

BENTHIC WALKING, CRYPTIC LOCOMOTION, AND EVOLUTIONARY
MORPHOLOGY OF BODY FORM IN FLATFISHES (PLEURONECTIFORMES)

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Flatfishes (Pleuronectiformes) are an ecologically and economically important group of benthic predators with unique adaptations to life on the bottom. Flatfishes' key innovations—eye-migration and lateralized behavior —result in evolutionary constraints as well as novel opportunities for this group (Chapter 1). How does an organism find food and evade predators when it is lying on one side? Using kinematic and comparative analyses (Chapter 2) I show that flatfishes use a mode of locomotion that is structurally and functionally unique amongst vertebrates, in which successive portions of median fins are co-opted to form functional “feet” that push against the substrate. This unusual form of locomotion allows the flatfish to maneuver, forage, and traverse across the substrate at slow to moderate speeds while maintaining a low profile. Using Digital Particle Image Velocimetry (Chapter 3) I measured the velocity magnitude and vorticity of water flow around walking and swimming flatfishes. I found that water disturbance was much lower during walking, without the trailing vortices produced during swimming, suggesting that benthic walking in flatfishes is hydrodynamically cryptic. To better understand the evolutionary morphology of this large and diverse group I analyzed body and fin shapes of 67 species from 12 flatfish families using geometric morphometric and phylogenetic methods (Chapter 1). Lastly, in fulfillment of my NSF Graduate Research Fellowship in K-12 STEM Education, I evaluated the impact of a new mobile science program using a combination of survey and interview methods (Chapter 4).

BIOGRAPHICAL SKETCH

Claire Hope Fox was born in Burlington, Vermont, and spent her childhood running around in the forest, catching animals, and splashing in the streams of the Blue Ridge Mountains, Virginia. She graduated from Cornell University with a B.A. in Visual Studies in 2001. Fascinated by the intersection of art and technology, she created an interdisciplinary course of study that integrated cultural theory, studio art, art history, technology studies, architecture, and the science of perception. Claire settled in Ithaca NY to raise two sons, Felix and Casper, with her husband Mark. A life-long learner at heart she has served as co-founder, director, and teacher at a series of educational nonprofit organizations including a cooperative learning center, community makerspace, mobile science program, and community science workshops. She is dedicated to building resources that enable anyone, especially from communities that have been historically oppressed and marginalized, to get excited about and enjoy science. It was her work with under-served youth that inspired her to pursue a PhD. In encouraging young people to dream big and overcome barriers she realized that she, too, could pursue her childhood dream of studying Zoology.

Dedicated to all the young people out there who are curious and fascinated by the natural
world around them, who remind me daily to find the joy
and never stop asking questions

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CHAPTER 1

DIVERSITY OF BODY FORM IN FLATFISHES (CARANGARIA:
PLEURONECTIFORMES) WITH COMMENTS ON FUNCTIONAL BIOLOGY AND
FEEDING BEHAVIOR ¹

¹ Claire Fox and William E Bemis

Abstract

Flatfishes (Pleuronectiformes) are a diverse and ecologically important group of benthic predators with unique morphological innovations for life on the bottom. How the unique *Bauplan* of flatfishes arose and diversified to fill ecological niches world-wide has been the subject of historic and recent interest. Here, to explore the morphological diversification of flatfishes, we use geometric morphometric analysis of radiographs and photographs in a phylogenetic context to investigate patterns of morphological diversity for 67 species (139 specimens) representing 13 of the 14 extant families of Pleuronectiformes. Separate PCA analyses of landmark-based data and meristic data are compared. Our analyses include transitional Eocene fossils, both species in the basal-most extant family of Pleuronectiformes, and outgroups within Carangaria. We resurrect and redefine three feeding types among flatfishes and use ancestral state reconstruction to evaluate the evolution of three ecologically important variables: feeding type, substrate type, and water type. We confirm family-level trends in eye size, interocular distance, jaw size, and abdomen size that relate to feeding type. The largest component of body shape evolution in flatfishes is elongation, which we show is correlated with increased vertebral counts. We discuss some functional implications of morphological differences in body, fin, and jaw shapes, and note limitations of our categories of feeding types because some flatfishes are broadly opportunistic predators.

