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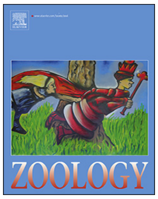

Benthic walking, bounding, and maneuvering in flatfishes (Pleuronectiformes: Pleuronectidae): New vertebrate gaits

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ABSTRACT

Video-based observations of voluntary movements reveal that six species of pleuronectid flatfishes use sequential portions of long-based dorsal and anal fins as "feet" (hereafter, fin-feet) to move on the substrate. All six species used a gait that we term "walking," which produced constant forward movement, and several of these species also used a second gait that we call "bounding" for intermittent movements over the substrate. We selected Pacific Sand Sole, *Psettichthys melanostictus*, and English Sole, *Parophrys vetulus*, for kinematic analyses of these two gaits. *Psettichthys melanostictus* consistently used walking for benthic locomotion; *Parophrys vetulus* primarily used a bounding gait. During forward walking, a fin ray swings up off the substrate, protracts and converges with neighboring fin rays to contribute to a fin-foot. The fin-foot pushes down on the substrate and rotates posteriorly by sequential recruitment of fin rays, a pattern known as a metachronal wave. As one fin-foot passes off the posterior end of the fin, a new fin-foot forms anteriorly. During bounding, undulations of the body and tail assist one or two waves of fin-feet, producing rapid but intermittent forward acceleration of the body. Flatfishes also use fin-feet to maneuver on the substrate. The Starry Flounder, *Platichthys stellatus*, performs near zero displacement rotation by running waves of fin-feet in opposing directions along the dorsal and anal fins. Although other teleosts use specialized pectoral fin rays for bottom walking (e.g., Sea Robins: Triglidae), the duplication of structures and patterns of movement in the median fins of flatfishes more closely resembles metachronal motions of millipede feet or the parapodia of polychaete worms. Sequential use of median fin rays in flatfishes resembles that of other teleosts that swim with elongate median fins, including Amiiformes, Gymnotiformes, and some Tetraodontiformes, but flatfishes offer a novel form of substrate locomotion based on dorsal and anal fins.

1. Introduction

Fishes as diverse as epaulette sharks (Hernandez et al., 2016), batoids (Koester and Spirito, 2003; Macesic and Kaijura, 2010; Macesic et al., 2013), lungfishes (King et al., 2011), sea robins (Jamon et al., 2007) and batfishes (Ward, 2002) walk on the substrate using paired appendages. Benthic walking may minimize pressure waves that could alert predators or potential prey to a fish's movements (Macesic and Kaijura, 2010; King et al., 2011). For fishes in fast-flowing or turbulent currents, such as the waterfall-climbing cave fish *Cryptotora thamicola*, walking enables the fish to adhere to the substrate (Flammang et al., 2016). Predatory benthic fishes may benefit from pushing against the substrate to produce a forward lunge or a rapid rotation when closing on a potential prey item.

Metamorphosed flatfishes (Pleuronectiformes) — including flounders, halibuts, sole, and plaice — are unusual within vertebrates because their median fins are in direct contact with the substrate. Bauplan remodeling and reorientation of the eyes during early development in pleuronectiforms results in an exceptionally asymmetrical body and cranium. As adults, flatfishes lie on one side on the bottom and survey the surrounding environment with eyes that protrude from their upward-facing side. When adult flatfishes rest on the substrate, the dorsal and anal fins are positioned such that they can interact with the substrate — a functional task usually assumed by vertebrate paired appendages. Flatfishes are a highly successful and abundant group of bottom-dwelling, ambush predators, with 14 extant families, 123 genera, and nearly 800 species (Nelson et al., 2016); additionally, many species are economically important as seafood and make up large portions of the benthic-living biomass in marine ecosystems (Munroe et al., 2015).

There has been extensive research on the development (Schreiber, 2013) and evolutionary origin of cranial novelties in Pleuronectiformes

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(Friedman, 2008; Harrington et al., 2016). Specializations for benthic life include: modifications for feeding on or near the substrate (Gibb, 1995, 1996, 1997); mechanisms that enable flatfishes to adhere to and rapidly separate from substrates (Brainerd et al., 1997); adaptations to facilitate color changes in the skin to enhance crypsis (Ramachandran et al., 1996); and burying behaviors (McKee et al., 2016). Webb (2002) evaluated swimming performance of Plaice (*Pleuronectes platessa*) above the substrate, finding that they swim only at moderate to high speeds, use symmetrical fin-beats on the upstroke and downstroke, maintain a positive but variable tilt angle (inclination of the body plane), and benefit from ground effects when swimming close to the substrate. There are some qualitative observations of walking behavior of flatfishes (Orcutt, 1950; Kruuk, 1963; Olla et al., 1969; Stickney et al., 1973) but no kinematic analyses.

We used high-speed video analyses of benthic locomotion in six species of flatfishes from the Pacific Northwest (all from the family Pleuronectidae) to address the following questions: Do flatfishes use their median fins to move along the substrate? If so, what patterns of fin movement are used to generate these behaviors? Do benthic gaits (patterns of fin movements) vary within and among species of flatfishes? How similar is the benthic walking of flatfishes to locomotor modes exhibited by other benthic fishes, the swimming gaits of mid-water fishes with elongate median fins, and invertebrate locomotor behaviors? Ultimately, we ask does benthic walking of flatfishes constitute an undescribed vertebrate gait?

2. Methods

2.1. Animal Collection and Care

We collected flatfishes from the Puget Sound near Friday Harbor Laboratories (Friday Harbor, WA) using bottom trawls and beach seines in July and August 2016, held them in flow-through seawater tanks (11–13 °C), and fed them every 1–2 days on mysid shrimp (University of Washington IACUC protocol 4208-03). We video-recorded fishes moving along the substrate within four weeks of collection, and, after the study was complete, either released or euthanized them.

2.2. Videography

We made high-speed digital-video recordings of 31 individual flatfishes representing six species in the family Pleuronectidae: Pacific Sand Sole, *Psettichthys melanostictus* (N = 7); English Sole, *Parophrys vetulus* (N = 12); Starry Flounder, *Platichthys stellatus* (N = 5); Slender Sole, *Lyopsetta exilis* (N = 2); Butter Sole, *Isopsetta isolepis* (N = 3); and Rock Sole, *Lepidopsetta bilineata* (N = 2). Prior to videography, we photographed each fish from above with a scale-bar in the frame. Specimens ranged from 6.3 cm–32.2 cm TL. Five of these six species are very closely related (Roje, 2010) and two hybridize (*P. vetulus* and *P. stellatus*; Eschmeyer and Herald, 1983).

During the trials, individuals were transferred to two tanks (61 cm × 33 cm × 43 cm and 122 cm × 20.5 cm × 30.5 cm) for filming sessions lasting 10–30 min. Water in the tanks was completely replaced once per hour. The substrate was a transparent, smooth, acrylic plate elevated 4 cm off the tank bottom to allow videography from the side to capture animal-substrate interactions. Locomotion was elicited by gently prodding fish with a soft plastic probe (46 cm × 0.9 cm). Two Panasonic Lumix DMC - FZ200 video cameras (120 frames per second, fps, at a resolution of 1280 × 720 pixels) were used to record five to ten locomotor sequences per individual from above and side.

Two species (*Psettichthys melanostictus* N = 3, *Parophrys vetulus* N = 7) also were filmed moving in a flow-through seawater tank with a fiberglass bottom (60 cm by 120 cm). The video camera was positioned above the tank to record self-initiated locomotion in a less constrained space. Filming took place during feeding sessions to capture intrinsically motivated movements.

Table 1
Analyses of Walking Speeds for *Psettichthys melanostictus* and *Parophrys vetulus*.

Species	Individual	TL (cm)	Sequences analyzed	Average Walking Speed (cm/s)	Average Wave Frequency (Hz)
Psettichthys melanostictus	5	19.6	9	5.37	1.69
	16	15.1	4	4.01	2.02
	18	8.8	4	3.73	1.62
	24	14.4	4	5.40	2.27
	30	12.2	3	4.60	1.52
	34	12.7	3	6.02	2.47
Parophrys vetulus	2	16.3	3	4.68	2.24
	19	6.8	2	3.17	3.83
	25	12.8	2	3.95	2.20
	28	9.2	8	3.65	2.86
	31	15.6	3	3.49	1.76
	32	6.3	4	3.53	2.69

We selected 67 sequences of benthic locomotion of *Psettichthys melanostictus* (N = 6: 27 walking sequences (see Table 1) and 3 walking-swimming transition sequences) and *Parophrys vetulus* (N = 9, 22 walking sequences (see Table 1) and 15 bounding sequences (see Table 2)) for detailed analyses because of the number of individuals available and their use of distinct walking and bounding gaits. We also quantified rotation in one sequence for *Parophrys vetulus* (N = 1) and three sequences for *Platichthys stellatus* (N = 3).

