

RESEARCH ARTICLE

Streamwise vortices destabilize swimming bluegill sunfish (*Lepomis macrochirus*)

Anabela Maia^{1,*}, Alex P. Sheltzer² and Eric D. Tytell²

ABSTRACT

In their natural environment, fish must swim stably through unsteady flows and vortices, including vertical vortices, typically shed by posts in a flow, horizontal cross-flow vortices, often produced by a step or a waterfall in a stream, and streamwise vortices, where the axis of rotation is aligned with the direction of the flow. Streamwise vortices are commonly shed by bluff bodies in streams and by ships' propellers and axial turbines, but we know little about their effects on fish. Here, we describe how bluegill sunfish use more energy and are destabilized more often in flow with strong streamwise vorticity. The vortices were created inside a sealed flow tank by an array of four turbines with similar diameter to the experimental fish. We measured oxygen consumption for seven sunfish swimming at 1.5 body lengths (BL) s^{-1} with the turbines rotating at 2 Hz and with the turbines off (control). Simultaneously, we filmed the fish ventrally and recorded the fraction of time spent maneuvering side-to-side and accelerating forward. Separately, we also recorded lateral and ventral video for a combination of swimming speeds (0.5, 1.5 and 2.5 BL s^{-1}) and turbine speeds (0, 1, 2 and 3 Hz), immediately after turning the turbines on and 10 min later to test for accommodation. Bluegill sunfish are negatively affected by streamwise vorticity. Spills (loss of heading), maneuvers and accelerations were more frequent when the turbines were on than in the control treatment. These unsteady behaviors, particularly acceleration, correlated with an increase in oxygen consumption in the vortex flow. Bluegill sunfish are generally fast to recover from roll perturbations and do so by moving their pectoral fins. The frequency of spills decreased after the turbines had run for 10 min, but was still markedly higher than in the control, showing that fish partially adapt to streamwise vorticity, but not completely. Coping with streamwise vorticity may be an important energetic cost for stream fishes or migratory fishes.

KEY WORDS: Oxygen consumption, Fish swimming, Respirometry, Turbulence, Kinematics

INTRODUCTION

Swimming is a metabolically costly activity and fish have developed different strategies to reduce energy expenditure (Bone, 1975; Pettersson and Hedenström, 2000). For example, oxygen consumption in fish increases at higher temperature or faster swimming speeds (Claireaux et al., 2006; Fuiman and Batty, 1997; Lee et al., 2003). Less is known about the effects of turbulence and unsteadiness in the flow, in part because turbulence itself is such a complex phenomenon. For example, vortex flows, which are

unsteady but not necessarily turbulent, can have vortices with different sizes and intensities, with varying degrees of predictability, or with different orientation (Lacey et al., 2012). Studies of fish behavior in vortex flows have had mixed results. Some fish species are able to take advantage of vertical columnar vortices to decrease metabolic costs, by entrainment and Kármán gaiting (Liao, 2007; Przybilla et al., 2010; Taguchi and Liao, 2011). These studies have focused mostly on vortices shed by horizontal and vertical stationary half-cylinders (Liao, 2007; Taguchi and Liao, 2011; Tritico and Cotel, 2010). Similarly, several groups have found increased fish abundance in areas of streams with high turbulence intensity (Smith et al., 2005; Van Zyll De Jong et al., 1997). Horizontal cylinders, in contrast, destabilize several species of fishes (Eidietis et al., 2002; Webb, 1998; Webb and Cotel, 2010). Finally, other studies have found that generalized turbulence has negative impacts on energetic demands of some fish species (Enders et al., 2003; Lupandin, 2005; Roche et al., 2014; Tritico and Cotel, 2010). Small turbulent eddies may also interfere with sensory receptors (Webb and Cotel, 2010).

Lacey et al. (2012) suggested that some of the diversity in the results could be organized by considering the intensity, periodicity, orientation and size of vortices in a wake, which they termed the 'IPOS' (intensity, periodicity, orientation and size) framework. This study fills a gap in the IPOS framework, by considering streamwise vortices, a class of vortex that has received very little attention. Streamwise vortices are shed as part of the 'horseshoe' vortex that forms in the wake of a bluff body such as a boulder (Roy et al., 2004; Smith et al., 2005). In fact, because of the boundary layer in streams, any vertical vortices will tend to tilt, becoming more streamwise in orientation. Therefore, this type of turbulence is likely to be very common in streams, and could have a greater impact in lentic freshwater fishes than other turbulent features. This class of turbulence is also commonly generated by boats, hydropower turbines and other propellers. Understanding how different classes of turbulent flows affect fish is essential to mitigate the effects of anthropogenic pressures in freshwater habitats (Lacey et al., 2012).

We chose to test the effect of streamwise vorticity on a species whose locomotion has been extensively studied, the bluegill sunfish *Lepomis macrochirus* Rafinesque 1819. Bluegill sunfish swim with a pectoral fin gait at low swimming speeds, switching to body and caudal fin locomotion at higher speeds (Gibb et al., 1994; Standen and Lauder, 2005; Tytell, 2006). We have data on the contributions of each fin to the wake structure during steady swimming locomotion (Drucker and Lauder, 2001; Tytell, 2006). This species has also been used in studies that examined maneuvering behaviors such as escape responses, maneuvering through complex habitats, braking and accelerating, as well as responses to small hydrodynamic perturbations (Drucker and Lauder, 2001; Ellerby and Gerry, 2011; Flammang and Lauder, 2009, 2013; Higham, 2007; Standen, 2010; Standen and Lauder, 2005; Tytell and Lauder, 2008). The cost of transport for this species has also been shown to

¹Eastern Illinois University, Department of Biological Sciences, 600 Lincoln Avenue, Charleston, IL 61920, USA. ²Tufts University, Biology Department, 200 Boston Avenue, Medford, MA 02155, USA.

*Author for correspondence (amresendedamaia@eiu.edu)

Received 22 September 2014; Accepted 23 December 2014

List of symbols and abbreviations

BL	body length
<i>f</i>	frequency
IPOS	intensity, periodicity, orientation and size
M_{O_2}	mass-corrected rate of oxygen consumption
PIV	particle image velocimetry

increase with the gait transition from pectoral fin to caudal fin locomotion (Kendall et al., 2007). However, it seems that fish of this species are not able to produce enough thrust with the pectoral fins alone at higher speeds, thus explaining the costly gait transition (Kendall et al., 2007).

