2c, 2d, and 2f) are similar, but they resulted in a very different distribution of larvae (Figure 10). Such differences indicate that a single flow parameter (whether mean velocity, or turbulence intensity) cannot be used to accurately predict larval response, but rather the combined effects of velocity, velocity gradients, turbulent kinetic energy, and type of turbulence (e.g., turbulent length scales and time scales) must be considered for more accurate predictions.
of velocity and larval distributions for the fastest velocity ($F = 25$ Hz, $U_c = 0.104$ m/s for $H = 0.2$ m or $U_c = 0.187$ m/s for $H = 0.4$ m), spatially averaged over the longitudinal coordinate $x$ along the field of view of our PIV sets, for both gravel bump and vegetation for both water depths (Figure 11) clearly show a different response, with larvae choosing to occupy the low-velocity region behind the gravel bump, while choosing to occupy the high-velocity region above the array of rigid vegetation, consistently for both water depths investigated.

Given the disparities in larval distribution with similar mean velocity profiles, we focus next on the turbulence metrics, $k$ and $\tau_{xz}$, which reveal further insight on the larval distribution across the water depth (Figure 11). Comparing the gravel bump (Figures 11c and 11i) to rigid vegetation (Figures 11d and 11j) we see that magnitudes of $k$ are about one order of magnitude higher for the gravel bump compared to the vegetation for both water depths. However, the maximum stress, $\tau_{xz}$, is about the same order of magnitude for both obstructions, and our data show that larvae avoid areas with peak turbulent stresses (Figures 11e, 11f, 11k, and 11l) for both the gravel bump and rigid vegetation cases. In the case of the rigid vegetation, larvae were distributed either below the mixing layer, or above it, even when it meant facing higher drag forces in the high-velocity zone (Figures 11f and 11l).

Data from the fastest flow condition (Figure 11) show a larval spatial distribution that is more correlated with values of $k$ and $\tau_{xz}$ than with mean velocity. In order to identify whether there is a threshold condition which could be associated with a response in larval behavior, we calculated the turbulence properties for all four flow conditions for the gravel bump (Figure 12) and rigid vegetation cases (Figure 13). For the gravel bump, we see that for $F = 10$ and 15 Hz ($U_c = 0.084$ and 0.113 m/s, respectively) larvae were distributed...
within and across the mixing layer (Figure 12a and 12b), presumably because turbulence levels were low enough to allow undisturbed passage between layers. However, for $F = 20$ Hz ($U_c = 0.150$ m/s) when peak turbulence levels reached $k = 1.0$ m$^2$/s$^2$ and $\tau_{xz} = 25$ N/m$^2$, there was a notable reduction in the percentage of larvae present in the mixing layer (Figures 12c and 12g). Such a decrease persists with higher flows (Figures 12d and 12h), and larvae entrapped in the lee of the bump remained swimming freely in this zone instead of lying on the bed as passive particles would do.

For rigid vegetation, even if the magnitudes of $k$ were one order of magnitude smaller than for the gravel bump, larvae clearly avoid the high shear region of the mixing layer once the peaks exceeded $k = 0.05$ m$^2$/s$^2$ or $\tau_{xz} = 10$ N/m$^2$ for $F = 20$ Hz ($U_c = 0.150$ m/s, Figures 13c and 13g). In contrast with gravel where most larvae opt for the low-flow recirculation bubble behind the obstacle, we see larvae opting for regions of high velocity far above the canopy with fewer in the low-speed area near the bed. Prada et al. (2020), showed that there is an actual active response from grass carp larvae to these types of flows. They compared trajectories from grass carp eggs, live larvae, and dead larvae, and showed that dead larvae were clearly present in the areas that live larvae tried to avoid (Figures 3–5 in Prada et al., 2020), concluding that an active response, rather than purely passive hydrodynamic transport, was responsible for the observed spatial distributions.

To further assess the turbulent coherent structures in each scenario, we estimate relevant turbulent time and length scales. As a first-order approximation, we can estimate the frequency of eddies generated behind a blunt body based on the Strouhal number, $S = fL/b / U_b$, where $f$ is the frequency of vortex shedding, $L_b$ is a representative length scale of the body (in our case, size of the gravel bump, diameter of the single pile, and diameter of individual cylinders), and $U_b$ is the velocity approaching the obstructions. The approaching velocity for the gravel bump and single pile can be considered as the unaltered velocity, $U_c$, from Table 1, since there are no other obstructions upstream. However, the array cylinders are already experiencing the altered velocity within the array, such that the reduced within-array velocity should be used as $U_b$. Assuming a value of $S = 0.21$ (Williamson, 1996), we have shedding frequencies in the range of 0.08–0.33 Hz for gravel bump, 0.18–0.77 Hz for the single pile, and 0.66–0.98 Hz for the vegetation array. Such frequencies correspond to ranges of time scales, $T_b = f^{-1}$, of 12.99–3.06 s for gravel, 5.52–1.30 s for

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**Figure 11.** Profiles of larvae distributions (dashed blue line) and flow properties ($U$, $k$, and $\tau_{xz}$, solid red lines) for gravel bump and vegetation cases at the highest free-stream velocities tested ($U_c = 0.104$ and 0.187 m/s, for $H = 0.2$ m (top panels) and 0.4 m (bottom panels), respectively). The panels show mean velocity $U$ for the gravel bump (a and g) and rigid vegetation (b and h), turbulent kinetic energy $k$ for the gravel bump (c and i) and rigid vegetation (d and j) and Reynolds’ stresses $\tau_{xz}$ for the gravel bump (e and k) and rigid vegetation (f and l).
single pile, and 1.52–1.02 s within the vegetation array. Hence, larvae are experiencing faster eddies generated by the smaller individual cylinders within the vegetation array, compared to slower eddies generated by the larger gravel obstruction, suggesting that these shorter, smaller eddies can have an effect on larval swimming capabilities, resulting in larvae avoiding these regions.

