

Living on the Bottom: Kinematics of Benthic Station-Holding in Darter Fishes (Percidae: Etheostomatinae)

Rose L. Carlson* and George V. Lauder

Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology,
Harvard University, Cambridge, Massachusetts 02138

ABSTRACT Darters represent a substantial radiation of freshwater fishes that live in close association with the substrate in North American streams and rivers. A key feature of any darter species is therefore its ability to stay in place or to “hold station” in flowing water. Here, we quantify the station-holding performance of two morphologically divergent darter species, the fantail darter *Etheostoma flabellare* and the Missouri saddled darter *Etheostoma tetrazonum*. We also characterize the primary kinematic responses of the two species when holding station in flow speeds ranging from 4 to 56 cm s⁻¹ in a flow tank on either plexiglas or small rock substrate. We then present a series of hypotheses about the potential hydrodynamic and functional consequences of the observed postural changes and the links among morphology, posture, and station-holding performance. On both substrates, *E. tetrazonum* was able to hold station at higher flow speeds than *E. flabellare*. On rocks, *E. tetrazonum* slipped at an average speed of 55.7 cm s⁻¹ whereas *E. flabellare* slipped at 40.2 cm s⁻¹. On plexiglas, *E. tetrazonum* slipped at an average speed of 24.7 cm s⁻¹ whereas *E. flabellare* slipped at 23.1 cm s⁻¹. We measured body and fin positions of the two species from individual frames of high-speed video while holding station on rocks and plexiglas. We found that on both substrates, the two species generally exhibited similar kinematic responses to increasing flow: the head was lowered and angled downward, the back became more arched, and the median and caudal fin rays contracted as water flow speed increased. The ventral halves of the pectoral fins were also expanded and the dorsal halves contracted. These changes in posture and fin position likely increase negative lift forces thereby increasing substrate contact forces and reducing the probability of downstream slip. *J. Morphol.* 271:25–35, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: boundary layer; *Etheostoma*; locomotion; paired fins

INTRODUCTION

Fishes in a wide variety of taxonomic groups have evolved the ability to remain stationary in rapidly flowing or moving water. Rheophilic members of the Percidae (darters) (Page and Swofford, 1984; Carlson, 2008) and all Loricariidae (armored catfishes) are capable of holding station in shallow turbulent streams or racing rapids (MacDonnell and Blake, 1990) as are young (or parr) of the

Atlantic salmon (Salmonidae; Arnold et al., 1991). Likewise, members of the Cottidae (sculpins) can successfully remain stationary in wave swept intertidal zones (Bolin, 1947; Norton, 1991) and species of Pleuronectidae (plaice, sole) can hold station in deeper flowing water (Arnold and Weihs, 1978; Webb, 1989). Despite the myriad taxa that exhibit some sort of station-holding behavior, little is known about the kinematics of the behavior. With the exception of a recent study by Blake (2006), the role of body posture and paired fin orientation in mitigating hydrodynamic forces has been poorly studied and as a result, the performance consequences of variation in body and fin morphology are unknown.

Species that benthic station-hold, especially those that inhabit flowing water, must contend with two primary dislodgement-inducing hydrodynamic forces: upward-directed (positive) lift and downstream-directed drag (Denny, 1988, 1993; Vogel, 1994). Small benthic fishes must also cope with the hydrodynamic instability of the substrate-associated boundary layer. These dislodgement-inducing forces are amplified when water flows over an uneven substrate and thus, fishes that commonly benthic station-hold are expected to exhibit behavioral and/or morphological characteristics that in some way counteract or otherwise contend with these forces (e.g., *Cottus bairdi*, various rheophilic catfishes; Blake, 2006; Webb et al., 1996).

Limited previous work on station-holding suggests that fishes use morphological features and/or behavior to mitigate positive lift and drag forces

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*Correspondence to: R.L. Carlson, Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138.
E-mail: rcarlson@oeb.harvard.edu

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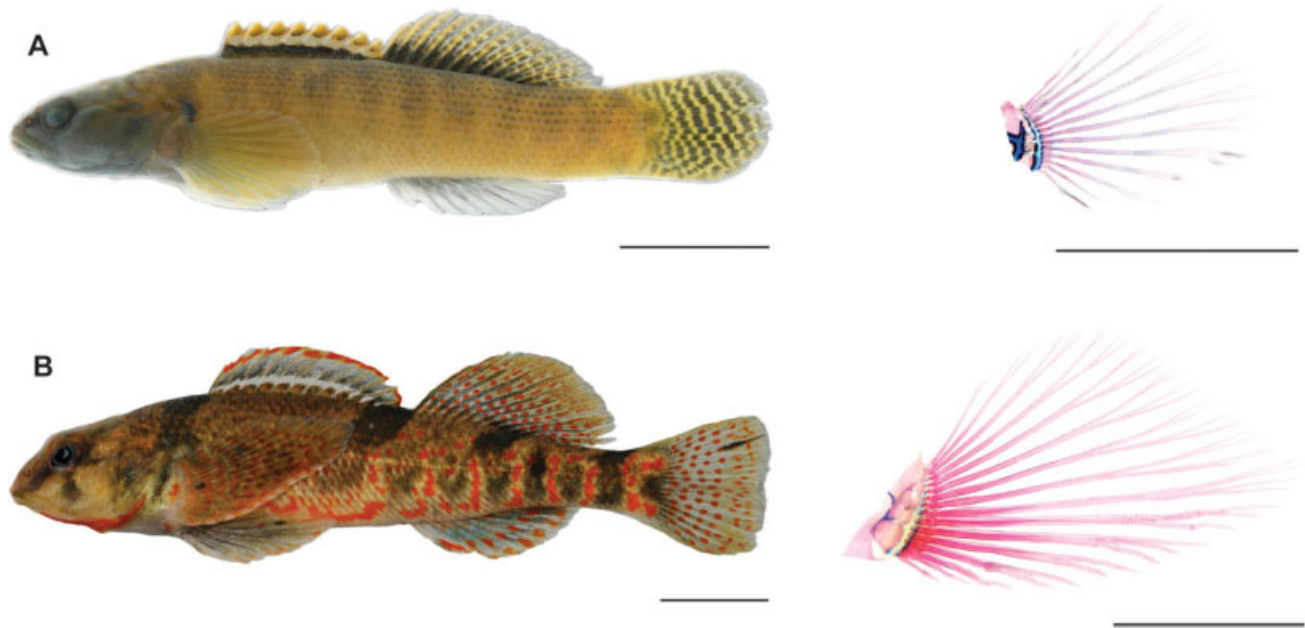