To interpret differences in substrate locomotion of flatfishes, we first had to characterize and clearly define locomotor modes; the first three of these modes are newly reported here. (1) Forward walking is based on convergence of fin-rays to form a “fin-foot” in which fin rays angle toward one another; the convergence of fin rays is most visible when viewed from above as a darker region of the fin that propagates in a wave, from anterior to posterior; three or more sequential fin waves produce continuous forward movement. (2) Forward bounding is generated by one or two fin waves with fin-ray convergence; this produces intermittent bouts of movement with the body coming to a complete stop between successive bounds. We defined a single bound as a push by median fin rays against the bottom, which launches the body into a glide phase during which there is no contact with the bottom. At the end of the glide, the fish comes to rest with the tips of its median fin rays in contact with the bottom. (3) Rotation against the substrate refers to a change in direction generated by out-of-phase waves of fin-ray convergence along the dorsal and anal fins. (4) Swimming is rapid and continuous forward movement in the water column generated by undulations of the body and median fin surfaces; no fin-ray convergence occurs. A final locomotor behavior not studied here is burying, which

Table 2
Analyses of Bounding for *Parophrys vetulus*.

Individual	TL	Sequence Length (s)	# bounds	Frequency (bounds/s)	Average Bounding Speed (cm/s)
19	6.84	29.2	5	0.23	1.35
		50.4	5	0.10	1.43
20	18.30	2.0	1	0.51	5.55
		20.6	3	0.15	0.67
		7.4	3	0.40	3.30
25	12.80	51.3	12	0.23	1.89
		10.5	6	0.57	2.87
		8.9	4	0.45	2.28
28	9.20	2.0	2	1.01	0.81
		3.1	2	0.64	1.59
35	5.32	46.4	16	0.34	1.89
		31.2	11	0.35	2.13
		30.0	11	0.37	1.88
37	10.21	36.4	11	0.30	2.67
		60.3	15	0.25	1.79

can be distinguished from other locomotor modes because the body is not displaced along the substrate; instead, the body rapidly undulates to displace substrate particles to cover the fish.

We studied locomotor preferences in a 7 min video of freely-moving *Parophrys vetulus* (N = 7) and *Psettichthys melanostictus* (N = 3) as they foraged in the flow-through seawater tank. We coded all movements during the 7 min period as walking, bounding, rotating, or swimming (using the definitions given above), and recorded the frequency and duration of each of these four locomotor modes.

For kinematic analyses of walking, bounding, and swimming, body velocity was calculated by auto tracking the eye in top-view video sequences using DLTdv5 digitizing tools for MATLAB (Hendrick, 2008); fin-foot velocity was measured using the same software in manual tracking mode. Each walking sequence used for kinematic analysis contained three or more successive fin-foot strides.

To determine if movements of the dorsal and anal fins were symmetrical, we measured the angle formed by the midline and the leading edge of the fin-foot at the beginning, middle, and end of one walk cycle per individual (N = 10) using the angle tool in Fiji (Schneider et al., 2012). To calculate wavelength (=distance between successive fin-feet), top-view video frames showing the beginning and end of a wave were measured in Adobe Illustrator by finding the length of a spline that follows the curvature of the fin base between fin-foot peaks.

To analyze rotation, we first observed metachronal waves of fin movement in top-view video footage and characterized them as uni-directional or bi-directional. Waves traveling in the same direction in the dorsal and anal fins produce either forward or backward walking; waves traveling in opposite directions produce rotation. For a subset of top-view rotation sequences (N = 3) the turning radius was calculated by selecting three frames from the beginning, middle and end of each rotation sequence, marking the centroid of the fish in each frame, drawing a circle that intercepts all three points, and calculating the radius.

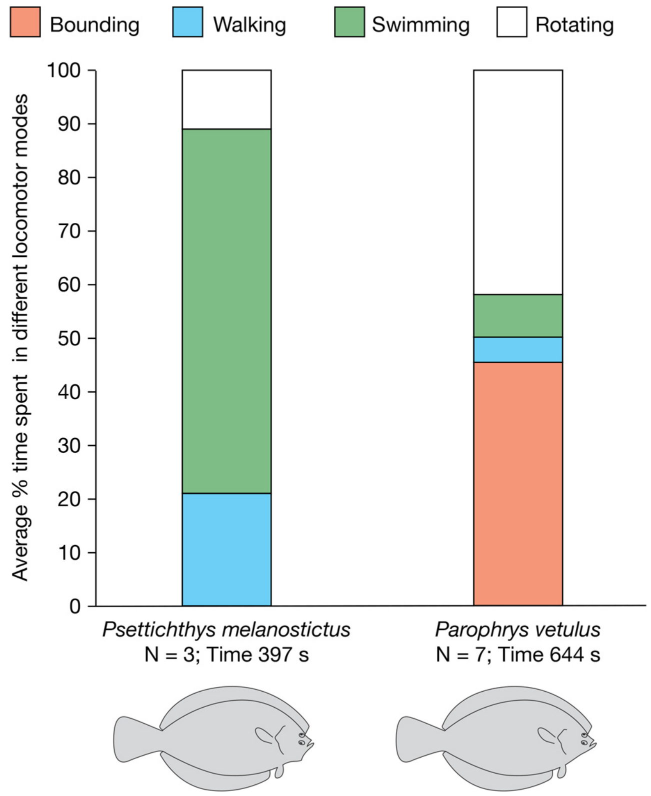

Fig. 1. Percentage time spent walking, bounding, rotating, and swimming for two species of flatfishes, *Psettichthys melanostictus* (N = 3) and *Parophrys vetulus* (N = 3) during voluntary movements in seven-minute observation period.

For fin ray angle analyses, we imported every 8th video frame from representative locomotor sequences into Adobe Illustrator; the bases of dorsal and anal fins were then traced with a spline. Two custom scripts were used. The first script divided the splines for dorsal fin and anal fin as described above, and placed a straight vector line at each point. The distal end of the line was moved manually to align with the end of the fin-ray whose base was closest to the anchor point. The second script calculated the angles for each fin-ray line.

2.3. Statistics

We used Microsoft Excel to calculate means, standard deviations, t-tests, and graph data. All values are reported with standard deviation.

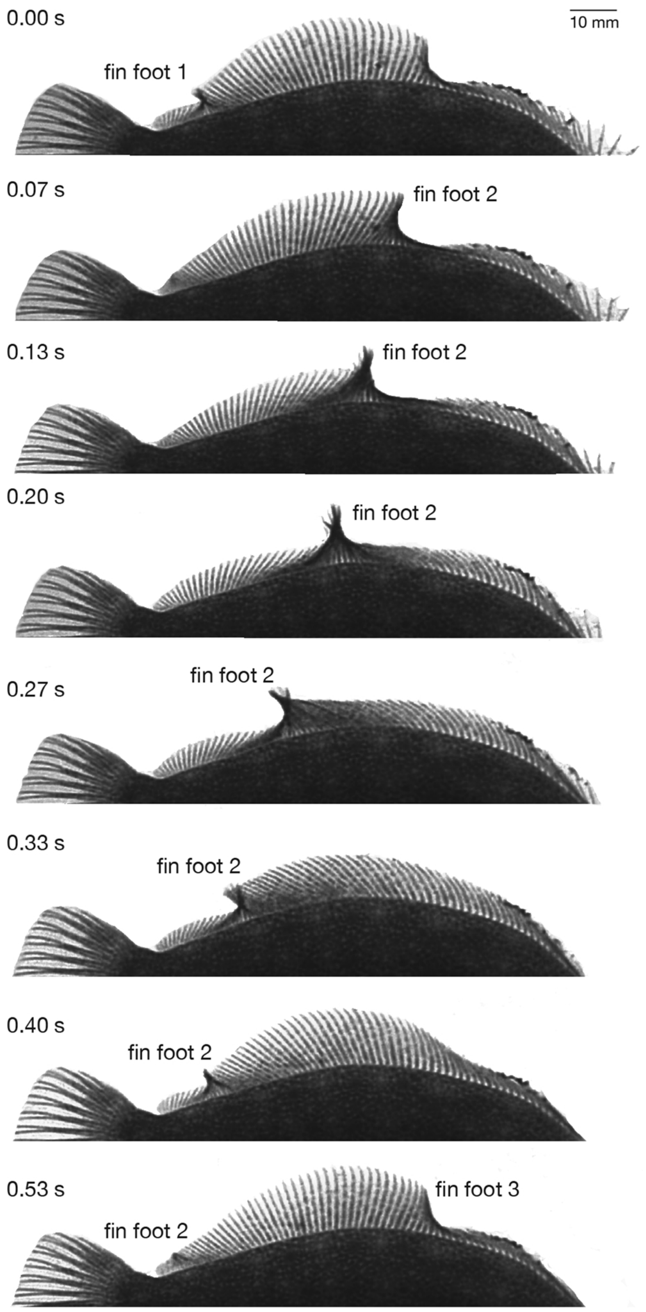

Fig. 2. Frames from a high-speed video (120 fps) showing positions of dorsal fin rays during a benthic walking sequence of the Pacific Sand Sole, *Psettichthys melanostictus*. A single stride in a walk cycle is shown.