To estimate characteristic length scales, we use the autocorrelation function, $\rho_{uu}(r) = \langle u'(x)u'(x+r) \rangle / \langle u'(x)^2 \rangle$ (where $x$ is the longitudinal location and $r$ is the spatial lag between samples), to calculate the turbulent integral length scale, $L_T = \int_0^\infty \rho_{uu}(r) dr$ (Pope, 2000), at each vertical location on our PIV data, to estimate the size of the predominant eddies in the flow. For the gravel obstruction, we get length scales up to 0.068 m, whereas length scales for the vegetated array case stay below 0.025 m. Comparing time and length scales of each scenario with the recorded spatial distributions, larvae are apparently more sensitive to three-dimensional small-scale turbulent eddies of similar size that their body length (i.e., stem-scale eddies from the

Figure 12. Profiles of larvae distributions (dashed blue line) and turbulence properties ($k$, and $\tau_{xz}$, solid red line) for the gravel bump case with $H = 0.4$ m for all free-stream velocities ($U_c$) tested (denoted above each column).
vegetation array, scaled with the diameter of individual cylinders, $d = 6.4 \text{ mm}$) than to large-scale vertical eddies generated by bulkier obstructions (e.g., gravel case), even if the latter results in higher turbulence intensities.

4. Conclusions

We subjected grass carp larvae to a variety of hydrodynamic forces of different magnitudes and scales in a laboratory flume with three different configurations representing common in-stream obstructions. In one case, a vertical cylinder in the flow created a turbulent wake ($Re_\lambda \sim 10^3$) for all of the flow velocities tested. This obstacle generated distributed turbulence throughout the water depth causing larvae to move downstream, avoiding the regions of increased turbulence (consistent with data from Figures 11–13 for gravel bump and vegetation cases). Larvae seemingly avoid turbulence, in a possible effort to minimize energy

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**Figure 13.** Profiles of larvae distributions (dashed blue line) and turbulence properties ($k$, and $\tau_{xz}$, solid red line) for rigid vegetation case with $H = 0.4 \text{ m}$ for all free-stream velocities ($U_c$) tested (denoted above each column).
expenditure (Müller et al., 2000). This is consistent with the other two cases (the gravel bump and rigid vegetation Figures 7-9) where we interpret the distribution of larvae within the water column to be indicative of the need for larvae to swim faster in areas of higher turbulence, resulting in higher expenditures of energy (Müller et al. 2000). In contrast to the gravel bump and rigid vegetation, the vertical cylinder did not create any low-velocity low-turbulence zone attractive for larvae.

The gravel bump generated a recirculation zone with low levels of drag and turbulent forces, where larvae accumulated once the mean flow increased to high levels. We interpret the accumulation of larvae to result from the increased turbulence in the mixing layer (k > 1.0 m²/s² or τ ≥ 25 N/m²) that larvae seek to avoid. A similar situation occurred for vegetation, where the mixing layer along the top of the canopy seems to have prevented larvae from swimming into the vegetation after turbulence exceeded k > 0.05 m²/s² or τ ≥ 10 N/m².

Despite the similarity of the mean velocity profile and mixing layers generated by the gravel bump and the rigid vegetation, differences in the coherent turbulent structures explain the differences in larval response to different turbulent metrics. In rigid vegetation, there are stem-scale eddies mostly located within horizontal (x-y) planes within the canopy that are of the same size as the larvae (6-7 mm). These coherent flow structures, combined with entrainment of canopy-scale eddies within vertical planes (since the array satisfies the dense condition ah > 0.1) create a complex three-dimensional flow that seems to be adverse to larval swimming capabilities. In contrast, the gravel obstacle provided full shelter from high drag and turbulent forces behind the obstacle. It created a sharper velocity gradient and more differentiated layers of flow. Larvae in the recirculation zone behind the gravel experienced turbulent eddies larger than their body length, shed by the flow passing above the gravel bump and contained mainly in vertical planes.

At early development stages, such as the ones tested in our study, larvae have not yet developed other senses (e.g., vision, hearing, chemosense). Our data showed that they are already responsive to mechanical stimuli driven by turbulence. Looking at larval response as we increase velocities (Figures 12 and 13), we notice that a change in swimming paths and preferential areas occurs at different magnitudes of turbulent kinetic energy and shear stress for the gravel and vegetation cases. In particular, our data show that larvae are sensitive to the small-scale, three-dimensional features created by the array of cylinders (eddies of similar size as the larvae), which they seemingly avoid. Planar, large-scale turbulent eddies from the gravel obstacle, were seemingly easier to navigate for the small larvae, consistent with previous studies (Lacey et al., 2012) on the effect of relative size between coherent flow structures and fish length. The observed response from Grass carp larvae presents a promising path to implement turbulent stimuli targeted to early stage larvae, for capture or redirection in streams.

Correlation between flow characteristics and larval distributions can be used in the design of in-stream structures that attract or repel larvae from specific zones as desired. Where they are invasive, grass carp larvae could be attracted to zones where they can be easily monitored or harvested, or repelled from zones with high food availability where they would otherwise thrive. Where grass carp are desirable components of the biota, but in decline, this understanding could be used to promote success of grass carp in finding and entering quality nursery habitat. Field collections of larvae are important for determining where grass carp are currently reproducing and recruiting new individuals to the population, and these data can help inform sampling techniques for greater success.

**Data Availability Statement**

Data used in the analyses described herein can be found online as a U.S. Geological Survey data release at the ScienceBase-Catalog at https://doi.org/10.5066/P9P81CUX.

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