Fig. 1. (A) Male fantail darter, *E. flabellare*, from Morgan Creek, Overton Co., TN. (B) Male Missouri saddled darter, *E. tetrazonum*, from the Meramec River, Franklin Co., MO. Cleared and stained pectoral fins from each species are shown to the right of each species. Note the relatively more pointed fin of *E. tetrazonum* compared with the rounded fin of *E. flabellare*. Scale bars: 1 cm.

by either reducing the magnitude of these forces or by producing significant opposing forces (Webb, 1989; Webb et al., 1996; Wilga and Lauder, 2001). Wilga and Lauder (2001) found that bamboo sharks use their pectoral fins to generate negative (substrate-directed) lift forces that counter the hydrodynamic lift and drag and effectively draw the fish toward the substrate. Arnold et al. (1991) described a similar behavior in station-holding Atlantic salmon parr. In another study, Gerstner and Webb (1998) found that, in addition to being an unusually dense fish, the plaice (flounder) has an especially drag-reducing body shape. At low speeds, the combination of the fish's high density, low-drag shape, and Stefan adhesive forces (Brainerd et al., 1997) are sufficient to counteract dislodgement forces. As flow speed increases, however, the magnitude of the drag force increases with the square of flow velocity and the fish must beat the posterior portion of its median fins to generate a sufficient opposing force and remain stationary (Arnold and Weihs, 1978). Benthic rays are known to use similar mechanisms to facilitate station-holding in flow (Webb, 1989).

Here, we describe the kinematics of station-holding in an additional group of fishes: the North American darters (Percidae). The name "darter" derives from the species' characteristic locomotor behavior: long periods of benthic station-holding punctuated by short, rapid forward, or lateral movements or "darts" (Page, 1983). All species of darters are benthic or hyperbenthic, living either

on or in close association with the substrate (Page and Swofford, 1984; Carlson, 2008). Darter species inhabit a range of freshwater habitats from small headwater streams to larger rivers and in a few cases, the shores of lakes. Most species are habitat specialists with specific water velocity and substrate size or type requirements as a result of either feeding ecology or reproductive biology (Carlson, 2008). Correlated with differences in habitat among darter species are differences in the morphology (shape and size) of the pectoral fins suggesting a link between fin morphology and function (Fig. 1).

Identifying the function of morphological characters, especially those that vary between species in diverse lineages, is an important first step toward understanding the mechanisms driving both the ecological and morphological diversification of those lineages (Wainwright, 2007). Along these lines, our goal with this study is to describe the kinematics of benthic station-holding in two species of darters that differ in both body and pectoral fin shape. Specifically, we determine the typical station-holding posture (body position and fin orientation) in the two species and ask whether there are similarities or differences between the two species. We also ask whether the species exhibit systematic changes in posture with increasing water velocity and whether the same species takes on different postures when station-holding on different substrate types. Finally, we ask whether differences in body shape and/or the size of the

pectoral fins can be linked to differences in station-holding performance.

MATERIALS AND METHODS

Study Species

We collected data on the kinematics of benthic station-holding from three adult fantail darters (*Etheostoma flabellare*; mean SL (standard length) \pm 1 SE (standard error) = 55.0 ± 3.2 mm) and three adult Missouri saddled darters (*Etheostoma tetrazonum*; mean SL \pm 1 SE = 55.6 ± 2.3 mm). We selected these two species because they are distantly related (Song et al., 1998) and never occur in the same ecological community (Lee et al., 1980) but inhabit similar microhabitats (rocky riffles), although the Missouri saddled darter is known to occupy areas of swifter flow and larger substrate than the fantail darter (Page, 1983; Carlson, 2008). The two species also differ in a number of relevant morphological features: body shape, in particular body depth, and pectoral fin shape (Fig. 1). Specifically, *E. tetrazonum* is relatively less laterally compressed than *E. flabellare* and has a broader, more dorso-ventrally flattened head. In addition, *E. tetrazonum* has relatively larger, more wing-like pectoral fins than *E. flabellare*.

Fantail darters were collected on August 14, 2008, from shallow riffles in Horse Creek at Cherry Chapel Road in Hardin County, TN ($35^{\circ} 7.477' N$, $88^{\circ} 2.576' W$). The mean diameter (i.e., longest dimension) of the substrate in the riffles was 1.93 ± 0.86 cm ($N = 25$), the mean water velocity was 1.07 ± 0.34 m s^{-1} , and the water depth ranged from 0.3 to 0.5 m. Missouri saddled darters were collected on August 20, 2008, from riffles and runs in the Gasconade River at St. Hwy 28 in Pulaski County, MO ($37^{\circ} 53.544' N$, $92^{\circ} 4.879' W$). The mean diameter of substrate in the run from which individuals were collected was 2.21 ± 1.6 cm, the mean water velocity was 1.6 ± 0.76 m s^{-1} , and the water depth ranged from 0.2 to 2.0 m. All individuals were collected under Scientific Collecting permits #1467 and #13923 from the states of Tennessee and Missouri, respectively. Darters from both sites were shipped live to Harvard University where they were maintained in 20 gallon aquaria with substrate and flow velocity mimicking their respective natural habitats at 19–20°C on a 12:12 L:D photoperiod.

Morphological Analysis

We gathered data on the shape (aspect ratio) of the pectoral fins from three cleared and double stained (bone and cartilage) adult *E. flabellare* and two *E. tetrazonum*. Fins were removed at the girdle from each specimen, expanded, pinned, and photographed using a digital camera. The area of the fin and the length of the longest fin ray were calculated using ImageJ64 image analysis software (National Institutes of Health, 2007). We used these data to calculate the aspect ratio of each fin as $(\text{length of the longest fin ray})^2 / (\text{area of the fin})^{-1}$ and the maximum length of the pectoral fin relative to the length of the body.

Behavioral Observations and Kinematics

We placed individual darters in a variable-speed flow tank on either plexiglas or small rock substrate (mean diameter = 0.74 cm). Flow within the tank was generated by an electric motor and made microturbulent using a series of thick plexiglas sheets and wide plastic baffles (Wilga and Lauder, 2000, 2001; Drucker and Lauder, 2003). We used several plastic baffles and opaque plexiglas to limit the fishes to a $16.5 \text{ cm} \times 20 \text{ cm}$ area of the tank. We filmed fishes holding station at a range of speeds using two or three high-speed video cameras. When filming fishes on rock substrate, we used two Photron PCI1024 Fastcam cameras (resolution: $1,024 \times 1,024$ pixels) to obtain lateral and dorsal views. When filming fishes on plexiglas, we

also used a third Photron APX camera (resolution: $1,280 \times 1,024$ pixels; Photron, San Francisco, CA) in addition to the other two cameras to obtain a ventral view. All cameras recorded at 250 frames s^{-1} and videos were electronically synchronized among the cameras.