“The Pleuronectidae, or Flat-fish, are remarkable for their asymmetrical bodies...the eyes offer the most peculiarity, for they are both placed on the upper side of the head. That the Pleuronectidae are remarkably adapted by their flattened and asymmetrical structure for their habits of life, is manifest from several species, such as soles, flounders, etc., being extremely common. The chief advantages thus gained seem to be protection from their enemies, and facility for feeding on the ground. *The different members of the family present, as Schiödte remarks, “a long series of forms exhibiting a gradual transition from Hippoglossus pinguis [Reinhardtius hippoglossoides] which does not in any considerable degree alter the shape in which it leaves the ovum, to the soles, which are entirely thrown to one side.”* - Charles Darwin

1872 p. 387

1. Introduction

Flatfishes (Pleuronectiformes) are an ecologically and economically important order of primarily marine fishes with about 800 species in 14 families occupying a wide range of ecosystems worldwide, structuring benthic communities and filling niches as keystone predators and prey, contributing largely to benthic biomass, and including commercially valuable food fish species (Munroe, 2015). Post-metamorphic flatfishes exhibit a key innovation, one that is unique among vertebrates, for life on the benthos: asymmetry and eye migration during ontogeny that allow flatfishes to lie flat on the substrate on one side of the body with both eyes pointing upwards. This innovation is accompanied by a suite of secondary adaptations for predation, locomotion, and camouflage, including: protrusible eyes (Chapleau, 1993); ability to alter body pigmentation to match the background (Ramachandran et al., 1996; Akkaynak, 2017); dorsal and anal fins with long bases (the dorsal fin originates on cranium) that are used for benthic locomotion (Wirtz and Davenport, 2017; Fox et al., 2018); asymmetric development of the lateral line (Voronina et al., 2019); asymmetry of trunk musculature; asymmetry of skin coloration; asymmetry of jaw morphology and jaw movement (Gibb, 2003); and often, asymmetry of pectoral fins. Although flatfishes share this broad suite of secondary adaptations, functional aspects of their morphology including body elongation, eye size, jaw size and shape, length of fin rays, and shapes of fins, vary greatly (Fig. 1.1).



Figure 1.1. **Diversity of body form in flatfishes.** Pleuronectiformes from six families display a range of body and fin shapes and features related to prey sensing and capture (eye size, eye location, jaw size and jaw symmetry). Images were created by layering a partial-transparency photograph of each fluid-preserved specimen over its inverted radiograph.

1.1. Historical and Evolutionary context

The flatfish *Bauplan* has long puzzled and fascinated evolutionary biologists, and periodically over the past 150 years flatfishes have been at the center of heated debates over evolutionary theory and methods. Transitional flatfish fossils were unknown in the 19th century, and discussion about the origin and history of Pleuronectiformes was mostly speculative. Mivart, an early advocate of Darwin's evolutionary theory who is notorious for later becoming one of his most vociferous critics, used flatfishes as a prime example of the problems he perceived with natural selection. Mivart (1871: 38) asserted that the gradual selection-driven change proposed by Darwin was not feasible because a partly-migrated eye “must rather have been injurious” to a pleuronectid in its intermediate form; he allowed that a sudden change in eye position could be beneficial but “an accidental occurrence of such a spontaneous transformation is hardly conceivable.” Sixty two years later, Goldschmidt (1933: 545) posited the spontaneous transformation of body form in flatfishes that Mivart had thought impossible. Pleuronectids were, according to Goldschmidt, likely examples of “Hopeful Monsters” – organisms in which a single mutation with a large effect results, randomly and rarely, in a trait with adaptive value that founds a new group. Goldschmidt believed that eye migration in flounders could be accomplished with a single mutation; all other cranial and neural transformations would be “necessary consequences of the first step” and smaller mutations would be gradually accumulated over time through natural selection.