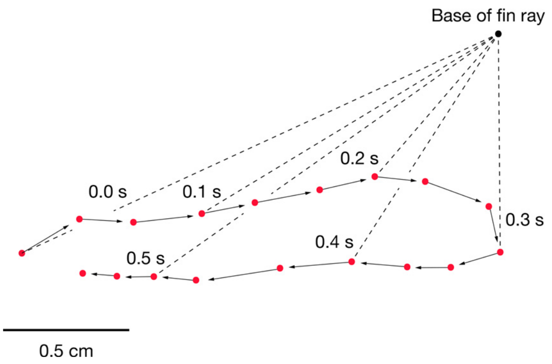

Fig. 3. Tracing of the tip of a single fin ray from the middle of the anal fin of *Platichthys stellatus* in side view during one stride cycle (120 fps). Dotted lines connect the position of the fin ray tip (red dots) to its base (black dot). Fin rays angle down from the fin base to contact the substrate. Beginning at time zero, the fin ray begins to lift off of the substrate. At 0.30 s, the fin ray again contacts the substrate. The distance moved in the horizontal (anterior-posterior) direction is greater than the vertical (lateral) distance moved between the substrate and the maximum elevation of the fin ray.(For interpretation of the references to colour in the text, the reader is referred to the web version of this article).

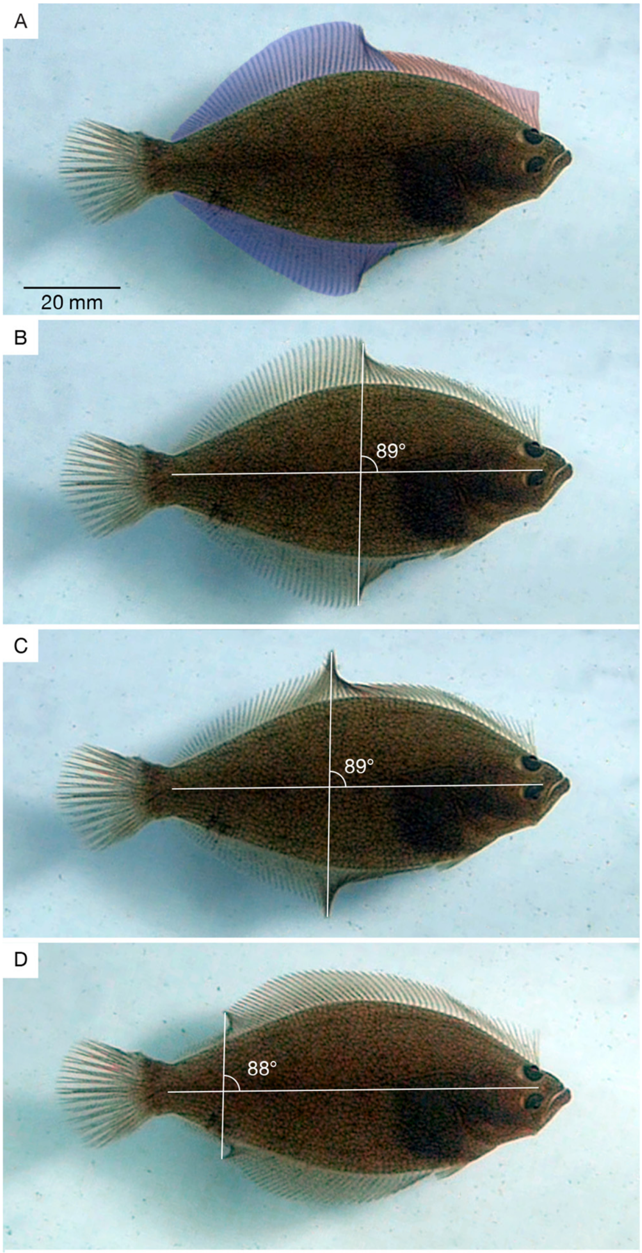

Fig. 4. Fin rays in the dorsal and anal fin move symmetrically to produce steady forward locomotion. A. The anterior-most 35% of the dorsal fin of *Psettichthys melanostictus*, shaded in red, is not used in the propulsive wave. Fin regions active in the propulsive wave, shaded in purple, are equal in the dorsal and anal fins. B. Beginning of the wave mid-way down the body. C. Middle of the wave approximately ¾ of the way along the body. D. End of the wave at the posterior of the body.

We used JMP Version 10 for a Wilcoxon two-sample test to compare gait preferences, a regression analysis, and an ANOVA to examine the bivariate fit of speed in BL/s as a function of wave frequency.

3. Results

3.1. Locomotor Preferences

A 7-minute video of foraging in two species of flatfishes freely moving in the sea table revealed species preferences in locomotor modes (Fig. 1). *Psettichthys melanostictus* ($N = 3$) moved forward on the substrate exclusively using a walking gait, while *Parophrys vetulus* ($N = 7$) moved forward by bounding and, less frequently, walking (Fig. 1). The percentage of time spent walking, swimming, and bounding differs between *P. melanostictus* and *P. vetulus* ($p < 0.05$ for each comparison, Wilcoxon two-sample tests), but species differences for rotation are not significant.

3.2. Forward Walking

Large anterior and posterior inclinations of fin rays occur during walking (Figs. 2 and 3). An exemplar forward walking cycle begins with an individual fin ray held approximately perpendicular (90°) to the body (Fig. 2). The fin ray lifts away from the substrate, toward the eyed side and into the water column. It moves anteriorly to converge with additional fin rays to form a fin-foot. When one fin ray starts to retract from its maximum anterior position, the fin ray immediately behind sweeps forward to reach its maximum anterior position. As it retracts posteriorly, a fin ray pushes down onto the substrate, causing forward movement of the body. During forward walking, more posterior rays are progressively recruited to the fin-foot, creating an anterior-to-posterior metachronal wave of fin-rays in contact with the substrate. In the sequence shown, a new fin-foot originates anteriorly just as the prior fin-foot reaches the posterior end of the fin (Fig. 2 0.00 s and 0.53 s; see also Supplemental Video 1). The wavelength, measured as the distance between two successive fin-feet, is slightly shorter than the length of the anal fin. This results in continuous support and propulsion: as the new fin-foot forms, the prior fin-foot is still present at the posterior end of the fin. In this way, steady locomotor progress is maintained. The fin ray concludes its posterior stroke, then is again lifted from the substrate and rotated forward to begin the next cycle, describing during its complete cycle a movement like a spoke in a wheel (Fig. 3). We observed this basic pattern of fin ray movements in the dorsal and anal fins during forward walking in all six species of flatfishes studied (Supplemental Video 2).

Waves of fin-foot propulsion along the median fins are in phase and operate in parallel, producing a synchronous, symmetrical gait (Fig. 4). As measured here, perfect symmetry would result in a 90° angle between the mid-line and the span line connecting the anterior-most portion of each fin-foot. For 10 strides analyzed, the average angle near the start of a wave was 89.6 ± 2.3°, at mid-wave 89.6 ± 4.2°, and end-wave 88.9 ± 4.1°. In analyses of 24 walk cycles from four *Psettichthys melanostictus* ($N = 12$) and four *Parophrys vetulus* ($N = 12$) wavelengths (= distance between fin-feet) were as follows: dorsal fin *P.*

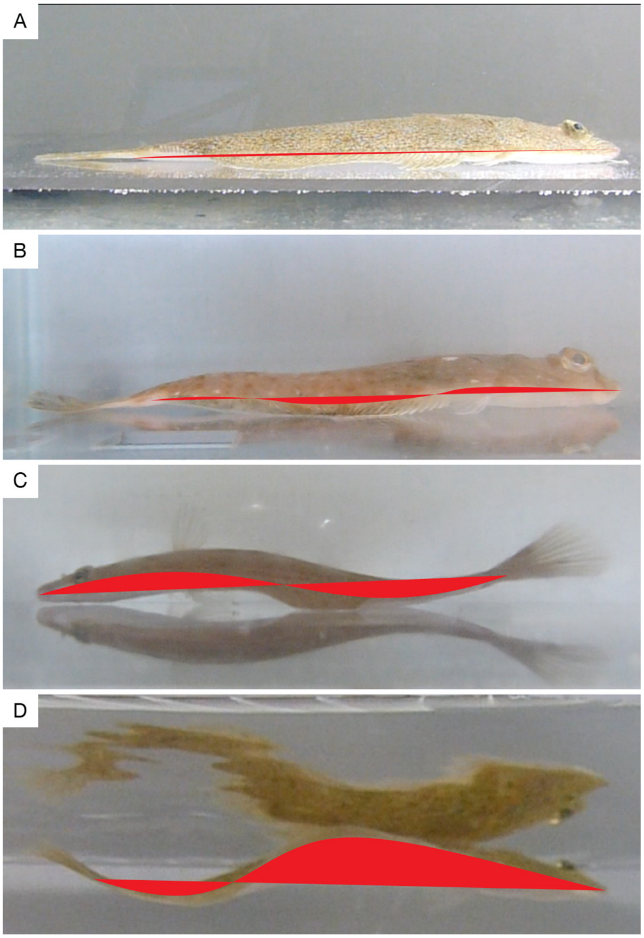

Fig. 5. Undulations of the body during walking and swimming. Areas above and below the mid-sagittal line are shaded red. A. Video frame from a walking sequence of *Psettichthys melanostictus* showing no body undulation; 0.34 BL/s. B. Video frame from a walking sequence of *Parophrys vetulus* with little body undulation; 0.28 BL/s. C. Video frame from a swimming sequence of *P. melanostictus* with some body undulation; 0.66 BL/s. D. Video frame from a swimming sequence of *P. melanostictus* with strong body undulation; 1.13 BL/s. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article).