We filmed each individual of the two species on both substrates. Individuals were added to the flow tank and allowed to acclimate for 10–15 min in low flow ($4.3 - 7.5$ cm s^{-1}) before filming. They were then filmed at a range of speeds from slow (4.3 cm s^{-1}) to the maximum speed at which the individual could hold station without slipping (see *Slip Speed Estimates* later). An individual was deemed to be holding-station if it was able to hold position in the flow for ≥ 5 s. Instead of filming individuals repeatedly over a narrow range of speeds, we focused on acquiring footage of each individual over the widest possible range of speeds to observe the maximum possible extent of flow-induced postural changes. As a result, our data capture inter-individual variation in posture across speeds rather than intra-individual (or across trial) variation in posture at a given speed.

Because individuals did not move when holding station, other than occasional rocking of the body or lateral fin fluttering, we digitized one frame from each station-holding sequence for analysis. The x (horizontal) and y (vertical) coordinates of 16 points on the lateral views were digitized using a custom MatLab program (The Mathworks, Natick, MA) developed by Hedrick (2008). The points were (Fig. 2A) as follows: 1, snout tip; 2, ventro-caudalmost point on the head; 3, origin of the spiny dorsal fin; 4, origin of the soft dorsal fin; 5, base of the caudal peduncle; 6, origin of the anal fin; 7, distal tip of the first pelvic fin spine; 8, distal tip of the ventral-most pectoral fin ray; 9, distal tip of the longest pectoral fin ray; 10, distal tip of the dorsal-most pectoral fin ray; 11, anterior-most visible point on the pelvic fin; 12, proximal end of the ventral-most pectoral fin ray; 13, proximal end of the longest pectoral fin ray; 14, proximal end of the dorsal-most pectoral fin ray; 15, 16, junction of the flow tank bottom and plexiglas barrier.

On the dorsal images, we digitized the x and z coordinates of 17 points (Fig. 2B): 1, snout tip; 2, center of the left eye; 3, origin of the spiny dorsal fin; 4, end of the spiny dorsal fin; 5, origin of the soft dorsal fin; 6, base of the caudal peduncle; 7, midbody surface; 8, distal tip of the ventral-most pectoral fin ray; 9, distal tip of the longest pectoral fin ray; 10, distal tip of the dorsal-most pectoral fin ray; 11, proximal end of the ventral-most pectoral fin ray; 12, proximal end of the longest pectoral fin ray; 13, proximal end of the dorsal-most pectoral fin ray; 14, 15, edge of plexiglas filming boat; 16, distal tip of the pelvic fin; 17, most proximal visible point on the pelvic fin.

Although ventral images were available for individuals on plexiglas, preliminary analysis indicated that no additional information about body posture and paired fin position could be gained from the images. Thus, we did not analyze any ventral image data.

Slip Speed Estimates

While filming individuals on both substrates, we also estimated the lowest speed at which the species began to slip either laterally (left to right) or downstream. After capturing video of the station-holding posture of each individual over a range of flow speeds, we then tested each individual's slip speed by slowly increasing the water flow speed until the individual was no longer able to maintain its position, contact with the substrate became reduced, and significant lateral or backward movement was observed. Even if an individual was able to hold station for several seconds and then subsequently slipped, we recorded the speed as one at which the individual slipped. We repeated these slip speed trials on all but one individual of *E. tetrazonum*. When we had multiple estimates of slip speed for a given individual, we used the lowest speed at which the individual slipped in our analysis of performance limits.

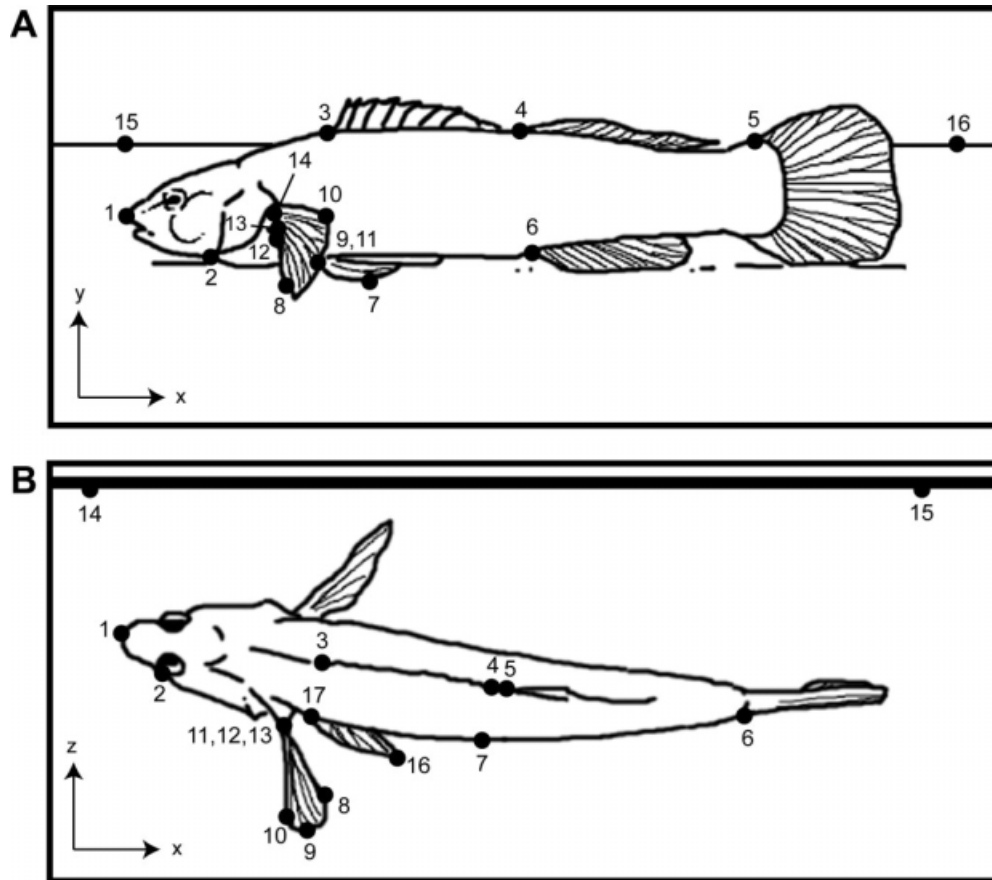


Fig. 2. Landmarks used when digitizing body and fin positions from video data on both substrates. (A) Landmarks in lateral and (B) dorsal view images. *E. flabellare* station-holding on plexiglas substrate is used as an example for both views. See text for details about each digitized point.