Streamwise vorticity is expected to cause mostly rolling disturbances, while cross-flow, horizontal vortices would cause pitching disturbances and vertical vortices would cause yawing disturbances. Fish have been observed to strongly avoid behaviors that lead to roll (Eidietis et al., 2002). Fish also respond more quickly to roll perturbations, compared with yaw, slip, heave and pitch disturbances (Webb, 2004). We therefore hypothesized that streamwise vorticity would cause a greater increase in oxygen consumption than other orientations of unsteady vortex flows.

In this study, we investigated oxygen consumption and kinematics of bluegill sunfish at various flow speeds and turbine speeds associated with distinct values of streamwise vorticity. Based on our hypothesis, we predicted that the vortices would cause spills and corrections of heading. Oxygen consumption was also expected to increase in the presence of such disturbances. In addition, because higher turbine frequencies will cause higher vortex intensities, resulting in stronger perturbations to the fish, we predicted that higher turbine frequencies would produce stronger corrective reflexes that require more oxygen.

RESULTS**Characterization of the vortices**

An array of four small turbines (6 cm diameter) was placed at the upstream end of a flow tunnel (Fig. 1). The turbulence generated by the turbines was characterized using particle image velocimetry (PIV) for the combinations of the three different speeds [0.5, 1.5 and 2.5 body lengths (BL) s^{-1} , based on the average length of the fish] and three turbine frequencies (0, 1, 2 and 3 Hz). Horizontal light sheets indicated that flow was not substantially reduced in the wake of the turbines. To measure the strength of the vortices, flow patterns were measured in a transverse (cross-flow) plane, 10 cm downstream of the turbines, over a period of 3.33 s (1000 frames). Two of the four vortices were imaged simultaneously. Fig. 2 shows the strength of streamwise vortices for different mean flow speeds and turbine frequencies. The mean circulation for the vortices seen in Fig. 2A over the time period is $106 \pm 13 \text{ cm}^2 \text{ s}^{-1}$ (mean \pm s.d.) and the mean radius is $2.2 \pm 0.4 \text{ cm}$. No systematic differences were observed among the four vortices. On average, the diameter of the vortices was $100 \pm 1\%$ of the body depth of the fish (not including the

height of the dorsal or anal fins). The mean peak vorticity (99th percentile) increased with both turbine frequency and flow speed. Circulation increased with increasing turbine frequency, but changed only marginally with increasing flow speed. In contrast, vortex radius decreased at higher flow speeds, but did not change much when turbine frequency changed. At low flow speeds, vortices seemed to be unstable, and would twist around one another. At higher flow speeds, the position was more consistent. Interactions between the four vortices being shed were only observed at low flow speeds.

Kinematics

Streamwise vortices cause bluegill to spill more often and to swim more unsteadily, spending a greater fraction of time accelerating forward or maneuvering from side to side. Spilling events (loss of heading accompanied by head turning, movement downstream and subsequent recovery; Fig. 3A), were virtually absent in control trials, but were common events in unsteady flow (see supplementary material Movies 1 and 2). Spills were more frequent when the turbines were on than in the control treatment at all speeds (Fig. 3A, $\chi^2=30.95$, $P=0.001$). At a swimming speed of 1.5 BL s^{-1} , spill rate increased more than 10-fold from the control ($0.3 \text{ spills min}^{-1}$) to the turbines on at 2 Hz ($4.5 \text{ spills min}^{-1}$), the same conditions as in our respirometry trials. Bluegill sunfish are generally fast to recover from roll perturbations and do so by moving their pectoral fins simultaneously to produce acceleration. The speed at which the fish were swimming did not affect spill rate (Fig. 3A, $\chi^2=2.135$, $P=0.344$). Individuals did not differ significantly for any of the variables tested ($P>0.05$ in all cases).

Fish accelerated forward and maneuvered more often when the turbines were running. As shown in Fig. 3B, accelerations were 50% more frequent under the turbulent regime (6% of total swimming time) than in the control (4%, $F=8.168$, $P=0.01$). The frequency of maneuvers also increased when the turbines were on (from 5% to 8% of total swimming time, $F=14.632$, $P=0.001$, Fig. 3B).

The unsteady movements were also stronger when the turbines were running (Fig. 4). The strength of accelerations, maneuvers and spills was assessed by measuring the average amplitude of any changes in velocity that took longer than one tail beat. We defined accelerations, maneuvers and spills as behaviors that lasted for longer than one tail beat, and thus had frequencies lower than the tail beat frequency. If such movements are present, they will be visible in the low frequency components of the Fourier power spectrum; the higher the average movement amplitude, the larger the low frequency power. For simplicity, we defined low frequency as less than 1 Hz. We estimated the mean amplitude of the behavior by taking the square root of the integral of the signal power at frequencies between 0 and 1 Hz. Accelerations corresponded to the amplitude of forward–back velocity fluctuations, maneuvers to the lateral velocity, and spills to the amplitude of angular velocity fluctuations. At the lowest swimming speed, additional streamwise vorticity increased the strength of accelerations and spills ($\chi^2=7.98$,

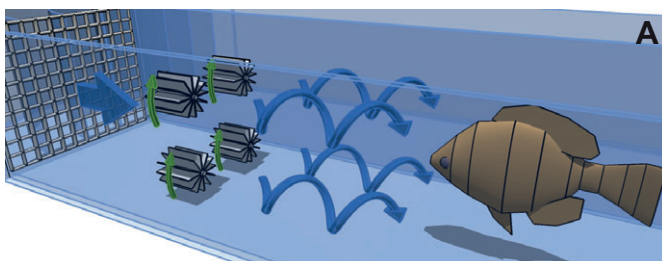


Fig. 1. Experimental setup for respirometry and kinematics experiments. (A) Schematic representation of the experimental design. Turbines are shown in gray with green arrows to indicate rotation. Streamwise vortices and mean flow are shown with blue arrows. (B) Detail of the turbine array.