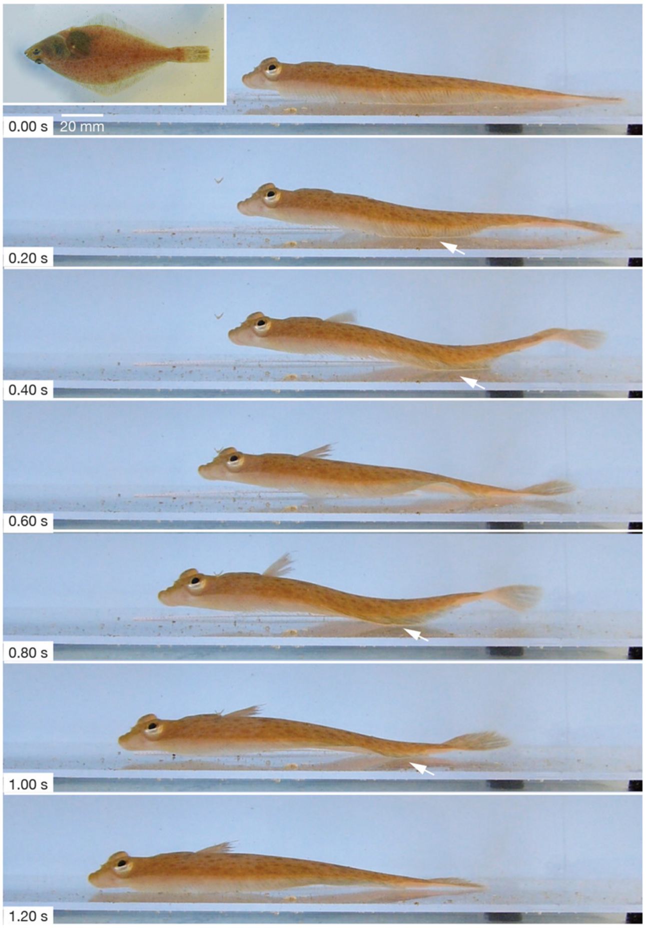

Fig. 6. Frames from high-speed videos of *Parophrys vetulus* showing a bound. White arrows indicate areas of fin-ray contact with the substrate. Fin rays successively push against the substrate, lift the body, and propel it forward. An initial wave of propulsion against the substrate is followed by a single undulation of the tail and body before the fish lands on the substrate. There is a glide phase at 0.60 s where the body is not in contact with the substrate.

melanostictus 0.47 ± 0.01 TL; dorsal fin of *P. vetulus* 0.49 ± 0.01 TL; anal fin *P. melanostictus* 0.46 ± 0.01 TL; and anal fin *P. vetulus* 0.47 ± 0.01 TL.

The dorsal and anal fins of all species studied are unequal in length and have unequal numbers of fin rays. Both fins end at the caudal peduncle, but the anterior end of the dorsal fin is near the anterior margin of the eye, whereas the anterior end of the anal fin is posterior to the pelvic fin. In four *Psettichthys melanostictus* the anal fin averaged 65% of the length of the dorsal fin, and in four *Parophrys vetulus* the anal fin averaged 69% of the length of the dorsal fin. Presumably because of the mismatch in overall fin lengths, the anterior-most portion of the dorsal fin does not form fin-feet during walking or bounding. Rather, the wave of movement that produces the fin-foot starts posterior to the head, in the same position in the transverse plane as the anterior-most end of the anal fin.

Walking requires little to no undulation of the body (Fig. 5A–B; also see Supplemental Video 1) whereas larger amplitude undulations are used in swimming (Fig. 5C–D). The amount of body undulation during walking and swimming of *Psettichthys melanostictus* and *Parophrys vetulus* (4 swimming sequences for two species, N = 3 individuals; 7 walking sequences for two species, N = 7 individuals) was significantly different (t-test $p < 0.005$).

3.3. Forward Bounding

During voluntary movements, *Parophrys vetulus* preferentially used a second substrate-based gait, which we term bounding (Fig. 6; also see Supplemental Video 3). During bounding, one or two rapid waves of fin propulsion are followed by a glide, during which the fish is no longer in contact with the substrate and body and tail undulation contribute to forward thrust (Fig. 6). During the landing phase, the fin rays are protracted and deflected towards the substrate to brake the body. A distinct pause with no forward movement follows the landing phase, before another bound is initiated.

Plots of locomotion in X-Y space highlight key differences between walking and bounding (Fig. 7). Walking is a continuous forward movement produced by successive waves of fin-feet (there are six waves in the sequence in Fig. 7A), each following immediately after the other. Bounding (Fig. 7B) involves intermittent forward movement with one to two waves of propulsion followed by distinctive short pauses when the body is at a full stop; these pauses lasted 0.48 s–3.61 s in the

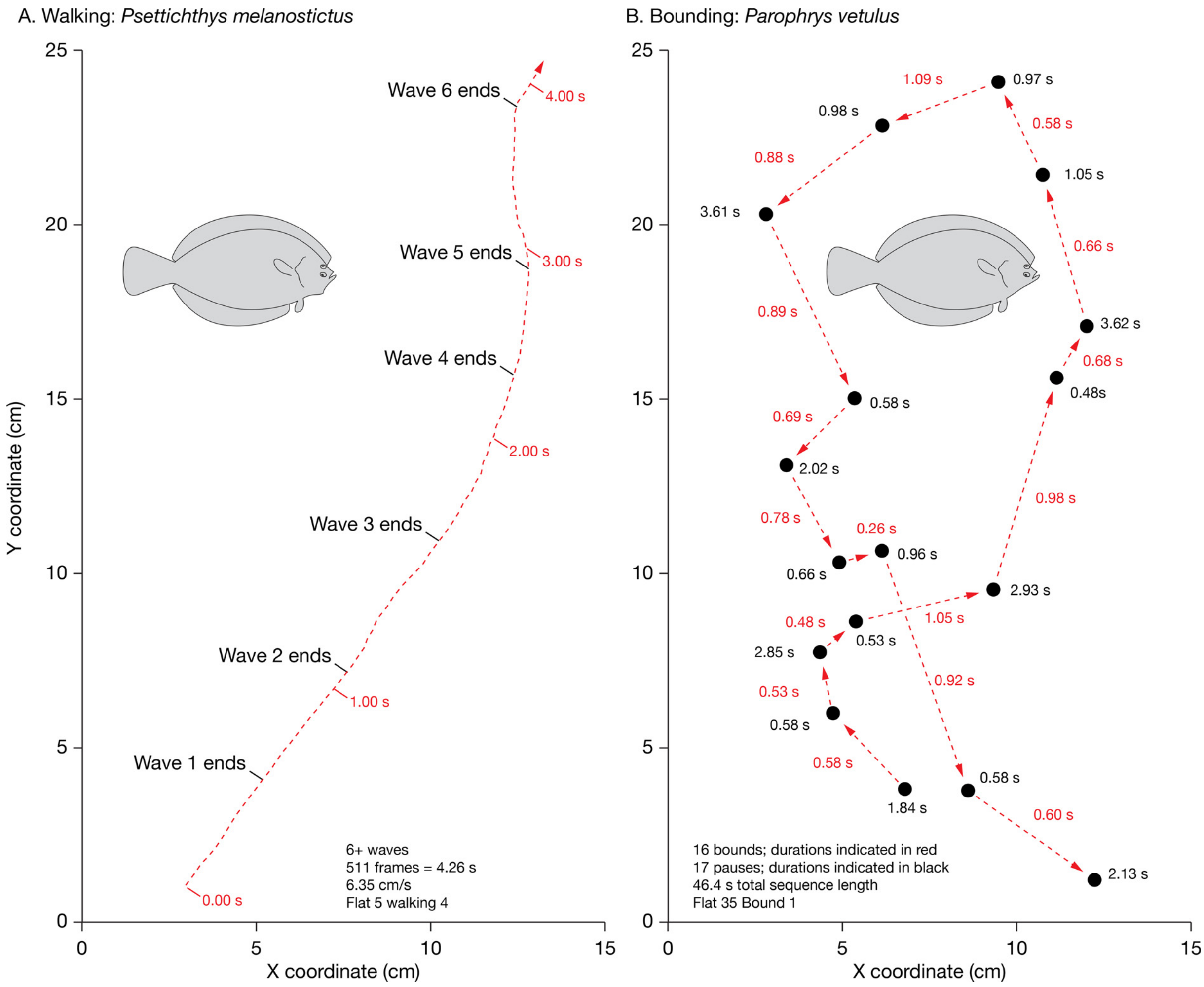

Fig. 7. Movements of two species of flatfish, *Psettichthys melanostictus* and *Parophrys vetulus,* as seen from above in X-Y space. Movement paths indicated with red dashed lines and arrows. A. Walking sequence of *Psettichthys melanostictus* (4.2 s total) with six successive waves and times noted. B. Bounding sequence of *Parophrys vetulus* (46.4 s total) with pauses (black dots) between bounds. Durations of pauses indicated with black numbers; direction of bounds indicated with red dashed lines, and durations of bounds indicated with red numbers. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article).

sequence shown in Fig. 7B (also see Supplemental Video 3). Frequent changes in direction highlight the maneuverability typical of bounding behavior (Fig. 7B).