Statistical Analyses

We calculated 15 variables using digitized data from the lateral view images and five variables using data from the dorsal view images. The variables reflected postural features of the head and body as well as aspects of the paired fins, including position and relative expansion of the pectoral fin rays. In the lateral view, we calculated the angle of the head, back, and ventral surface of the body relative to the horizontal, the extent of back arch (two variables), the angle of the ventral-most, center, and dorsal-most fin rays of the left pectoral fin relative to the horizontal, the angle between the ventral-most and middle pectoral fin rays (a measure of the relative expansion of the ventral half of the fin), the angle between the middle and dorsal-most pectoral fin rays (a measure of the relative expansion of the dorsal half of the fin), total pectoral fin expansion (the sum of the previous two variables), the angle of the left pelvic fin's longest ray relative to the horizontal, and the absolute elevation of the head. Because the angles of the head and back were calculated relative to the horizontal, higher (i.e., less negative) values indicate a more downward-oriented head and back, respectively. In the dorsal view, we calculated the angle of the central pectoral fin ray relative to the body midline, the angle of the pelvic fin relative to the body midline, and the expansion of the ventral and dorsal portions of the pectoral fin and total pectoral fin expansion as described earlier.

For datasets from both substrates, we used linear regression to test for significant changes in body posture and paired fin orientation with increasing speed using the JMP statistical

package (SAS Institute, Inc.). We then used ANCOVA (analysis of covariance), with flow speed as the covariate, to compare the magnitude and direction of observed postural changes and fin orientations between the two species and within a single species on different substrates. When species showed no significant change in posture or fin orientation with speed, we compared the means for those variables between species and substrates using a *t*-test.

To concurrently compare multiple aspects of station-holding posture between the two species, we performed a pair of principal component (PC) analyses with nine variables (six from the lateral view images and three from the dorsal view images). We selected variables that describe unique postural features and performed separate analyses on data from individuals' holding station on each substrate.

Finally, we used a *t*-test to compare station-holding performance (minimum slip speed) between the two species on plexiglas and small rocks.

RESULTS

In the most typical station-holding posture, regardless of substrate, speed, or species, the fish is pointed directly or nearly directly upstream and is supported primarily by the pelvic and the caudal fins (Fig. 3). The head is raised and the back is at least moderately arched with the first and second

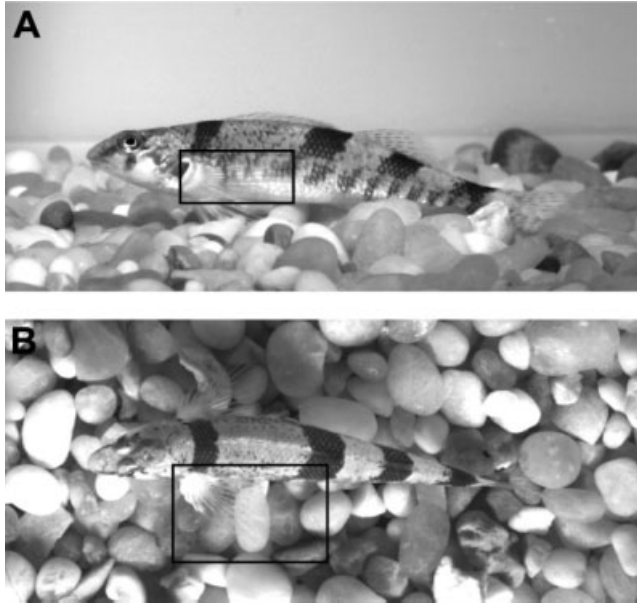


Fig. 3. High-speed video images from simultaneous (A) lateral and (B) dorsal views of the Missouri saddled darter *E. tetrazonum* (52.4 mm SL) holding station in water flowing at 47.5 cm s^{-1} over rocks. Boxes enclose the left pectoral fin in both images. Note that in (A), the left pelvic fin extends below the pectoral fin.

dorsal fins moderately to entirely depressed. The pectoral fins are protracted slightly with only the ventral-most fin ray tips in contact with the substrate.

The Effect of Flow Speed on Posture

On both plexiglas and rocks, both *E. flabellare* and *E. tetrazonum* slightly altered their posture

and changed the orientation of the paired fins as flow speed increased. However, the specific changes in posture and fin orientation differed slightly between the two species (Figs. 4A,B and 5) and between the two substrates. Because rocks provide a more realistic and ecologically relevant substrate than plexiglas, we will focus on postural changes on rocks. It is notable, however, that at very high speeds on plexiglas, individuals of both species assumed a similar posture: fishes rolled the main axis of the body slightly to the left or right, leaned heavily on the pectoral fin on the side of the body to which the body was rolled, pelvic fins, and caudal fin, all the while raising the remaining pectoral fin off the substrate and holding it nearly parallel to the substrate and in the flow.

At low speeds on rocks (i.e., the first panel of Fig. 4A,B), individuals of both species elevated the head above the substrate and pitched it either slightly upward or held it parallel to the substrate. The back was typically kept straight or nearly straight and the caudal fin was expanded. As the flow increased (i.e., the third and fourth panels of Fig. 4A,B), the head was lowered and pitched (Fig. 5A). The back became more arched dorsally (Fig. 5B) and expansion of the median and caudal fins decreased, effectively lowering the caudal fin into the substrate. The ventral halves of the pectoral fins were expanded and the dorsal halves contracted (Figs. 4A,B and 5C,D). At very high speeds (i.e., the fourth panel in Fig. 4A, B), the body was lowered into the substrate and the back more strongly arched dorsally, forming a convex dorsal surface. The ventral portion of the pectoral fins was further expanded and the entire fin was abducted and held into the flow.