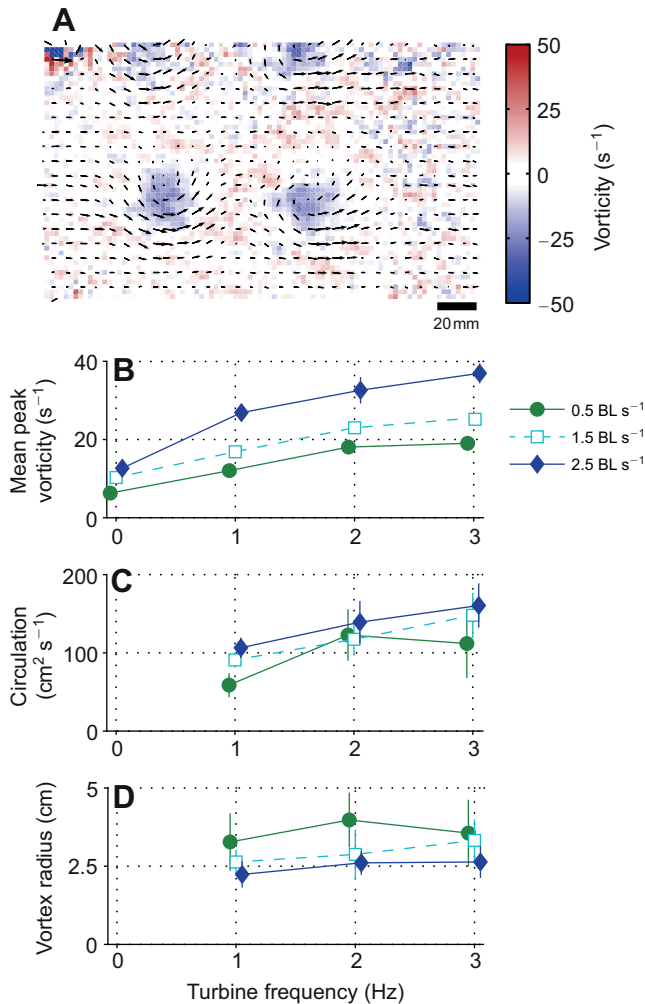


Fig. 2. Vortex patterns caused by the turbines. (A) A representative flow field, 10 cm downstream of the turbines [2 Hz turbine speed; 1.5 body lengths (BL) s⁻¹ flow speed]. (B) Mean peak vorticity (99th percentile) at different turbine rotational frequencies and mean flow speeds (see key). (C) Mean circulation of the vortices. (D) Mean radius. Error bars are s.d. ($N=10$ frames); where they are not visible, they are smaller than the symbols.

$P=0.463$; $\chi^2=9.07$, $P=0.0284$, respectively). Accelerations and maneuvers were both stronger at higher swimming speeds, with little effect due to turbine frequency (Fig. 4A,B). At 1.5 BL s⁻¹, spill strength increased when the turbines were on, but it did not change substantially at the highest swimming speed (Fig. 4C).

To test for adaptation, we compared the frequency of spills immediately after the turbines started with the spill frequency after the turbines had run for 10 min (Fig. 5). The spill frequency decreased from 5.6 to 3.6 spills min⁻¹ after the turbines had run for 10 min ($\chi^2=4.684$, $P=0.03$), but was still markedly higher than in the control (0.5 spills min⁻¹). Therefore, fish can adapt to streamwise vorticity, but not completely (Fig. 5).

Respirometry

Oxygen consumption in the control trials (with no added turbulence) averaged 309 mg O₂ kg⁻¹ h⁻¹. Assuming that fish can convert oxygen into mechanical energy at a rate of 14.1 J mg⁻¹ O₂ during aerobic metabolism (Videler, 1993), this corresponds to 1.21 W kg⁻¹. Fig. 6A shows the decrease in oxygen in the tank for the same fish in control and vortex flow, demonstrating a more rapid decrease in oxygen when the streamwise vortices were present. In

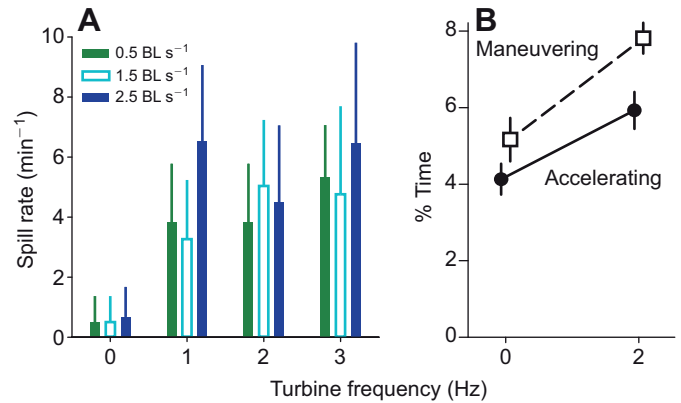


Fig. 3. Spills, yaw maneuvers and forward accelerations increased in frequency when the turbines were running. (A) Spill rate (\pm s.d.) as a function of turbine frequency and swimming speed (see key). (B) Percentage of total swimming time spent maneuvering or accelerating when the turbines were off or on.

the presence of turbulence, the mean mass-corrected rate of oxygen consumption (\dot{M}_{O_2}) increased by 18 mg O₂ kg⁻¹ h⁻¹ (0.07 W kg⁻¹) on average compared with the control ($t=-2.294$, $P=0.027$, Fig. 6B), but by as much as 58 mg O₂ kg⁻¹ h⁻¹ (0.23 W kg⁻¹) in 25% of cases.

There was a positive correlation between the frequency of accelerations and the rate of oxygen consumption in turbulence (Fig. 7A), but not in the no added turbulence control. Each point in Fig. 7 is from a single trial, with different individuals represented with specific symbols. Four individuals were selected at random for this detailed analysis. Certain individuals were able to compensate for the vorticity better than others, with fewer accelerations and spills, and thus a lower energy cost. Others accelerated a lot, nearly doubling their oxygen consumption. In contrast, the frequency of maneuvers did not affect oxygen consumption in either condition (Fig. 7B).