Recent paleontological evidence shows that evolution of the flatfish *Bauplan* was gradual and incremental, with ocular asymmetry being one of the first derived features to arise. In his description of †*Heteronectes chaneti* and re-examination of †*Amphistium*

paradoxum, both from localities near Bolca, Italy, Friedman (2008, 2012) recognized intermediate eye migration along with a mixture of ancestral and derived character states that place them unequivocally as stem Pleuronectiformes. Partial eye migration found in large-sized, fully ossified adult †*Heteronectes* and †*Amphistium* and their presence in the fossil record over two geological stages proves that the intermediate condition was not maladaptive in this group. Piscivory, most likely through ambush, is likely to be the feeding mode of stem flatfishes, and †*Amphistium* and †*Heteronectes* were piscivorous based on morphological features and stomach contents preserved in the fossils (Friedman 2008, 2012). Experimental studies of diet and behavioral observations for the basal-most extant genus of Pleuronectiformes, *Psettodes*, exclusively foraged by ambush predation, accepting only live demersal fish (Kizhakudan et al., 2013). Juvenile and adult *Psettodes* lie buried in the sand waiting for prey fish to approach and “snap the fish with wide open flexible jaws bearing large number[s] of teeth and swallow it”, typically consuming one or two before retreating to the sand (Kizhakudan et al., 2013: 28).

Which came first, the behavior or morphology? Other examples of extant fishes outside the flatfish clade that employ similar predation strategies include the cichlid *Haplochromis livingstoni*, which lies on one side on the substrate, apparently feigning death, until small fishes come close enough to ambush (McKaye, 1981); similar behavior has been noted in another cichlid (Tobler, 2005) and a serranid, the comb grouper *Mycteroperca acutirostris* (Gibran and Armbruster, 2004). For fish already exhibiting this mode of ambush behavior, improvements in visual perception of prey could be advantageous. Schreiber (2005) showed that lateralized behavior initiates before eye

migration in larval flatfishes and is decoupled from it. It may be that behavior preceded morphology in this case.

Despite recent progress in understanding the evolutionary history and interrelationships of Pleuronectiformes, many questions remain (Munroe, 2015). Even the fundamental question of whether flatfishes are monophyletic has been controversial (Campbell et al., 2013; Betancur-R. and Ortí 2014) with early molecular support inconclusive, most likely due to long-branch attraction (Wei et al., 2018); the subsequent debates spurred both criticisms of and refinements to molecular phylogenetic methods. Here, we regard Pleuronectiformes as monophyletic and sister to Pleuronectoidei based on anatomical synapomorphies (Chapleau, 1993), and molecular analyses with sound hypothesis-testing procedures and adequate taxonomic coverage (Betancur-R. et al., 2013b; Betancur-R. and Ortí 2014), and a large number of ultraconserved DNA element loci and broader clade sampling (Harrington et al., 2016). Molecular phylogenetic studies place Pleuronectiformes within Carangaria (Carangimorpha, *sensu* Li et al. 2009, Harrington et al. 2016; Carangimorpharia, Betancur-R. et al., 2013a,b), which includes archerfishes, Nile perches, cobias, remoras, swordfishes, moonfishes, barracudas, and jacks. Pleuronectiformes originated in the Paleocene and rapidly diversified in an adaptive radiation (Harrington et al., 2016; Ribeiro et al., 2018), a pattern of diversification common within Carangaria and acanthomorph clades more generally (Friedman, 2010). The earliest anatomically modern flatfish, †*Eobothus minimus* (minimum age 63 Ma, Rabosky et al., 2018) has morphological apomorphies characteristic of the bothid lineage of Pleuronectoidei; †*Eobothus minimus* is abundant at Monte Bolca along with †*Amphistium paradoxum* (Chanet, 1999). One of the earliest

soles (Soleidae), †*Eobuglossus eocenicus* from a locality near Cairo, also dates from this period (Chanet, 1994).