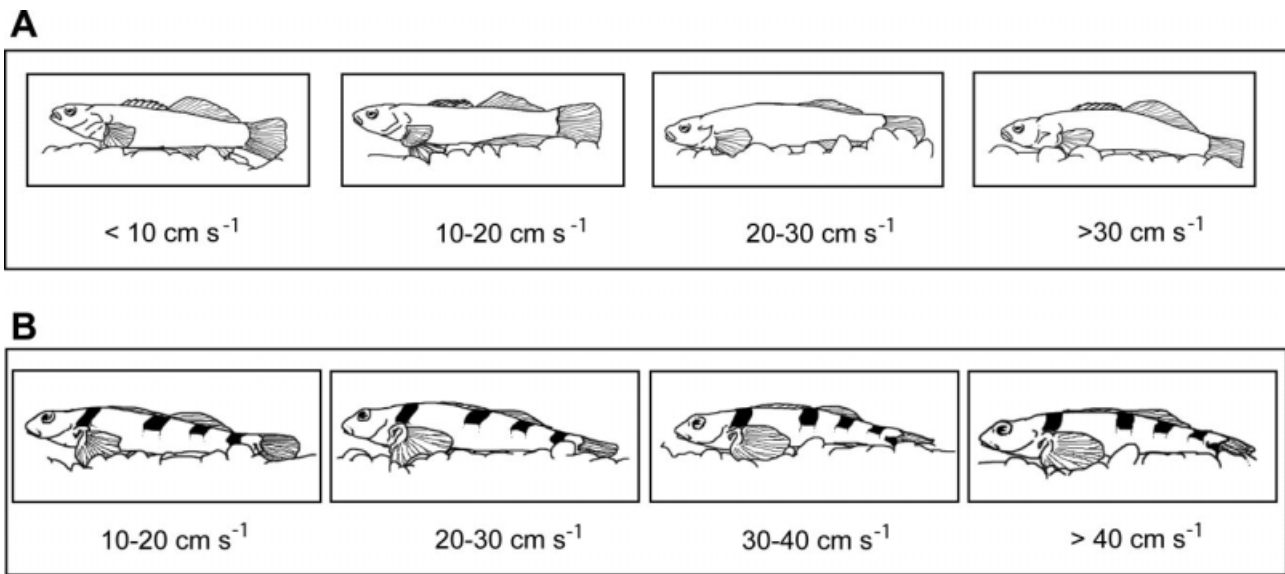


Fig. 4. Representative drawings from high-speed video images of (A) *E. flabellare* and (B) *E. tetrazonum* holding station on rocks across a range of flow speeds.

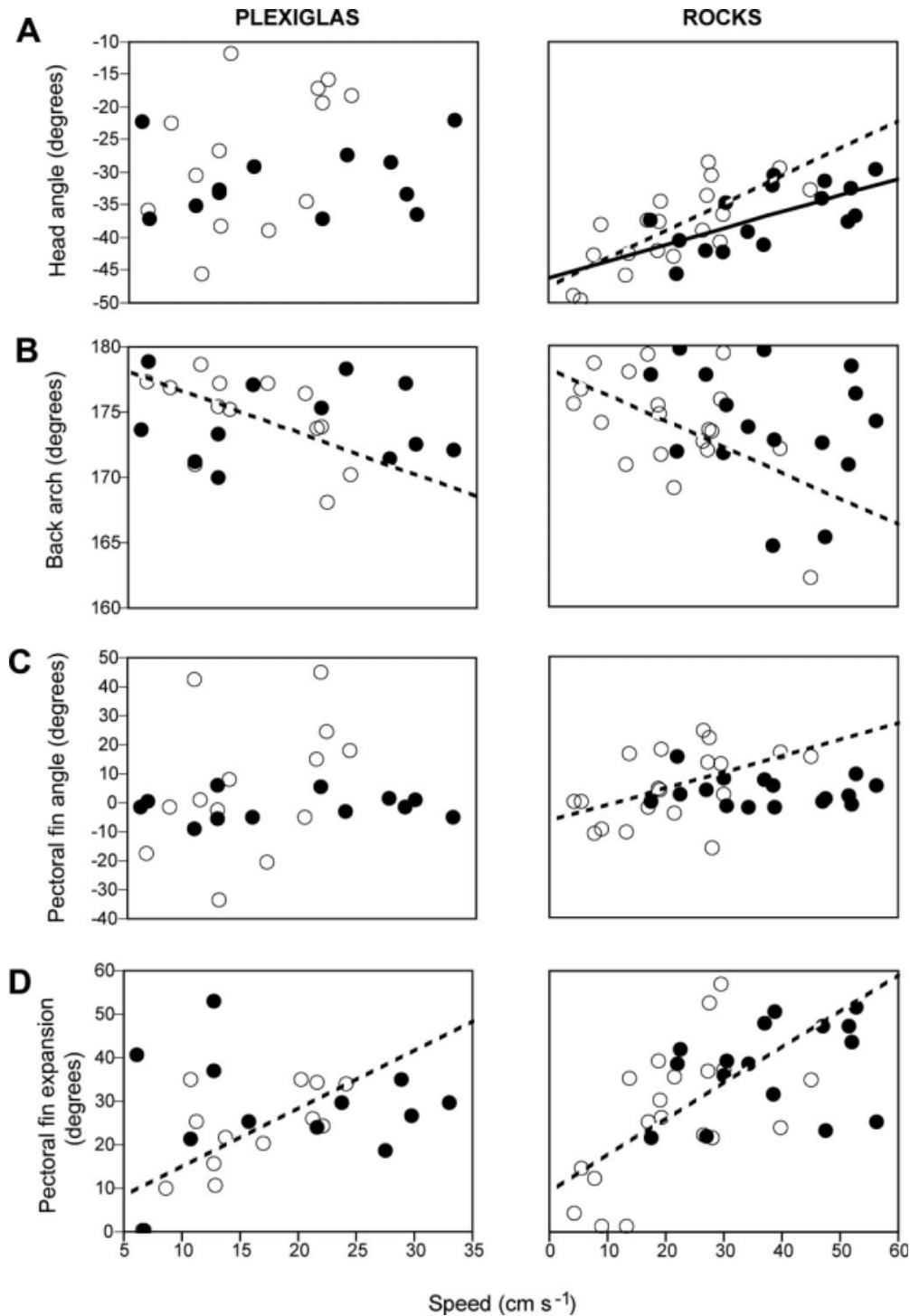


Fig. 5. Scatterplots of individual kinematic variables on rock and plexiglas substrates across a range of flow speeds for the two species (*E. flabellare* = \circ , *E. tetrazonum* = \bullet). Regression lines are fit to the data only when one or both of the species exhibits a significant change in posture or pectoral fin orientation with increasing speed ($\alpha = 0.05$) (*E. flabellare* = dashed line, *E. tetrazonum* = solid line). (A) Angle of the head in lateral view relative to the horizontal, (B) extent of back arch in lateral view, (C) angle of the dorsal-most pectoral fin ray in lateral view, and (D) relative expansion of the dorsal half of the pectoral fin in dorsal view. Estimates of intercepts and slopes for the seven significant regressions are presented in Table 1.