DISCUSSION

In the wild, fish encounter unsteady flows frequently. In some cases, fish can extract energy from vortices (Liao, 2007; Liao et al., 2003a; Taguchi and Liao, 2011), but in other cases, maintaining heading in unsteady flows increases energy cost (Cotel et al., 2006; Enders et al.,

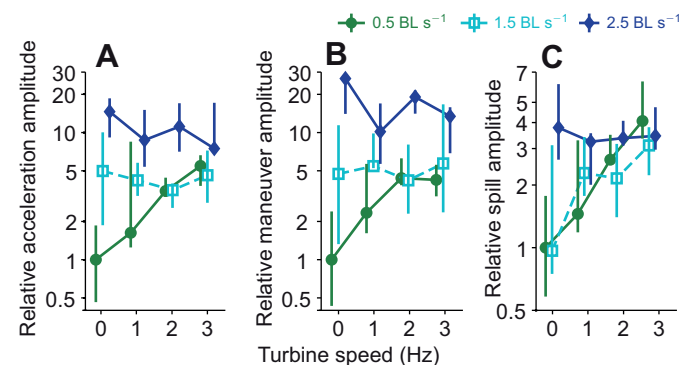


Fig. 4. Amplitude of accelerations, maneuvers and spills at different swimming speeds and turbine speeds. The panels show the square root of the integrated power in the low frequency ($0 < f \leq 1$ Hz) portion of the fast Fourier transform (FFT) power spectrum for forward velocity (A), lateral velocity (B) and body angular velocity (C). In each case, the values are shown on a logarithmic scale relative to the mean signal power for swimming at 0.5 BL s⁻¹ with no streamwise vorticity. Error bars connect the 25th and 75th percentiles.

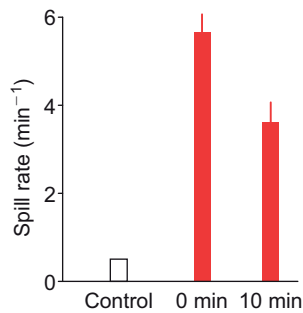


Fig. 5. Spill rate decreased after 10 min of accommodation. Spill rate for control and for all speeds (0.5, 1.5, 2.5 BL s⁻¹) and turbine frequencies combined (1, 2, 3 Hz) immediately after turning the turbines on and 10 min later. Error bars represent s.d.

2003; Roche et al., 2014). Because fish respond strongly to roll perturbations (Eidietis et al., 2002), we hypothesized that streamwise vortices would destabilize swimming fish and increase the energy cost. Here, we have shown that bluegill sunfish do indeed use more energy when forced to swim in streamwise vortices. We observed a 6% average increase in oxygen consumption due to the vortices in contrast with the control condition (no added turbulence), but with a wide individual variation, from -19% to 82%. Energy cost increases partly because the fish must maintain an upright posture despite perturbations from the vortices, but also because the fish swim more unsteadily: streamwise vortices increase the rate of maneuvers and accelerations, as well as losses of heading (spills).

Most previous work on the interaction of fish with vortices has focused on either vertical or horizontal cross-flow vortices in the wake of cylinders (Liao et al., 2003b; Taguchi and Liao, 2011; Tritico and Cotel, 2010; Webb, 1998). Creek chub (*Semotilus atromaculatus*) spill more often in the wake of horizontal cylinders than in that of vertical cylinders, and the chub's critical swimming speed was lower when the turbulence was high (Tritico and Cotel, 2010). Behind vertical cylinders, some fish can reduce the cost of swimming by harnessing energy from the vortices using a swimming pattern called the Kármán gait (Liao et al., 2003b; Taguchi and Liao, 2011).

Our study is innovative compared with these previous ones that used cylinders, because the turbines actively generate vorticity, but do not reduce the overall flow speed. Therefore, the effects that we see can be attributed specifically to the interaction with the vortices, and not to other behavioral strategies. In contrast, near cylinders, fish can avoid interacting with the vortices by behaviors such as

drafting, riding the bow wake, or entraining, all strategies that reduce their energy consumption (Liao, 2007). When fish draft or ride the bow wake, they take advantage of the slow flow immediately behind and in front of the cylinder (Taguchi and Liao, 2011; Webb, 1998). When they entrain, they use the shear layer on the edge of the cylinder's wake to reduce energy consumption (Przybilla et al., 2010; Taguchi and Liao, 2011).

Other studies that examined disorganized turbulence have also found a detrimental effect of turbulence in general. Turbulence reduces critical swimming speed for perch (*Perca fluviatilis*), particularly when average eddy diameter is close to the size of the fish (Lupandin, 2005). It also increases energy consumption for juvenile Atlantic salmon (*Salmo salar*) (Enders et al., 2003). Unsteady wave-like motion also increases costs in shiner surfperch (*Cymatogaster aggregata*) (Roche et al., 2014).

One reason why swimming takes more energy in streamwise vorticity is that many of the fish swim much less steadily (Figs 3 and 4). Forward accelerations, side-to-side maneuvers and spills were all more frequent, and often larger in amplitude, when the turbines were on. Tritico and Cotel also found that large horizontal vortices, similar in size to our streamwise vortices, caused chub to spill more often than in steady flow (Tritico and Cotel, 2010). We found that fish that accelerated more often used significantly more energy (Fig. 7). Indeed, the upper 25% of oxygen consumption measurements shown in Fig. 6 includes the individuals with the highest percentage time for accelerations in Fig. 7A.

A second reason for increased energy costs in the vortices is that fish must maintain their upright orientation. Streamwise vortices will tend to cause the fish to roll. However, we did not observe any unusually rolling behavior. Maintaining upright posture despite the perturbations from the vortices therefore likely contributes to the increased energy cost. Indeed, we often observed unusual, unilateral pectoral fin use (see supplementary material Movie 3), indicating that the fish must coordinate their fins in unusual, and possibly energetically costly ways to maintain upright posture.

We saw few substantial differences in the effects of the vortices as we increased the turbine speed (Figs 3 and 4). This lack of difference may be a consequence of the turbine design; circulation and vortex radius did not change much as we increased the turbine speed (Fig. 2). It may be that the turbines had too many blades. As rotation speed increased, they may not have been able to entrain enough fluid to impart additional rotational momentum.