3.4. *Maneuvering and Rotation*

All six species used variations in the relative wavelength, duration, and direction of the waves of fin-feet along their dorsal and anal fins to produce rotating and pivoting maneuvers (N = 31 rotation sequences total). Fig. 8 shows a locomotor sequence of *Platichthys stellatus* with fin-rays moving in opposite directions against the substrate on opposite sides of the body to achieve a near-zero turn rotation (Fig. 8). Analysis of three such rotations of *Platichthys stellatus* had an average turning radius of 1.1 ± 0.4 cm. The blind-side pectoral fin of *P. stellatus* is near the center of mass, and appears to serve as a rotation point (Supplemental Video 4). Rotations with larger turning radii occurred when a wave of forward propulsion passed along one median fin, with the other median fin held stationary as a pivot point. Rotation combined with forward lunging occurred during predatory strikes.

3.5. *Comparisons of Locomotor Behaviors*

The angles subtended by individual fin rays differ during three locomotor behaviors: bounding, walking, and swimming (Fig. 9). We focus here on analyses of the two dorsal fin rays and two anal fin rays nearest to the mid-point of the body because we could clearly measure them throughout all sequences and highlight the differences between the locomotor modes. To assess the ranges of fin-ray motion, we calculated the differences between the smallest angle (anterior inclination) and the largest angle (posterior declination) reached by the focal fin rays. The anterior to posterior range of fin-ray motion during walking was as great as 93° (i.e., the fin-ray moved from 41° anterior inclination to 134° posterior declination). The mean range of motion during walking = 74 ± 14 °N = 44; for bounding: mean = 37 ± 9 °N = 36; for swimming: mean = 20 ± 7 °N = 44. The range of motion for walking was significantly greater than for bounding (t-test; $p < 0.005$), and significantly greater than for swimming (t-test; $p < 0.001$). The range of motion for bounding was significantly greater than for swimming (t-test; $p < 0.001$).

To assess the convergence of fin rays, we calculated differences in angles between adjacent focal fin rays. For example, during the walking sequence shown in Fig. 9B 0.13 s, the convergence of dorsal fin rays is 102° (i.e., 145°–43°); this was the greatest fin ray convergence observed across all locomotor behaviors. Mean convergence angles were: walking 30 ± 39°; bounding –20 ± 19°; and swimming –15 ± 9°. Convergence during walking differed significantly from convergence during swimming (t-test; $p < 0.001$).

As in Fig. 4, fin rays in the anterior portions of the dorsal fin do not

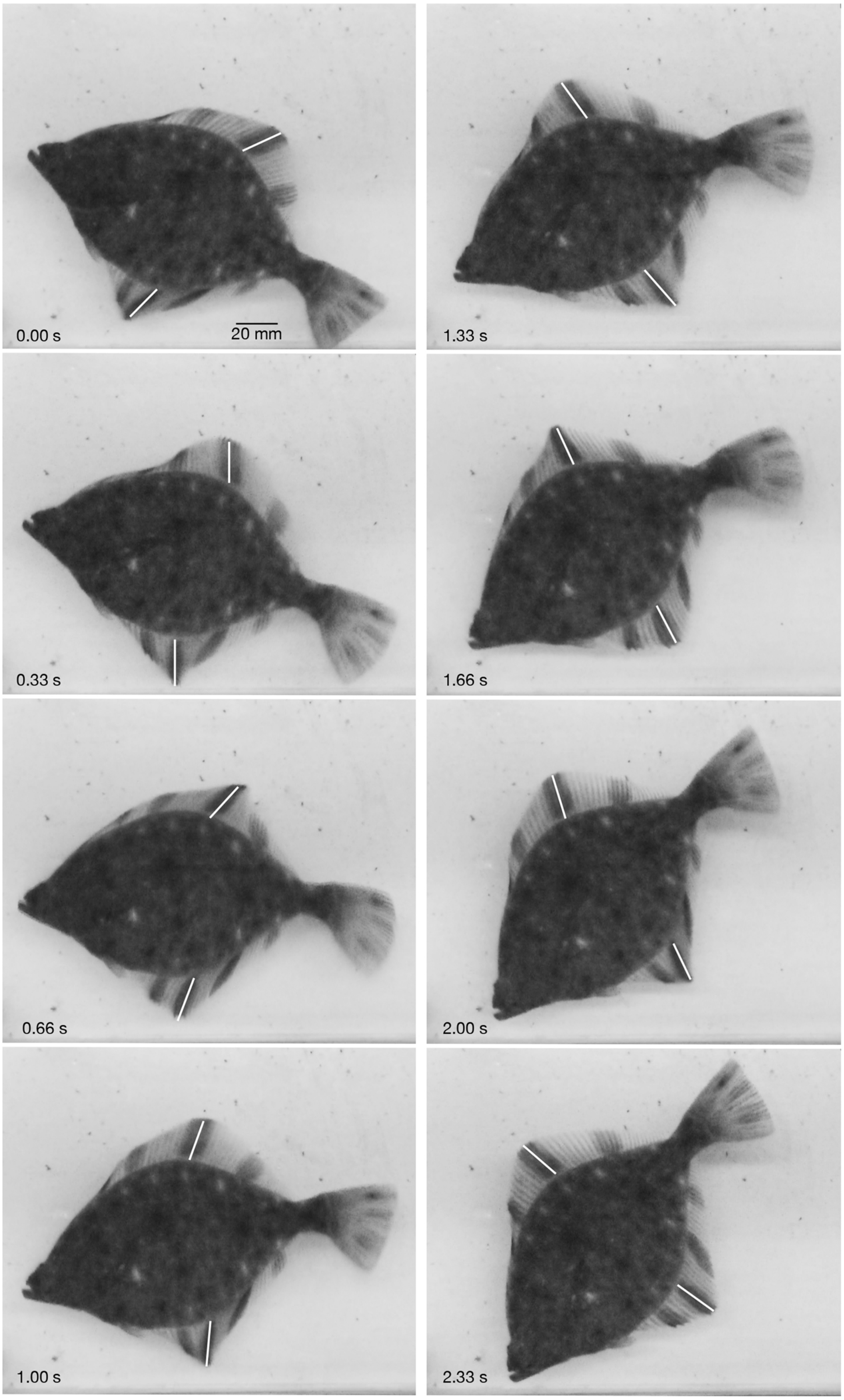

Fig. 8. Frames from high-speed videos of *Platichthys stellatus* showing rotation in place. White lines highlight the fin-ray angles. The fin rays push against the substrate in opposite directions to produce rotation.

contribute to the formation of fin-feet and show a limited range of motion in all four locomotor behaviors (Figs. 9 and 10). In particular, the anterior-most dorsal fin rays of *Psettichthys melanostictus* extend in a fixed position nearly perpendicularly from their insertion above the eye during walking and swimming (Fig. 9B–C).

Fin ray angles during rotation of *Parophrys vetulus* show a large range of motion and are asymmetrical in the dorsal and anal fins (Fig. 10). The mean range of motion of the four focal fin rays during rotation was 65 ± 18° (N = 36). The mid-body fin rays on dorsal and anal fins reach asymmetrical obtuse and acute angles at the beginning