On rocks, *E. tetrazonum* exhibited a statistically significant increase in head angle (linear regression: $R^2 = 0.41$, $P = 0.0075$) with increasing speed

(Fig. 5A, Table 1). Four changes in posture or pectoral fin orientation were significant in *E. flabellare* on rocks: a decrease in head angle relative to

TABLE 1. Statistics for the seven significant regressions shown in Figure 5

Substrate	Variable	Species	Slope	Intercept	R^2	P-value
Plexiglas	Back arch	<i>E. flabellare</i>	-0.321	179	0.33	0.040
	Pectoral fin expansion	<i>E. flabellare</i>	1.32	1.87	0.48	0.0088
Small rocks	Head angle	<i>E. flabellare</i>	0.421	-47.5	0.57	0.0002
		<i>E. tetrazonum</i>	0.252	-46.2	0.41	0.0075
	Back arch	<i>E. flabellare</i>	-0.198	178	0.29	0.017
	Pectoral fin angle	<i>E. flabellare</i>	0.561	-6.72	0.26	0.026
	Pectoral fin expansion	<i>E. flabellare</i>	0.828	9.48	0.35	0.0081

All statistics are from a linear regression of the listed variable versus flow speed for the species and substrate indicated.

the horizontal (linear regression: $R^2 = 0.57$, $P = 0.0002$), an increase in back arch (linear regression: $R^2 = 0.29$, $P = 0.017$), an increase in the angle of the pectoral fin (linear regression: $R^2 = 0.26$, $P = 0.026$), and an expansion of the dorsal portion of the pectoral fin (linear regression: $R^2 = 0.20$, $P = 0.064$; Fig. 5, Table 1). On plexiglas, *E. tetrazonum* did not exhibit any significant changes in posture with increasing flow speed. In contrast, *E. flabellare* increased its back arch (linear regression: $R^2 = 0.33$, $P = 0.040$) and expanded its pectoral fins (linear regression: $R^2 = 0.48$, $P = 0.0088$) with increasing speed (Fig. 5B,D; Table 1).

The Effect of Substrate on Posture

The two species exhibited slightly different body postures on the two substrates. *E. flabellare* increased the angle of its head more quickly with increasing flow speed on plexiglas (ANCOVA: substrate effect, $F = 25.2$, $P < 0.0001$). Similarly, fantail darters increased the extent of the back bend more quickly on rocks than on plexiglas (ANCOVA: substrate effect, $F = 4.55$, $P = 0.042$). Differences in the rate of postural changes between rocks and plexiglas were similar for *E. tetrazonum*. The species raised its head more quickly on rocks than on plexiglas (ANCOVA: substrate effect, $F = 15.6$, $P = 0.0006$).

Interspecific Comparisons of Posture and Performance

The two species were similar in most aspects of station-holding posture including the back arch (Fig. 6B), the angle of the pectoral fin in lateral view (Fig. 6C), and expansion of the dorsal half of the pectoral fin (Fig. 6D) on both plexiglas and rocks. However, there were several differences in posture between the two species on rocks: *E. flabellare* angled its head upward slightly more (ANCOVA: species effect, $F = 4.57$, $P = 0.04$; Figs. 5A and 6A) leading to a more elevated head (ANCOVA: species effect, $F = 5.36$, $P = 0.027$) but kept its back flatter (ANCOVA: species effect, $F = 19.2$, $P = 0.0001$) than *E. tetrazonum*. The combined result of these postural differences was a

more smoothly curved rather than sharply bent body (on average) in *E. tetrazonum* than in *E. flabellare* (Fig. 4). On plexiglas, the two species differed only in the angle of the back (ANCOVA: species effect, $F = 11.3$, $P = 0.0029$). Again, *E. tetrazonum* typically angled its back more steeply than *E. flabellare* on this substrate.

When postural space was reduced to the major axes of variation between the two species, differences in body position and fin orientation were more apparent (Fig. 7). On plexiglas, the first four PC axes explained 87.2% of the variation in the data. Only the first two axes, however, had eigenvalues greater than one. The first axis (46.7% of the total variation) described variation in the angle of the pectoral fin and the second axis (20.6% of the total variation) captured variation in body orientation (the angle of the body relative to the horizontal) and pectoral fin expansion (Fig. 7A). The two species were not significantly different from one another on PC axis 1 but did differ on PC axis 2 (ANOVA: $F_{1,22} = 29.35$, $P < 0.0001$). Thus, overall posture but not fin angle differed between the two species when they held station on plexiglas.

The distribution of points was very similar in the postural space described by the first two PC axes from the species station-holding on rocks (Fig. 7B). Here, PC axes 1–4 captured 79.7% of the total variation among the data and all four axes had eigenvalues greater than one. For consistency with the plexiglas analysis, we focus on the first two axes. The first PC axis captured variation in pectoral fin angle and dorsal expansion and body angle relative to the horizontal (29.7% of the total variation) and the second axis captured variation in the extent of back arch (19.7% of the total variation). The two species did not differ significantly in PC axis 1 scores but did differ in scores on PC 2 (ANOVA: $F_{1,30} = 12.86$, $P = 0.0012$). Thus, as suggested by the univariate analyses described earlier, the station-holding postures of the two species differed primarily in the extent of back arch and not in the orientation of the fins or body on rocks.