Although our study showed that streamwise vorticity increases swimming costs in a similar way to that found in other studies

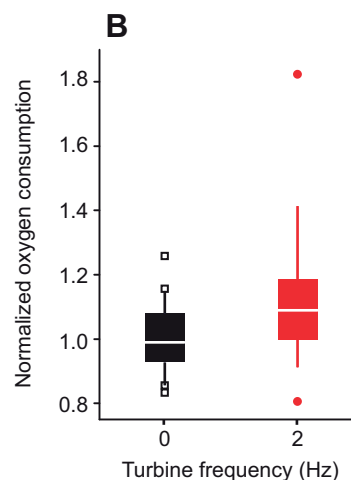
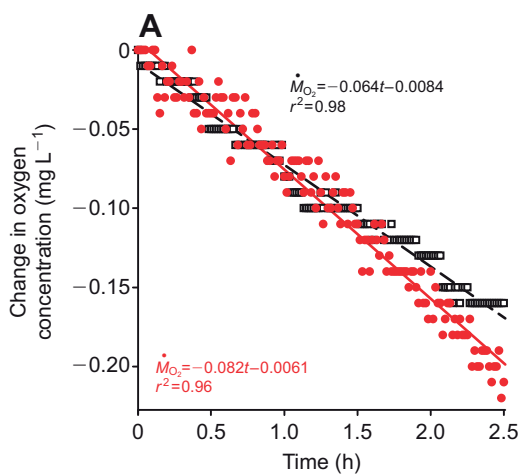


Fig. 6. Oxygen consumption under turbulent and control flows. (A) Representative traces of different trials for the same individual showing raw oxygen concentration data fitted with linear regressions with the turbines off (0 Hz frequency, open squares) or on (2 Hz frequency, red circles). M_{O_2} , mass-corrected rate of oxygen consumption. (B) Mass-corrected oxygen consumption standardized by the control mean for each fish separately. Bars represent 25th and 75th percentiles.

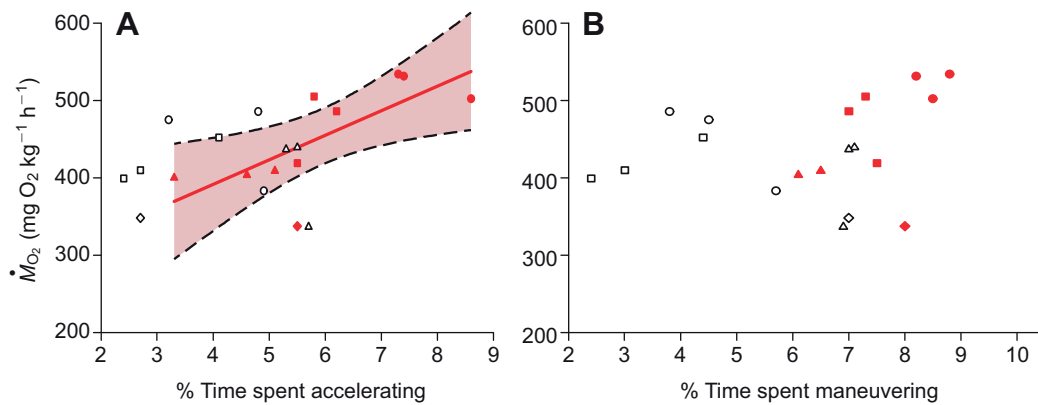


Fig. 7. Relationship between oxygen consumption and kinematic events. \dot{M}_{O_2} plotted against the percentage of time spent accelerating (A) or maneuvering (B) with turbines off (0 Hz frequency, open symbols) and running at 2 Hz (red symbols). Different symbols represent different individuals. The only significant relationship is with accelerations under turbulence, shown with a red line in A. The shaded region in A represents the standard error in the estimate of the slope.

(Enders et al., 2003; Roche et al., 2014; Tritico and Cotel, 2010), the increases were smaller on average than those seen in other studies. We measured an average 6% increase in oxygen consumption in streamwise vorticity. Enders et al. (2003) showed that disorganized streamwise turbulence can cause trout to increase oxygen consumption by 31–60%, depending on the speed of the base flow. Roche et al. (2014) found that surf perch increase oxygen consumption in wave flow by 25.3% on average.

We hypothesized that streamwise vorticity might be more detrimental to fish than horizontal or vertical vortices. Instead, our results suggest that streamwise vortices may have a relatively small impact on bluegill sunfish. The vortices were probably large enough to cause an effect on the fish, because they were the same diameter on average as the fish's body depth, not including the depth of the dorsal and anal fin. Increasing the vortex size so that the vortices were the same diameter as the body and fin depth could cause a larger effect. Several studies have found that vortices with diameters less than about 30% of the fish's size have no effect, and that large effects were not observed until the vortices reached 75% of the fish's size (reviewed in Lacey et al., 2012).

Another possibility is that the streamwise orientation of the vortices may limit their impact. Horizontal cross-flow vortices or vertical vortices are shed periodically (Lacey et al., 2012). Thus, their effects may be different in time and position along a fish's body, as the vortices move downstream. Streamwise vortices, in contrast, should have effects that are similar all along a fish's body. Because fish generate force by accelerating fluid along their bodies, the differential effects of horizontal and vertical vortices may interfere more with the physical processes that fish use for generating thrust. Flow visualization will be necessary to understand exactly how fish are interacting with the vortices.

Individual fish also differ substantially in their ability to compensate for the vortices (Fig. 7). Some fish accelerate relatively little (Fig. 7A), and do not require much more energy than in the control case, while others accelerate a lot and use dramatically more energy (see outlier point in Fig. 6B, which corresponds to a fish that used 82% more energy in the vortices). All fish also learned to compensate better, although not completely, over time (Fig. 5). The differences in compensation and learning may be related to the unusual asynchronous fin motion that we often observed (supplementary material Movie 3). Perch subjected to comparable sized vortices also use asynchronous fin motion (Lupandin, 2005). Asynchronous fin movement has been shown

to be used by a variety of fish while maneuvering (Bartol et al., 2003; Standen, 2010; Webb, 2006).

Streamwise vorticity occurs in the wild both from anthropogenic pressures such as boats, energy turbines and other propellers and naturally from river confluences, meandering and interactions with obstacles in the river bed (Harrison et al., 2011; Roy et al., 2004; Smith et al., 2005). In the wild, streamwise vorticity might be more detrimental as it is not frequently constant or predictable. The metabolic costs we observed may be amplified in wild populations as wild fish may not be able to acclimate to constant vorticity like fish in the laboratory did (Fig. 5).