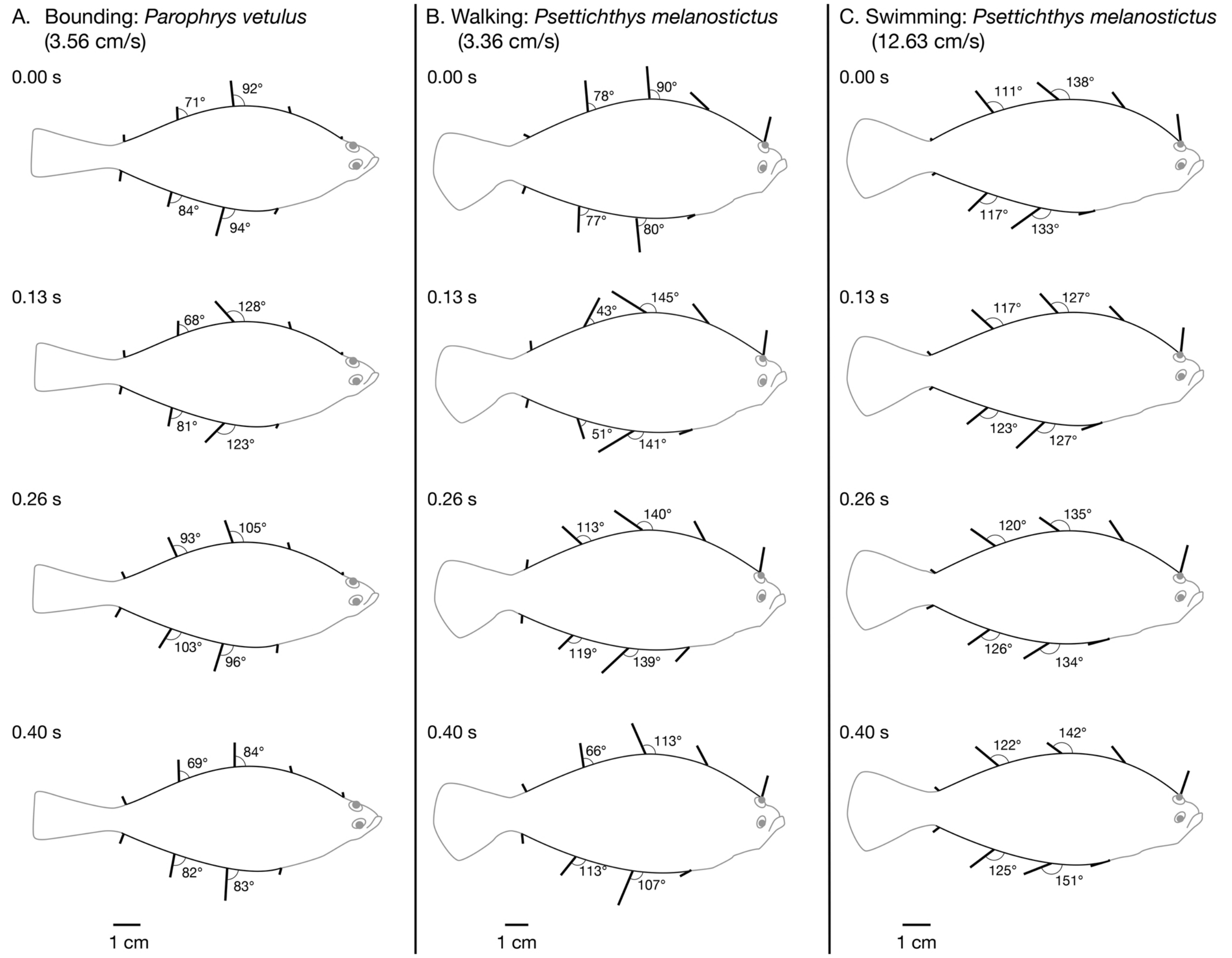

Fig. 9. Comparison of fin ray angles for three locomotor behaviors at 0.4 s frame sampling. Heavy solid black lines indicate the five dorsal fin rays and four anal fin rays; angles are reported for two focal fin rays in the dorsal fin and two focal fin rays in the anal fin. A. Fin ray angles during walking of *Psettichthys melanostictus*. Fin rays converge to form fin-feet (0.00-0.13 s) that rapidly progress backwards (0.26 s) and begin their return to the start condition (0.40 s). B. Fin ray angles during bounding of *Parophrys vetulus*. Fin rays converge to push away from the substrate (0.13 s) followed by a glide (0.26 s) and fin protraction (0.40 s) before initiating landing. C. Swimming in *P. melanostictus*. Fin rays show minimal convergence.

and end of the rotation maneuver (0.00 s 64° and 95°; 0.20 s 112° and 32°; 0.27 s 117° and 45°; 0.47 s 65° and 98°) and are symmetrical in the middle of the rotation maneuver (0.07 s 74° and 75°; 0.40 s 101° and 89°). Posterior fin rays parallel the position of mid-body fin rays as they reach maximum inclination and declination (0.27 s anal fin 45° and 43°; 0.33 s dorsal fin 115° and 115°) and show convergence in the middle of the rotation maneuver (0.20 s dorsal 48° and 112°; anal 98° and 42°).

3.6. Speed Analyses

Walking speed in BL/s increases significantly for both *Psettichthys melanostictus* and *Parophrys vetulus* as wave frequency increases (Fig. 11; F < 0.0001).

We compared the speed of 106 fin-feet (54 for *Psettichthys melanostictus*, 52 for *Parophrys vetulus*) moving posteriorly during walking to the average forward speed of the body (Fig. 12). Average fin speed was greater than body speed with a slip ratio (ratio between forward speed and wave speed) of 0.53 ± 0.1 for *P. melanostictus* and 0.36 ± 0.1 for *P. vetulus.*

Comparisons of bounding, walking, and swimming speeds show significant differences between the two substrate-based benthic gaits and swimming (Fig. 13). Because of the pauses between each set of one or two waves, bounding is a slow locomotor mode, with *Parophrys vetulus* achieving an average bounding speed of 0.22 ± 0.1 BL/sec. Walking was slightly faster than bounding, with an average walking speed in *Psettichthys melanostictus* of 0.34 ± 0.1 BL/s (27 sequences N = 6) and average walking speed for *P. vetulus* of 0.39 ± 0.1 BL/s. In both species, swimming speeds were more than twice as fast as walking: 0.91 ± 0.3 BL/s for *Psettichthys melanostictus* and 0.96 ± 0.3 BL/s for *Parophrys vetulus*. Three transition sequences lasting 4.37 to 27.6 s in length for three individual *Psettichthys melanostictus* showed a smooth transition between walking and swimming and a significantly faster swimming speed than walking speed ($p < 0.04$). Average walking speed during transition sequences was 0.29 ± 0.1 BL/s, and average swimming speed was 0.96 ± 0.3 BL/s.

4. Discussion

Six species of pleuronectids studied use median fins to walk and maneuver in direct contact with the substrate using successive recruitment of fin rays to form a fin-foot that travels down the body in a metachronal wave. This metachronal locomotion resembles the

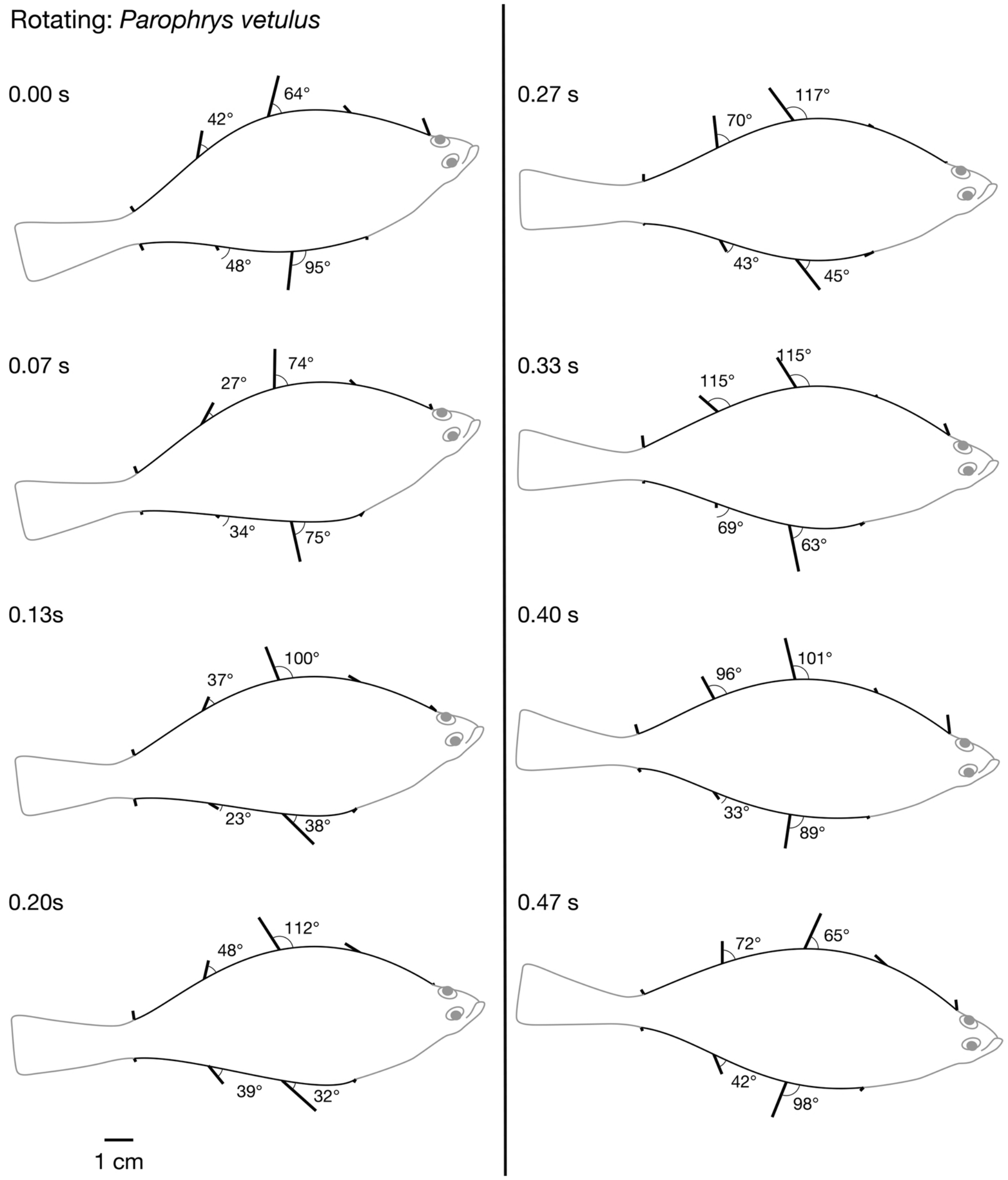

Fig. 10. Fin ray angles during a rotation of *Parophrys vetulus* at 0.4 s frame sampling. Heavy solid black lines indicate the five dorsal fin rays and four anal fin rays; angles are reported for two focal fin rays in the dorsal fin and two focal fin rays in the anal fin.

movements of feet of millipedes and parapodia of polychaetes (Sleigh and Barlow, 1980; Hesselberg, 2007) and enables a previously undescribed mode of substrate-walking in vertebrates. Because transformation of body orientation during metamorphosis of flatfishes results in dorsal and anal fins positioned on the functional lateral edges of the fish, flatfishes use median appendages for substrate-based locomotion. Flatfishes walk on the substrate using fins that are not homologous to the paired appendages of other vertebrates, yet, like vertebrates that use paired appendages, flatfishes coordinate movements of their median fins to produce symmetrical gaits.