Early taxonomic efforts grouped flatfishes by the morphological trait of “handedness”, i.e., to which side of the body eyes migrated, a trait now understood to be an unreliable indicator of relatedness because of familial-, generic-, and individual-level reversals (Palmer, 2009). Monophyly of six families of Pleuronectiformes is well established by both morphological and molecular phylogenetic analyses: Psettodidae (Chapleau, 1988), Pleuronectidae (Cooper and Chapleau, 1998), Cynoglossidae (Chapleau, 1988), Samaridae (Sakamoto, 1984), Achiridae (Ramos, 1998), and Soleidae (Chapleau and Keast, 1988). Four groups traditionally regarded as subfamilies of Pleuronectidae have been elevated to families: Paralichthodidae, Rhombosoleidae, and Poecilopsettidae (Chapleau and Keast, 1988; Chapleau, 1993). Scopthalmids, traditionally regarded as a sub-family of Bothidae (Norman, 1934), are now confirmed to be monophyletic and are broadly recognized as Scopthalmidae (Hensley and Ahlstrom, 1984; Chapleau 1993; Chanet 1998). Achiropsettidae, Paralichthodidae and Rhombosoleidae are small families whose affiliations and familial status remain uncertain. Paralichthyidae is problematic: in addition to a lack of morphological synapomorphies (Chapleau, 1993; Hoshino, 1999), molecular phylogenies consistently divide the family into two or more groups. Monophyly of Citharidae and its recognition as a family was reinforced by identification of new morphological synapomorphies (Hoshino, 2001) following decades of contention over shared derived characters and the inclusion of genera with opposite “handedness”.

Recent molecular phylogenetic studies provide enough taxon sampling within Pleuronectiformes to begin exploring phenotypic evolution in this important and curious clade of fishes (Byrne et al., 2018, Rabosky et al., 2018). In this study we use geometric morphometrics to examine body form variation of flatfishes in a phylogenetic context, investigate possible ecological correlates of body form, and assess conservation and convergence of aspects of feeding and habitat within Pleuronectiformes using ancestral character state reconstruction.

2. Methods

2.1. Specimen Selection

Specimens were selected from the fluid ichthyology collections of the Smithsonian Institution, American Museum of Natural History, and Cornell Museum of the Vertebrates; selection was informed by specimen condition, morphological diversity, and availability of phylogenetic and ecological data. We studied 139 specimens representing 67 species of Pleuronectiformes from 13 families: Psettodidae: 2 species (N=9), Citharidae: 5 species (N=12), Samaridae: 3 species (N=12), Cynoglossidae: 5 species (N=11), Achiridae: 6 species (N=12), Soleidae: 5 species (N=9), Pleuronectidae: 7 species (N=15), Paralichthyidae: 9 species (N=17), Bothidae: 9 species (N=16), Scophthalmidae: 5 species (N=12), Poecilopsettidae: 4 species (N=4), Rhombosoleidae: 5 species (N=5), Achiropsettidae: 2 species (N=2). We chose the pilotfish, *Naucrates ductor* (Carangaria: Carangidae), as an outgroup. We included two genera of fossil flatfishes (N=2) with exceptional preservation: †*Amphistium paradoxum* (MNHNFBOL86) and †*Heteronectes chaneti* (NMNH_1974-1639-24); we used

photographs of fossils for landmark-based morphometrics and interpretive drawings and descriptions in Friedman (2008, 2012) for meristic counts. We estimated eye sizes and position based on the ocular opening. We grouped species into families for PCA, ANOVA, and ASR analyses according to the 14 families recognized by Munroe (2015) and Nelson et al. (2016). We obtained current counts for the number of species in each family from Eschmeyer's Catalog of Fishes (Eschmeyer et al., 2020). We did not include three small families (Leptobramidae, 2 species, Oncopteridae, 1 species, Paralichthodidae, 1 species) recognized by Eschmeyer et al. (2020).

2.2. Radiography and Photography

NMNH specimens were radiographed at the Smithsonian Museum Support Center using a Varian PaxScan 4030 E panel with Kodak Lanex Fine Screen scintillator and 40 x 28 cm dimension of capture; Xray source was Thermo Scientific Kevex PXS5-927EA, Focal spot 4 microns @ 2 watts, Target-Tungsten-Window Be, Beam angle 45 deg.; Image capture by VIVA K.03 Image Acquisition/Control Software. Additional specimens were radiographed at the AMNH (Carestream DRX, Rochester, NY) and CUMV specimens at the Cornell Veterinary Clinic (imaging system: Computed Radiography System, AGFA NX, Canton MA; radiography tube: Linear MC 150, North American Imaging, Camarillo, CA). A Cannon EOS Rebel t3 was used to photograph the eyed and blind side of each specimen. Radiographs and photographs of the eyed side of each specimen were overlaid in Photoshop, and the opacity of the top layer (the photograph) was reduced to allow visibility of both soft tissue and skeletal landmarks, resulting in two-layer images saved in .tiff format.