Conclusions

Fish must use more energy to swim in flow with streamwise vortices, which are shed off both rough elements on a streambed (Roy et al., 2004) and man-made devices like turbines and propellers (Whale et al., 2000), likely because they swim less steadily and move their pectoral fins in an unusual asymmetric way. The additional energy required is relatively low on average (6% over baseline swimming costs), but can be dramatically higher for certain individuals (up to an 82% increase). Some individuals swam more steadily in the vortices, using less energy, probably because they learned better how to compensate for the vortices; others swam less steadily and used more energy. Future work must examine how prevalent such vortices are naturally and how the effects change as the size of the vortices changes, so that we can better understand the role of vorticity and turbulence in the energy budget of fish in nature.

MATERIALS AND METHODS

Animals

Seven bluegill sunfish, *L. macrochirus*, were captured by beach seine in White Pond, Concord, MA, USA. The bluegill were housed individually in 10 gallon tanks at 19.5°C with pH 7.4 and fed live worms daily. Lights were kept on a day–night cycle that matched the time of year. The same animals were used first in the respirometry experiments and then in the kinematics experiments, with at least 1 week interval in between. Fish body length (BL) ranged from 12.5 to 15.1 cm (mean \pm s.d. = 13.3 \pm 1.0 cm), and mass ranged from 45.1 to 65.2 g (55.6 \pm 8.6 g). Fish body depth (dorsoventral, not including dorsal or anal fin depth) was 0.34 \pm 0.01 BL (4.4 \pm 0.1 cm) and width was 0.14 \pm 0.01 BL (1.8 \pm 0.2 cm). Husbandry and experiments complied with federal animal care and use standards and were approved by the Tufts University Institutional Animal Care and Use Committee.

Characterization of the vorticity

Four custom-designed turbines were printed in ABS plastic using a 3D printer (Stratasy Dimension 1200, Stratasy, Eden Prairie, MN, USA). The turbines

had a diameter of 6 cm with a blade length of 5 cm. We chose turbines of this size so that the vortices generated would be similar in diameter to the depth of the fish. Each turbine was spaced 2 cm from the surrounding turbines, blade to blade, 5 cm away from the tank walls (Fig. 1). The set of four turbines was placed at the upstream end of the working section of a flow tunnel (25×26×150 cm). PIV was used to characterize the turbulence generated by the turbines (Tytell, 2010). A continuous diode laser (Opus model, 5 W; Laser Quantum, Cheshire, UK) was used at 4 W together with optics to create transverse and horizontal sheets to characterize the turbulence shed by the turbines spinning at 1, 2 and 3 Hz with background flows of 0.5, 1.5 and 2.5 BL s⁻¹ (based on average bluegill sunfish length). Horizontal sheets were placed at the mid-turbine blade, the blade center and in between the turbines. The transverse sheet was placed across the flow, 10 cm behind the turbines, which was the area where fish spent most of their time. A Phantom Miro M120 (Vision Research, Wayne, NJ, USA) was used to gather high speed video at 500 frames s⁻¹ and PIV data were analyzed using Insight 4.1 (TSI Inc., Shoreview, MN, USA). The camera was placed downstream of the light sheet at ~30 deg angle to the flow. A full-field calibration was performed to correct distortion ('Off-axis PIV'; TSI Insight). To quantify the strength of the vortices, vortex centers were identified manually and circulation Γ was calculated, using the following equation: $\Gamma = \oint_S \mathbf{u} \cdot d\mathbf{l}$, where \mathbf{u} is the velocity vector and $d\mathbf{l}$ is the tangent vector along the circular contour S (Tytell, 2010). Circulation was calculated in contours of increasing radius until a maximum circulation value was reached; the vortex radius is the one with the maximum circulation. The 99th percentile of counter-clockwise vorticity was also calculated, as an estimate of the peak vorticity. Mean circulation, peak vorticity and vortex radius were calculated by averaging across 10 frames, taken every 0.5 s.

Respirometry

Fish were starved for 48 h before respirometry trials to account for specific dynamic action (Beamish, 1974). The fish were weighed and measured, then placed in a 293 l recirculating flow tank respirometer (Loligo Systems, Tjele, Denmark), equipped with a Pro Odo dissolved oxygen probe (YSI, Yellow Springs, OH, USA). The temperature in the tank was maintained between 19.5 and 20.5°C. The fish were confined to a 30 cm long section of the 25×26×150 cm (height×width×length) working section. For respirometry experiments, a DRS Lighting High-Speed RDT1 camera (DRS Technologies, Parsippany, NJ, USA) was used at 25 frames s⁻¹ to capture a ventral view. In order to sample kinematics consistently over the full trial duration, 6 s of video were recorded to disk for each 20 s of experimentation. For each individual fish, three trials were performed for each of two conditions: turbines off (control) and turbines rotating at 2 Hz, in a randomized order. Flow speed was kept at 1.5 BL s⁻¹. The trials were of 2.5 h duration, which was the minimum time to register a 2% decrease in oxygen concentration. Bluegill sunfish had no problem swimming for the entire duration of the trials and did not exhibit benthic station-holding behaviors or resting against the back of the working section. After each trial, the tank was flushed with oxygenated water for 1 h to return oxygen levels back to saturation. Background respiration rate was measured with no fish in the tank before and after the experiments and was found to be negligible.

Linear regressions were fitted to the oxygen concentration data over time and trials when the regression coefficient was lower than 0.95 were discarded. The slope of the regression was then multiplied by the tank volume and divided by the fish mass to obtain \dot{M}_{O_2} . The values were further normalized by the mean value for each individual in control trials.

Video from the respirometry experiments was analyzed by quantifying the fraction of time spent accelerating and maneuvering. Four individuals were selected randomly for detailed analysis. For the purpose of this study, we defined accelerations as periods when the fish swam faster than the incoming flow, resulting in a net movement upstream in the video frame, and we defined maneuvers as instances when the fish's head deviated more than 30 deg from parallel to the flow.

Kinematics experiments

Lateral and ventral views were recorded with two synchronized high speed cameras (Phantom Miro M120, Vision Research; and Lightning RDT1, DRS Technologies, Parsippany, NJ, USA), both recording at 100 frames s⁻¹. Fish swam in the working section at a constant speed of 0.5, 1.5 or 2.5 BL s⁻¹.