We also found that similar body and fin morphologies can produce functional diversity, highlighting the importance of examining both morphology and behavior to assess locomotor capabilities. Flatfishes can use their unpaired fins to produce both walking and bounding gaits, and they also use the dorsal and anal fins to reorient their bodies during benthic locomotion. However, species of flatfishes with only subtle morphological differences prefer different locomotor modes. For example, *Psettichthys melanostictus* routinely used the tips of its median fin rays to walk with a continuous forward motion and minimal body undulation, reserving rapid and intermittent acceleration for direct attacks on prey. *Parophrys vetulus*, in contrast, foraged using a distinct bounding gait characterized by rapid and intermittent acceleration with pauses between bounds to reorient and arch the body upwards, effectively placing the eyes in a higher visual plane above the substrate. Although all six species studied exhibited lunging and gliding behaviors, none employed a bounding gait as consistently as *P. vetulus*. Bounding is a conspicuous and therefore potentially risky behavior depending on environmental conditions. For example, Lemke and Ryer (2006: 267) showed that juvenile *P. vetulus* are subject to greater predation in clear water than are two other flatfishes studied (Pacific Halibut, *Hippoglossus stenolepis*, and Northern Rock Sole, *Lepidopsetta polyxystra*), a difference they attributed to the more conspicuous foraging behaviors of *P. vetulus*. When tested in turbid water that simulates the estuarine habitats to which juvenile *P. vetulus* recruit, the predation rate decreased and was comparable to those found for *H. stenolepis* and *L. polyxystra* in clear water. Subsequently, Ryer et al. (2012) reported a higher intrinsic

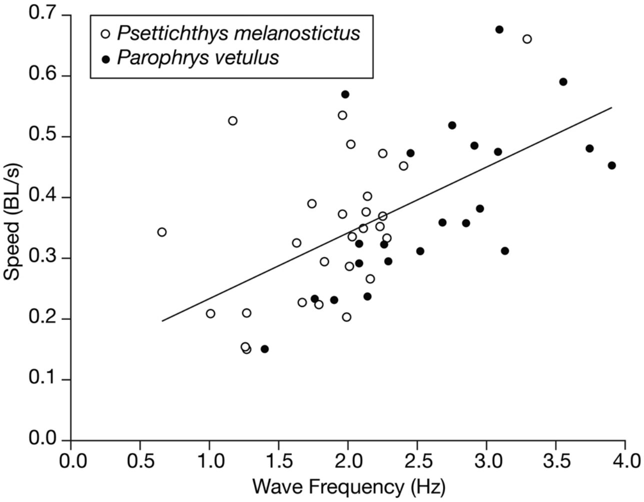

Fig. 11. Speed in BL/s as a function of wave frequency for *Psettichthys melanostictus* (N = 27 sequences) and *Parophrys vetulus* (N = 22 sequences).

growth rate for *P. vetulus* than for *H. stenolepis* or *L. polyxystra*, which may be linked to the more active foraging behavior of *P. vetulus*. Thus, the different gaits we observed in morphologically similar species may reflect trade-offs related to water clarity, foraging strategy, and intrinsic growth rates.

How does benthic locomotion of flatfishes compare to that of other fishes that walk on the bottom? Batoids such as skates and rays punt along the substrate using their pelvic fins in a symmetrical gait, with or without assistance from their pectoral fins (Macesic et al., 2013); the ceratotrichia of chondrichthyans are incapable of the convergence observed in the fin rays that form the fin-feet of flatfishes. Lungfishes and epaulette sharks use diagonal sequence gaits, while sea robins move via sequential movements of three pairs of free (that is, not connected by integument) pectoral fin rays (Jamon et al., 2007). Like the median fins of flatfishes, the pectoral fin rays of sea robins move symmetrically, using successive rays for propulsion. However, unlike flatfishes, sea robins are functionally hexapods because they use only six propulsive rays and lack a membrane between the rays. It will be interesting to examine movements of fin-rays in other benthic fishes that have enlarged pectoral and pelvic fins, such as Hillstream Loaches (Balitoridae), to learn if they also use metachronal waves of fin rays to move on the substrate.

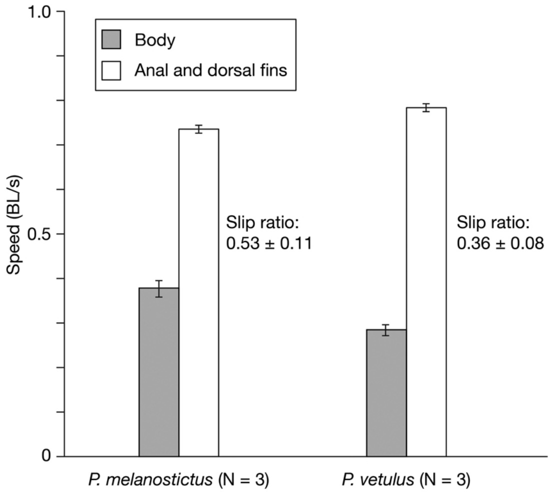

Fig. 12. Average speed of body and median fins for *Psettichthys melanostictus* (N = 3) and *Parophrys vetulus* (N = 3). For each walking sequence analyzed 6 or 7 waves were included. Ratio between forward speed and fin speed is reported as Slip Ratio.

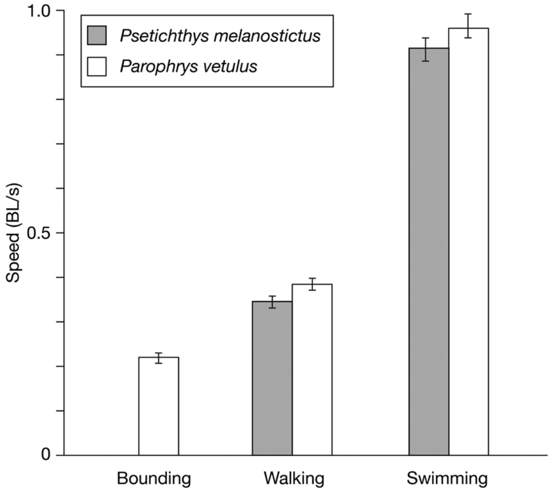

Fig. 13. Comparisons of speeds of bounding, walking, and swimming in *Psettichthys melanostictus* and *Parophrys vetulus*. Note that none of 67 locomotor sequences captured for *P. melanostictus* included bounding. For *P. vetulus* bounding, 15 sequences from 6 individuals were analyzed; for *P. melanostictus* walking, 27 sequences from 6 individuals were analyzed; for *P. vetulus* walking, 22 sequences from six individuals were analyzed; for *P. melanostictus* swimming, 6 sequences from 5 individuals were analyzed; for *P. vetulus* swimming, 3 sequences from 3 individuals were analyzed.

Metachronal waves that flatfishes use for substrate locomotion are both similar to, and different from, those seen in other teleosts that use elongate median fins for swimming. For example, the Bowfin (*Amia calva*) has 48–51 dorsal fin rays (Grande and Bemis, 1998) undulated laterally in waves to swim forward or backward; faster swimming speeds are produced by adding body and caudal fin undulations (Jagnandan and Sanford, 2013). The Black Ghost Knifefish, *Apteronotus albifrons*, has approximately 140–160 anal fin rays (Albert, 2001) and is capable of exceptionally rapid movements and maneuvers —forward, backward, up, and down— achieved by holding the body immobile and modulating wave direction and amplitude, as well as curving the fin rays (Ruiz-Torres et al., 2014). Balistiform locomotion of triggerfishes similarly involves a rigid body and undulation of median fins; specializations of the fin skeleton and associated inclinator and declinator muscles allow strong lateral movements of the fin rays (Sorenson, 2007). Metachronal waves of pleuronectid flatfishes differ in at least two ways: 1) the tips of the fin rays contact the substrate, differentially slowing and distorting the waveform; 2) fin rays converge to form a fin-foot, which requires both mobility and control in which individual fin rays can be protracted, retracted, inclined, or declined with respect to adjacent rays in a joystick-like rotation.