In terms of station-holding performance, *E. tetrazonum* was able to hold station at higher flow

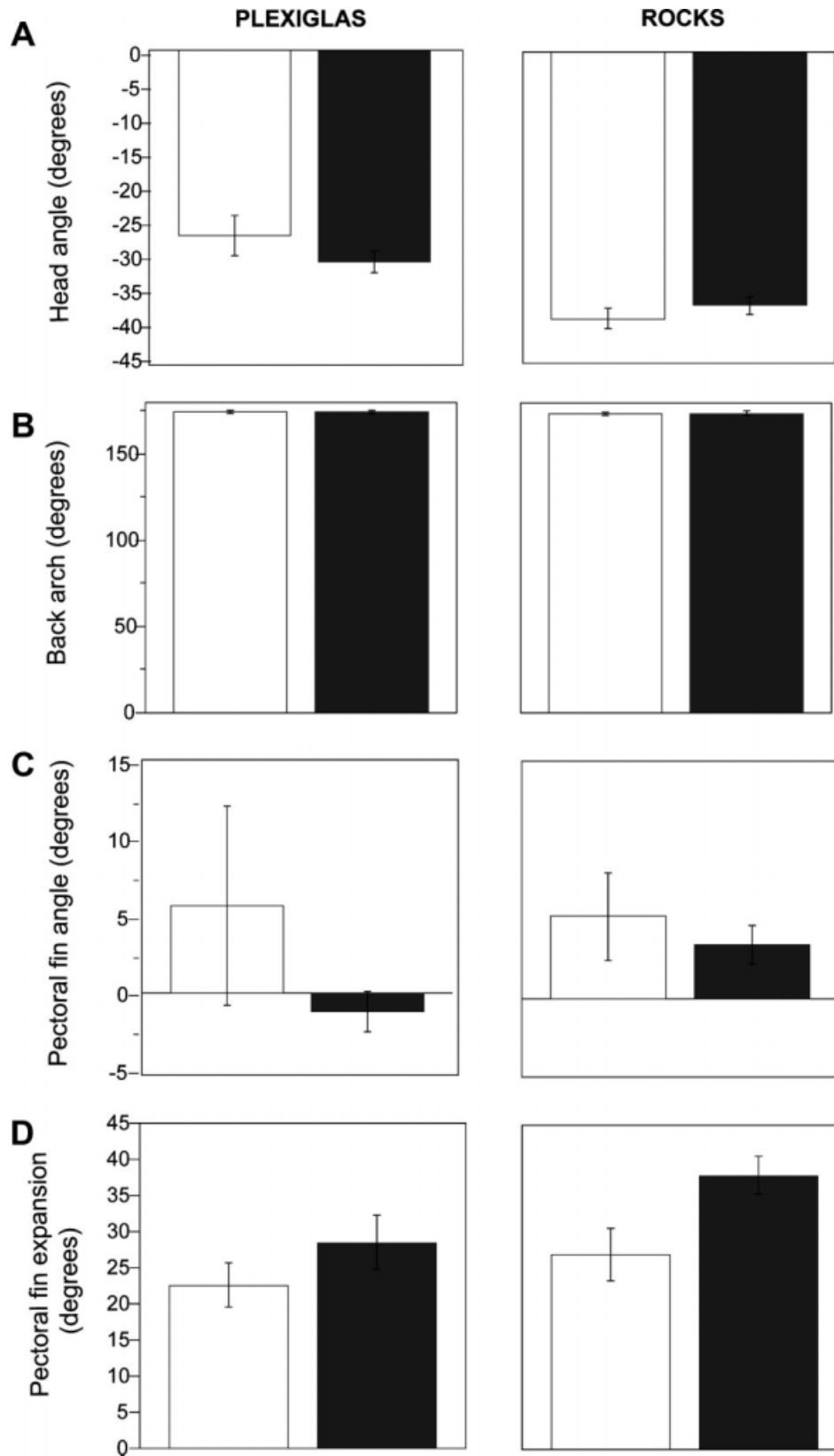


Fig. 6. Mean (± 1 SE) across all flow speeds of four postural or fin orientation variables (Fig. 5) by the two species on plexiglas or small rocks (*E. flabellare* = open bars, *E. tetrizonum* = filled bars). (A) Angle of the head in lateral view relative to the horizontal, (B) extent of back arch in lateral view, (C) angle of the dorsal-most pectoral fin ray, in lateral view, and (D) relative expansion of the dorsal half of the pectoral fin in dorsal view.

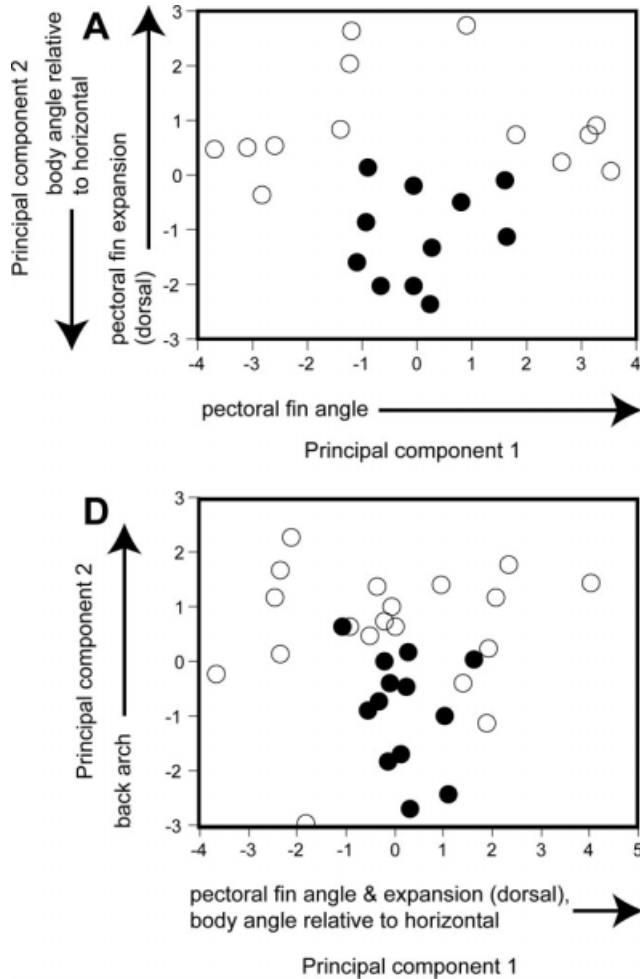


Fig. 7. Scatterplots of scores on the first two principal component axes for *E. flabellare* (○) and *E. tetrazonum* (●) on (A) plexiglas and (B) rocks.

speeds than *E. flabellare* on both substrates. However, this difference was not significant on plexiglas (*t*-test assuming unequal variance, $t = -1.220$, $P = 0.371$). On rocks, *E. tetrazonum* slipped at an average speed (± 1 SE) of $55.7 \pm 1.26 \text{ cm s}^{-1}$ whereas *E. flabellare* slipped at an average speed of $40.2 \pm 4.58 \text{ cm s}^{-1}$ (*t*-test assuming unequal variance, $t = -3.405$, $P = 0.0616$). On plexiglas, *E. tetrazonum* slipped at an average speed of $24.7 \pm 1.1 \text{ cm s}^{-1}$ whereas *E. flabellare* slipped at an average speed of $23.1 \pm 0.61 \text{ cm s}^{-1}$.