Turbines were then turned on at one of three frequencies (1, 2 and 3 Hz), and video was recorded for the next 10 s. Fish then swam in the vortex flow for 10 min, and video was recorded a second time for 32 s to look for adaptation. A total of four trials at each speed and turbine frequency (0, 1, 2 and 3 Hz) were obtained for four of the fish used in the respirometry experiments, in a randomized order of presentation.

Fish were considered to spill when (1) they lost their heading, as evident by a head angle of more than 20 deg to the midline and (2) they were pushed downstream in the flow tank by at least 1 cm. Yaw maneuvers, termed 'maneuvers' here, were identified when the fish changed its side-to-side position in relation to the turbines. Yaw maneuvers were distinguished from spills through a lack of downstream movement. Positive accelerations, here termed 'accelerations', were registered when the fish increased forward speed and moved forward in the working section by at least 1 cm.

From each video, the location of the snout, mid-body (between the pelvic fins) and tail tip were manually identified using Matlab (R2012b; Mathworks, Natick, MA, USA). A smoothing spline was used to smooth the data and estimate derivatives (following Walker, 1998). We defined accelerations, maneuvers and spills as behaviors that took longer than one tail beat. To quantify the amplitude of the behaviors, we arbitrarily set a cut-off at 1 Hz and analyzed the low frequency portion (0 < f ≤ 1 Hz) of the power spectra of the forward velocity, lateral velocity and body angular velocity. The intensity of each type of behavior was estimated by taking the square root of the integral of the power spectra over this frequency range.

Statistical analysis

To analyze the effect of the turbines on the rate of oxygen consumption, an ANOVA test was performed with turbine frequency and fish as the independent variable and normalized \dot{M}_{O_2} as the dependent variable. As no individual effects were observed, a *t*-test was used to compare the control and vortex flows at 2 Hz; *t*-tests were also used to compare the frequency of maneuvers and accelerations during control and turbulence conditions. Linear regressions were also used to determine the relationships between \dot{M}_{O_2} and frequency of accelerations and maneuvers. Frequency of spills, accelerations and yaw maneuvers were tested against turbine frequency (0, 1, 2 or 3 Hz), flow speed (0.5, 1.5 or 2.5 BL s⁻¹) and individual with ANOVA when the data were normally distributed and with Kruskal–Wallis tests when the assumptions could not be met. Adaptation to the turbines was also tested using Kruskal–Wallis to compare spill frequency in the three conditions: no turbulence, right after turning the turbines on and after 10 min. Statistical tests were performed using JMP software (SAS, Cary, NC, USA). Values reported are means and s.d., unless indicated otherwise.

Acknowledgements

The authors would like to thank Tarundeep Singh and Georgina Govostis for help with turbine design and video analysis, respectively. We would also like to thank the Museum of Comparative Zoology, Harvard University for field collection equipment.

Competing interests

The authors declare no competing or financial interests.

Author contributions

A.M. designed the experiments, conducted all experiments, coordinated the data analysis and wrote the manuscript. A.P.S. conducted part of the experiments and analyzed kinematic data. E.D.T. designed the experiments, conducted PIV experiments, analyzed data and revised the manuscript.

Funding

Funding for this research was provided by Tufts University, the National Science Foundation (NSF; RCN-PLS 1062052 to Lisa Fauci, P.I.) and an American Philosophical Society Franklin Research Grant (to A.M.).

Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.114363/-/DC1>

References

Bartol, I. K., Gharib, M., Weihs, D., Webb, P. W., Hove, J. R. and Gordon, M. S. (2003). Hydrodynamic stability of swimming in ostraciid fishes: role of the carapace in the smooth trunkfish *Lactophrys triqueter* (Teleostei: Ostraciidae). *J. Exp. Biol.* **206**, 725–744.