The diverse body shapes and dorsal and anal fin morphology within Pleuronectiformes are undoubtedly suited for different locomotor functions, but relationships between form and function are yet unclear. It is likely that the range of body and fin morphologies exhibited by flatfishes reflects morphological trade-offs to facilitate walking, burying, and swimming behaviors. Members of the most basal lineage of flatfishes (Psettodidae) have fewer vertebrae and fin rays than more derived flatfishes and swim vertically in the water column. There are anecdotal reports of enhanced body-substrate adhering abilities in the disk-shaped American soles (Achiridae). Preliminary observations of benthic walking in tonguefishes (Cynoglossidae), which have elongate bodies, a diphycercal caudal fin, and uniformly short dorsal and anal fin-rays, suggest that they are crawling specialists. Investigating the morphology and locomotor capabilities of diverse flatfish groups will increase our understanding of functional diversity and evolutionary trade-offs in this group, and shed light on the potential locomotor capabilities of transitional flatfishes in the fossil record.

5. Conclusion

The convergence of flatfish fin rays to form a fin-foot that travels down the length of the body and pushes directly against the substrate is a previously undescribed locomotor mode in fishes. The anatomical and functional specializations that underlie benthic locomotion in flatfishes and the diversity of locomotor capabilities across flatfish phylogeny are poorly understood and warrant future investigation. To our knowledge, no other vertebrate uses non-paired appendages to produce a walking gait. Thus, benthic locomotion performed by flatfishes appears to be structurally and functionally unique among vertebrates.

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This project started as part of the Functional Morphology of Fishes course at Friday Harbor Laboratories in summer 2016; many course participants provided early and helpful input. Amy McCune, John Hermanson, and Peter Wainwright offered comments on locomotion in other teleosts and gaits. Frances Lux, Isaiah Gutman, Betty McGuire, and Mark Zifchock assisted with data collection and analyses. Stephen Parry offered statistical support. CHF was supported by NSF GRFP 2016231353 and a Wainwright Fellowship for study at FHL. APS and the fish class were supported by NSF grant IOS -1256602. Additional support from the Tontogany Creek Fund.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.zool.2018.07.002.

References

Albert, J.S., 2001. Species diversity and phylogenetic systematics of American knifefishes (Gymnotiformes, Teleostei). Misc. Publ. Mus. Zool. Univ. Michigan 190, 1–127.

Brainerd, E., Page, B., Fish, F., 1997. Opercular jetting during fast-starts by flatfishes. J. Exp. Biol. 200, 1179–1188.

Eschmeyer, W.N., Herald, E.S., 1983. A Field Guide to Pacific Coast Fishes of North America. (Peterson Field Guides). Houghton Mifflin, New York.

Flammang, B.E., Suvarnaraksha, A., Markiewicz, J., Soares, D., 2016. Tetrapod-like pelvic girdle in a walking cavefish. Sci. Rep. 6, 23711.

Friedman, M., 2008. The evolutionary origin of flatfish asymmetry. Nature 454, 209–212.

Gibb, A., 1995. Kinematics of prey capture in a flatfish, *Pleuronichthys verticalis*. J. Exp. Biol. 198, 1173–1183.

Gibb, A., 1996. The kinematics of prey capture in *Xystreurys liolepis*: Do all flatfish feed asymmetrically? J. Exp. Biol. 199, 2269–2283.

Gibb, A., 1997. Do flatfish feed like other fishes? A comparative study of percomorph prey-capture kinematics. J. Exp. Biol. 200, 2841–2859.

Grande, L., Bemis, W.E., 1998. A Comprehensive Phylogenetic Study of Amiid Fishes (Amiidae) Based on Comparative Skeletal Anatomy. An Empirical Search for Interconnected Patterns of Natural History. J. Vert. Paleontol. 18 (Supplement) Special Memoir #4.

Harrington, R.C., Faircloth, B.C., Eytan, R.I., Smith, R.I., Near, T.J., Alfaro, M.E., Friedman, M., 2016. Phylogenomic analysis of carangimorph fishes reveals flatfish asymmetry arose in a blink of the evolutionary eye. BMC Evol. Biol. 16, 224.

Hendrick, T.L., 2008. Software techniques for two and three dimensional kinematic measurements of biological and biomimetic systems. Bioinspir. and Biomim. 3, 034001.

Hernandez, A.V., Gervais, C.R., Rummer, J.L., Porter, M.E., 2016. Comparing submerged walking and swimming kinematics in epaulette sharks. Integr. Comp. Biol. 56 E301 P2. 185.

Hesselberg, T., 2007. Biomimetics and the case of the remarkable ragworm. Sci. Nat. 94, 613–621.

Jagnandan, K., Sanford, C.P., 2013. Kinematics of ribbon-fin locomotion in the bowfin, *Amia calva*. J. Exp. Zool. 319A, 569–583.

Jamon, M., Renous, S., Gasc, J.P., Bels, V., Davenport, J., 2007. Evidence of force exchanges during the six-legged walking of the bottom-dwelling fish, *Cheidonichthys lucerna*. J. Exp. Biol. 307A, 542–547.

King, H., Shubin, N., Coats, M., Hale, M., 2011. Behavioral evidence of the evolution of walking and bounding before terrestriality in sarcopterygian fishes. Proc. Natl. Acad. Sci. U.S.A. 2011 (108), 21146–21151.

Koester, D.M., Spirito, C.P., 2003. Punting: An unusual mode of locomotion in the little skate, *Leucoraja erinacea* (Chondrichthyes: Rajidae). Copeia 2003, 553–561.

Kruuk, H., 1963. Diurnal periodicity in the activity of the common sole, *Solea vulgaris* Quensel. Neth. J. Sea Res. 2, 1–28.

Lemke, J.L., Ryer, C.H., 2006. Relative predation vulnerability of three juvenile (Age-0) North Pacific flatfish species: Possible influence of nursery-specific predation pressures. Mar. Ecol. Prog. Ser. 328, 267–273.

Macesic, L.J., Kaijura, S.M., 2010. Comparative punting kinematics and pelvic fin musculature of benthic batoids. J. Morphol. 271, 1219–1228.

Macesic, L.J., Mulvaney, D., Blevins, E.L., 2013. Synchronized swimming: Coordination of pelvic and pectoral fins during augmented punting by the freshwater stingray *Potamotrygon orbigny*. Zoology 116, 144–150.

McKee, A., MacDonald, I., Farina, S., Summers, A., 2016. Undulation frequency affects burial performance in living and model flatfishes. Zoology 119, 75–80.

Munroe, T., 2015. Systematic diversity of the Pleuronectiformes. In: Gibson, R., Nash, R., Geffen, A., Veer, van der, John, H. (Eds.), Flatfishes: Biology and Exploitation, 2nd ed. Wiley and Sons, Ltd., New York, pp. 13–51.

Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. Fishes of the World, 5th edition. Wiley and Sons, Inc, Hoboken.

Olla, B.L., Wicklund, R., Wilk, S., 1969. Behavior of winter flounder in a natural habitat. Trans. Am. Fish. Soc. 98, 717–720.

Orcutt, H.G., 1950. The life history of the Starry Flounder, *Platichthys stellatus*. Cal. Dept. Nat. Res. Div. Fish Game Bur. Mar. Fish. Fish Bull., pp. 1–68 No. 78.

Ramachandran, V.S., Tyler, C.W., Gregory, R.L., Rogers-Ramachandran, D., Duensing, S., Pillsbury, C., Ramachandran, C., 1996. Rapid adaptive camouflage in tropical flounders. Nature 379, 815–818.

Roje, D.M., 2010. Incorporating molecular phylogenetics with larval morphology while mitigating the effects of substitution saturation on phylogeny estimation: A new hypothesis of relationships for the flatfish family Pleuronectidae (Percomorpha: Pleuronectiformes). Mol. Phylogenet. Evol. 56, 586–600.

Ruiz-Torres, R., Curet, O.M., Lauder, G.V., MacIver, M.A., 2014. Kinematics of the ribbon fin in hovering and swimming of the electric ghost knifefish. J. Exp. Biol. 216, 823–824.

Ryer, C.H., Boersma, K.S., Hurst, T.P., 2012. Growth and distributional correlates of behavior in three co-occurring juvenile flatfishes. Mar. Ecol. Prog. Ser. 460, 183–193.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of Image analysis. Nat. Methods 9, 671–675.

Schreiber, A.M., 2013. Flatfish: An asymmetric perspective on metamorphosis. Curr. Top. Dev. Biol. 103, 167–194.

Sleigh, M.A., Barlow, D.I., 1980. Metachronism and control of locomotion in animals with many propulsive structures. In: Elder, H.Y., Trueman, E.R. (Eds.), Society for Experimental Biology Seminar Series: Volume 5, Aspects of Animal Movement. Cambridge University Press, Cambridge.

Sorenson, L., 2007. Examining morphological characteristics of triggerfish: A three-dimensional reconstruction of skeletal and muscular features of *Rhinecanthus rectangulus*. J. Young Investig. 16, 3.

Stickney, R.R., White, D.B., Miller, D., 1973. Observations of fin use in relation to feeding and resting behavior in flatfishes (Pleuronectiformes). Copeia 1973, 154–156.

Ward, A.B., 2002. Kinematics of the pectoral fins in batfishes (Ogcocephalidae) during aquatic walking. Integr. Comp. Biol. 42 1331–1331.

Webb, P., 2002. Kinematics of *Pleuronectes platessa*, and Cod, *Gadus morhua*, swimming near the bottom. J. Exp. Biol. 205, 2125.