2.3. Selection and Digitization of Landmarks

We used 2-D landmarks because the highly compressed body forms of flatfishes allow 2-D morphometrics to capture shape variation for co-planar landmarks (Buser et al., 2018). We defined 25 homologous fixed landmarks and 16 sliding semi-landmarks, which are used to create point to point correspondence along homologous curves (Bookstein 1997) (Fig. 1.2). Skeletal and soft-tissue landmarks deemed of functional and ecological relevance were selected: landmarks 1-12 capture eye size and eye position, jaw size and jaw orientation (relevant to prey-capture); landmarks 13 and 17 define the abdominal region (relevant to feeding mode); landmarks 16-20 and 31, 32 capture fin length and placement (relevant to locomotion); semi-landmarks 23-30 and 33-40 capture the width and curvature of the body (relevant to locomotion). Landmarks related to features that were not present in all species, such as pectoral fins, were excluded from the morphometric data set. Measurements of caudal fin rays were excluded because damage to tails is common in fluid fish collections. We used sliding semi-landmarks to define the exterior margin of the body form where homology was impossible to determine. All landmarks were placed on specimen images by one individual (senior author) to minimize errors of interpretation.

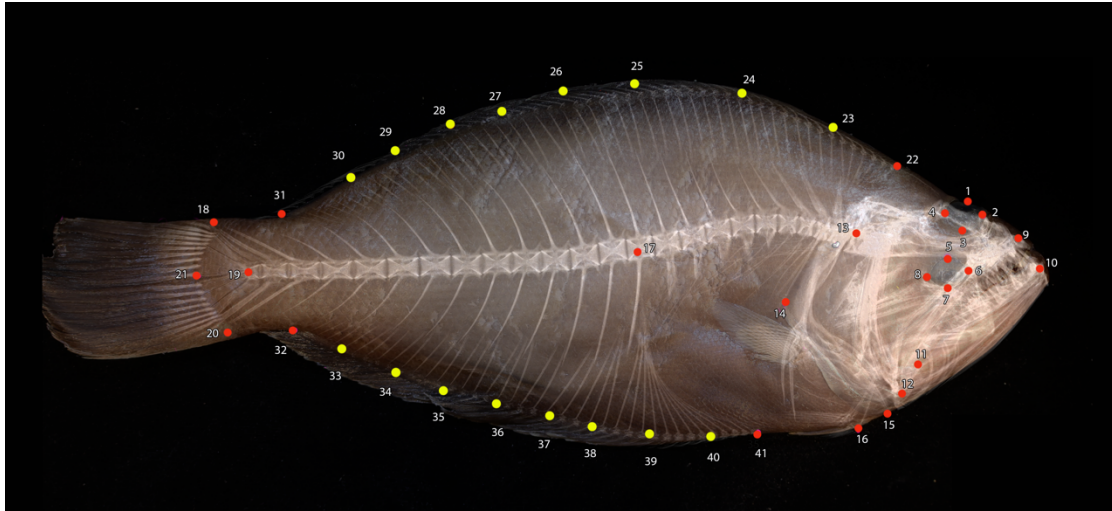


Figure 1.2. **2-D Landmarks:** (1-4) exterior margin of left eye (dorsal-most eye); (5-8) exterior margin of right eye (ventral-most eye); (9) anterior tip of premaxilla; (10) anterior tip of dentary; (11) posterior end of maxilla/corner of oral opening; (12) articular-quadrato jaw joint; (13) posterior of cranium; (14) posterior-most point of operculum; (15) interopercular joint; (16) origin of pelvic fin; (17) anterior-most caudal vertebrae; (18) dorsal-most intersection of caudal fin rays with the body; (19) hypural joint; (20) ventral-most intersection of caudal fin rays with the body; (21) mid-point of posterior edge of urostyle; (22) origin of dorsal fin; (23-30) sliding semi-landmarks along base of dorsal fin; (31) last anal fin ray (32) last anal fin ray; (33-40) sliding semi-landmarks along base of anal fin; (41) origin of anal fin. Note that right-facing (“right-handed” or dextral) specimens were transformed horizontally for geometric morphometric analyses, to match left-facing (“left-handed” or sinistral) specimens.