- Beamish, F. W. H.** (1974). Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. *J. Fish. Res. Board Canada* **31**, 1763-1769.
- Bone, Q.** (1975). Muscular and energetic aspects of fish swimming. In *Swimming and Flying in Nature*, Vol. 2, pp. 493-528. New York: Springer.
- Claireaux, G., Couturier, C. and Groison, A.-L.** (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). *J. Exp. Biol.* **209**, 3420-3428.
- Cotel, A. J., Webb, P. W. and Tritico, H.** (2006). Do brown trout choose locations with reduced turbulence? *Trans. Am. Fish. Soc.* **135**, 610-619.
- Drucker, E. G. and Lauder, G. V.** (2001). Wake dynamics and fluid forces of turning maneuvers in sunfish. *J. Exp. Biol.* **204**, 431-442.
- Eidietis, L., Forrester, T. L. and Webb, P. W.** (2002). Relative abilities to correct rolling disturbances of three morphologically different fish. **2163**, 2156-2163.
- Ellerby, D. J. and Gerry, S. P.** (2011). Sympatric divergence and performance trade-offs of bluegill ecomorphs. *Evol. Biol.* **38**, 422-433.
- Enders, E. C., Boisclair, D. and Roy, A. G.** (2003). The effect of turbulence on the cost of swimming for juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **60**, 1149-1160.
- Flammang, B. E. and Lauder, G. V.** (2009). Caudal fin shape modulation and control during acceleration, braking and backing maneuvers in bluegill sunfish, *Lepomis macrochirus*. *J. Exp. Biol.* **212**, 277-286.
- Flammang, B. E. and Lauder, G. V.** (2013). Pectoral fins aid in navigation of a complex environment by bluegill sunfish under sensory deprivation conditions. *J. Exp. Biol.* **216**, 3084-3089.
- Fuiman, L. A. and Batty, R. S.** (1997). What a drag it is getting cold: partitioning the physical and physiological effects of temperature in fish swimming. *J. Exp. Biol.* **200**, 1745-1755.
- Gibb, A., Jayne, B. C. and Lauder, G. V.** (1994). Kinematics of pectoral fin locomotion in the bluegill sunfish *Lepomis macrochirus*. *J. Exp. Biol.* **189**, 133-161.
- Harrison, L. R., Legleiter, C. J., Wyzga, M. A. and Dunne, T.** (2011). Channel dynamics and habitat development in a meandering, gravel bed river. *Water Resour. Res.* **47**, W04513. (<http://onlinelibrary.wiley.com/doi/10.1029/2009WR008926/epdf>)
- Higham, T. E.** (2007). Feeding, fins and braking maneuvers: locomotion during prey capture in centrarchid fishes. *J. Exp. Biol.* **210**, 107-117.
- Kendall, J. L., Lucey, K. S., Jones, E. A., Wang, J. and Ellerby, D. J.** (2007). Mechanical and energetic factors underlying gait transitions in bluegill sunfish (*Lepomis macrochirus*). *J. Exp. Biol.* **210**, 4265-4271.
- Lacey, R. W. J., Neary, V. S., Liao, J. C., Enders, E. C. and Tritico, H. M.** (2012). The IPOS framework: linking fish swimming performance in altered flows from laboratory experiments to rivers. *River Res. Appl.* **28**, 429-443.
- Lee, C. G., Farrell, A. P., Lotto, A., MacNutt, M. J., Hinch, S. G. and Healey, M. C.** (2003). The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. *J. Exp. Biol.* **206**, 3239-3251.
- Liao, J. C.** (2007). A review of fish swimming mechanics and behaviour in altered flows. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 1973-1993.
- Liao, J. C., Beal, D. N., Lauder, G. V. and Triantafyllou, M. S.** (2003a). Fish exploiting vortices decrease muscle activity. *Science* **302**, 1566-1569.
- Liao, J. C., Beal, D. N., Lauder, G. V. and Triantafyllou, M. S.** (2003b). The Kármán gait: novel body kinematics of rainbow trout swimming in a vortex street. *J. Exp. Biol.* **206**, 1059-1073.
- Lupandin, A. I.** (2005). Effect of flow turbulence on swimming speed of fish. *Biol. Bull.* **32**, 461-466.
- Pettersson, L. B. and Hedenström, A.** (2000). Energetics, cost reduction and functional consequences of fish morphology. *Proc. R. Soc. B Biol. Sci.* **267**, 759-764.
- Przybilla, A., Kunze, S., Rudert, A., Bleckmann, H. and Brucker, C.** (2010). Entraining in trout: a behavioural and hydrodynamic analysis. *J. Exp. Biol.* **213**, 2976-2986.
- Roche, D. G., Taylor, M. K., Binning, S. A., Johansen, J. L., Domenici, P. and Steffensen, J. F.** (2014). Unsteady flow affects swimming energetics in a labriform fish (*Cymatogaster aggregata*). *J. Exp. Biol.* **217**, 414-422.
- Roy, A. G., Buffin-Blanger, T., Lamarre, H. and Kirkbride, A. D.** (2004). Size, shape and dynamics of large-scale turbulent flow structures in a gravel-bed river. *J. Fluid Mech.* **500**, 1-27.
- Smith, D. L., Brannon, E. L. and Odeh, M.** (2005). Response of juvenile rainbow trout to turbulence produced by prismatic shapes. *Trans. Am. Fish. Soc.* **134**, 741-753.
- Standen, E. M.** (2010). Muscle activity and hydrodynamic function of pelvic fins in trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **213**, 831-841.
- Standen, E. M. and Lauder, G. V.** (2005). Dorsal and anal fin function in bluegill sunfish *Lepomis macrochirus*: three-dimensional kinematics during propulsion and maneuvering. *J. Exp. Biol.* **208**, 2753-2763.
- Taguchi, M. and Liao, J. C.** (2011). Rainbow trout consume less oxygen in turbulence: the energetics of swimming behaviors at different speeds. *J. Exp. Biol.* **214**, 1428-1436.
- Tritico, H. M. and Cotel, A. J.** (2010). The effects of turbulent eddies on the stability and critical swimming speed of creek chub (*Semotilus atromaculatus*). *J. Exp. Biol.* **213**, 2284-2293.
- Tytell, E. D.** (2006). Median fin function in bluegill sunfish *Lepomis macrochirus*: streamwise vortex structure during steady swimming. *J. Exp. Biol.* **209**, 1516-1534.
- Tytell, E. D.** (2010). Experimental hydrodynamics. In *Encyclopedia of Fish Physiology: From Genome to Environment* (ed. Farrell, A. P., Stevens, E. D. and Shadwick, R. E.), pp. 535-546. San Diego: Elsevier.
- Tytell, E. D. and Lauder, G. V.** (2008). Hydrodynamics of the escape response in bluegill sunfish, *Lepomis macrochirus*. *J. Exp. Biol.* **211**, 3359-3369.
- Van Zyll De Jong, M. C., Cowx, I. A. N. G. and Scruton, D. A.** (1997). An evaluation of instream habitat restoration techniques on salmonid populations in a Newfoundland stream. **614**, 603-614.
- Videler, J. J.** (1993). *Fish Swimming*. New York: Chapman and Hall.
- Walker, J. A.** (1998). Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerical differentiation algorithms. *J. Exp. Biol.* **201**, 981-995.
- Webb, P.** (1998). Entrainment by river chub *Nocomis micropogon* and smallmouth bass *Micropterus dolomieu* on cylinders. *J. Exp. Biol.* **201**, 2403-2412.
- Webb, P. W.** (2004). Response latencies to postural disturbances in three species of teleostean fishes. *J. Exp. Biol.* **207**, 955-961.
- Webb, P. W.** (2006). Stability and maneuverability. In *Fish Physiology* (ed. Shadwick R. E. and Lauder G. V.), pp. 281-332. San Diego: Elsevier.
- Webb, P. W. and Cotel, A. J.** (2010). Turbulence: does vorticity affect the structure and shape of body and fin propulsors? *Integr. Comp. Biol.* **50**, 1155-1166.
- Whale, J., Anderson, C. G., Bareiss, R. and Wagner, S.** (2000). An experimental and numerical study of the vortex structure in the wake of a wind turbine. *J. Wind Eng. Ind. Aerodyn.* **84**, 1-21.



Movie 1 – Sunfish swimming at $1.5 \text{ BL}\cdot\text{s}^{-1}$ in control conditions. Flow is from left to right and the stopped turbines can be seen upstream



Movie 2 – Sunfish swimming at $1.5 \text{ BL}\cdot\text{s}^{-1}$ under moderate streamwise vorticity. The turbines can be seen rotating upstream at 2Hz



Movie 3 – Sunfish swimming at $1.5 \text{ BL}\cdot\text{s}^{-1}$ under streamwise vorticity and after 10 minutes of exposure. Turbines are located upstream to the left of the fish. Note the asynchronous pectoral fin movement.