E. flabellare and *E. tetrazonum* differ in a number of morphological features that may be linked to the observed differences in station-holding posture and fin orientation. The species differ in body shape (mean ratio of body depth to standard length (± 1 SE): *E. flabellare* = 0.195 ± 0.004 , *E. tetrazonum* = 0.206 ± 0.008), pectoral fin shape (mean pectoral fin aspect ratio (± 1 SE): *E. tetrazonum* = 1.82 ± 0.001 , *E. flabellare* = 1.34 ± 0.03), and relative pectoral fin length (mean ratio

of length of the longest fin ray to standard length (± 1 SE): *E. flabellare* = 0.212 ± 0.0006 , *E. tetrazonum* = 0.301 ± 0.004 ; Fig. 1).

DISCUSSION

E. flabellare and *E. tetrazonum* exhibit broad similarities in station-holding posture and fin orientation between substrates and across flow speeds. Both species rest on their pelvic and caudal fins in a tripod-like posture and point directly or nearly directly upstream, moderately protract and expand their pectoral fins, and either elevate the entire pectoral fin above the substrate or rest the ventral-most rays lightly on the substrate. In addition, both species hold their pectoral fins at an incline to the oncoming flow with the ventral edge anterior to the dorsal edge. This posture is nearly identical to that taken by Atlantic salmon parr (*Salmo salvar*) under similar flow conditions (Arnold et al., 1991).

Specific aspects of posture, however, such as the angle of the head relative to the horizontal, the extent to which the back is arched, and the degree of expansion of the pectoral fins differ between the two species. The observed differences may be related to differences in body and fin morphology or may simply be due to difference in behavior. In addition to the differences in posture, we also found evidence of interspecific differences in station-holding ability (measured as the lowest speed at which individuals began to slip): on both substrates, *E. tetrazonum* was able to hold station up to higher speeds than *E. flabellare*. It is notable that each species' performance limit is well-matched to its natural environment, with *E. tetrazonum* occurring in areas of rivers and streams characterized by higher flow velocities than *E. flabellare* (Carlson, 2008).

Both species altered their posture and/or the orientation of their fins as flow speed increased. Although the exact manner of postural alteration differed slightly between the two species, each set of changes likely provided a benefit, in terms of stability, drag reduction, or force production, to the species. For example, as water speed increased, both *E. flabellare* and *E. tetrazonum* lowered their heads and increased the arch in their backs to produce a convex dorsal surface. *E. tetrazonum* also lowered the posterior portion of its body (and in particular its caudal fin) further into the substrate with increasing speed. The arching of the back combined with the lowering of the head and body likely act to increase the magnitude of negative (substrate-directed) lift forces generated by the fish. These forces in turn increase friction between the body and the substrate and help to prevent downstream slipping. In addition to these changes in body position, we also found that both species expanded and more sharply inclined the pectoral

fins with increasing flow speed. This action is also expected to increase the magnitude of the negative lift forces generated by the fins.

Although we can only make hypotheses about the hydrodynamic consequences of each species' body posture and fin orientation, it is likely that subtle postural differences between the two species contribute to the dramatic difference in station-holding performance. *E. tetrazonum* was able to hold station at speeds of up to 55 cm s^{-1} whereas *E. flabellare* was able to remain stationary only up to 40 cm s^{-1} . In addition, given that body and pectoral fin shape and size differ markedly between the species, it is also possible that these factors contribute to differences in station-holding performance. If the pectoral fins are used in a manner similar to that of bamboo sharks (i.e., to generate negative lift forces; Wilga and Lauder, 2001), then species with a larger fin area relative to body size, such as *E. tetrazonum*, would present increased surface area to oncoming flow resulting in larger magnitude negative lift forces and thereby facilitate station-holding up to higher flow velocities. The higher aspect ratio pectoral fins of *E. tetrazonum* may also permit modulation of lift forces by allowing the fishes to extend the distal tip of the fin away from the substrate and into higher velocity flows without exposing the entire fin to higher velocities. Although we did not quantify them here, differences in fin ray structure (thickness, branching) between *E. flabellare* and *E. tetrazonum* may also contribute to differences in station-holding ability between the two species (Taft et al., 2008).

We found strong evidence to suggest that substrate type affects the station-holding performance of the two darter species. Both species were able to hold station at higher speeds on rocks than on plexiglas. *E. tetrazonum* slipped at speeds of approximately 25 cm s^{-1} on plexiglas compared with 55 cm s^{-1} on rocks whereas *E. flabellare* slipped at 23 cm s^{-1} on plexiglas compared with approximately 40 cm s^{-1} on rocks. Although the difference in performance between substrates was not as dramatic for *E. flabellare* as for *E. tetrazonum*, the difference was still notable.

There are several possible explanations for the observed difference in performance of the fishes on the two substrates. First, by nature of their variable topography, rocks provide many surfaces onto which fish can grasp with dexterous pelvic fins. Second, the uneven surface of a rock substrate produces pockets of reduced flow into which a fish can insert itself. Fishes have been known to take advantage of areas of flow recirculation behind larger rocks or groups of smaller rocks because such zones have been shown to reduce the muscular effort needed to hold station in high flows (Liao et al., 2003a,b). In contrast to rocks, plexiglas offers only a smooth surface on which to rest;

there is nothing to either produce areas of lower flow or protect the fish from on-coming water. In addition to these structure-based differences, fish that occur in close proximity to the substrate, such as darters, will likely feel the effects of differences in the thickness of the boundary layer between the two substrates. Again, due to the irregularity of the rock substrate, the boundary layer, a region of reduced and otherwise disturbed flow, is expected to be significantly thicker above this substrate than over plexiglas.

In this study, we characterized the kinematic responses of two species of darter fishes to imposed flow velocity on plexiglas and rock substrates. We then presented several hypotheses about the effect of these changes in posture and fin orientation on flow and on the magnitude of the generated negative lift forces. To date, however, details about the fluid velocity gradient the fishes encounter on rocky substrates are unknown as are the specific fluid dynamic effects of the observed body and fin postural changes. Studies that image flow in the boundary layer of rocky substrates and over the body and fins of darters in a variety of flow regimes are therefore the next step to understand how these species contend with high-velocity flows and flow gradients.

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