We used tpsUtil ver. 1.76 x64 to build tps files from images and append tps curves to landmarks. Landmarks and semi-landmarks were placed using tpsDig2 ver. 2.31 (Rohlf, 2004).

2.4. Landmark-based PCA Analyses

We used landmark-based morphometric methods (R ver. 4.0.2 and package geomorph ver. 3.3.1, Adams et al., 2020) to quantify shape variation in the 13 families of flatfishes together with representatives of two genera of fossil flatfishes (†*Amphistium paradoxum* and †*Heteronectes chaneti*) and one outgroup to Pleuronectiformes, the

Pilotfish *Naucrates ductor*. We used rotate.coords flipX to flip “right-eyed” specimens to face left; without this transformation the overwhelming driver of the morphospace would be the character state of sinistral vs. dextral, which is fixed at the family level for nearly all flatfishes, with a few exceptions such as Psettodidae and *Platichthys stellatus* (Munroe, 2015). Generalized Procrustes Analysis (Gower, 1975) was performed by gpagen (Adams et al., 2013) to align specimen coordinates and correct for differences in specimen sizes, rotation, and location in the frame. gpagen was used to slide semi-landmarks along their tangent directions to a position optimized by algorithms that minimize bending energy (Bookstein 1997). Principal component analyses (PCA) were performed on the aligned Procrustes coordinates using gm.prcomp. We plotted PCA functions with family and species labels and then drew and colored convex hulls in Adobe Illustrator 16.0.0 for easy visualization of the 13 families within the morphospace.

2.5. Meristic Counts and Measures

Values for the meristic PCA were counted and measured on radiographs imported into Adobe Photoshop. Total length was measured from the tip of the rostrum to the posterior edge of the caudal fin, and body width was measured perpendicular to the spine at the deepest spot on the body. To represent variation in dorsal and anal fin morphology, we counted the number of dorsal and anal fin rays and measured the longest and shortest fin rays on both fins (some species of flatfishes have very long fin rays that they use to prop up their bodies off the substrate, while others are uniformly short). Fin-ray lengths were measured with a straight line from the base of the fin ray to its distal tip, excluding fin rays that were broken or unnaturally bent. Vertebrae and fin rays were counted in the image using a small tic mark to keep track of progress. Because intraspecific variability

in vertebral counts is well documented due to developmental plasticity in response to temperature (Hubbs, 1922) and water turbulence (Corral and Aguirre, 2019) and opposing direct and indirect selection (Swain, 1992; Tibbin et al., 2016), our meristic analyses incorporated multiple individuals for most species that had been collected at different locations and times. JMP Pro 14 was used to perform a PCA on the meristic data; lengths were converted to ratios (ratio of length to width, ratio of shortest to longest fin rays) to control for specimen size. The resulting plot, with points colored by family, was imported into Adobe Illustrator and hulls were drawn and filled with colors to match those of the landmark PCA.

We used *interlmkdist* in *geomorph* (Adams et al., 2020) to obtain specific inter-landmark measures relevant to feeding type (post-Procrustes transformation). Inter-landmark measure included in boxplots were jaw length (landmarks 10 to 12, anterior tip of dentary to jaw joint); eye size (landmarks 1 and 3, diameter measured in the vertical orientation of the migrated eye); interocular spacing (landmarks 3 and 5, distance between eyes); and abdominal length (landmarks 13 and 17 from posterior of cranium to first caudal vertebrae).

2.6. Feeding Type Categorization

We obtained diet data from peer-reviewed journal articles and federally-sponsored literature for 33 of our study species (for sources and categorization, see Supplementary Table 1). Diet and life history information are unknown for many species of flatfishes, particularly smaller-bodied species with tropical distributions and those that are uncommon or have low commercial value (Munroe, 2015). For each species with

data, we determined the major prey categories e.g., “Fishes and Small Crustaceans” (see Supplementary Table 1).

We defined three feeding types, inspired by categories proposed by Yazdani (1969) and de Groot (1969): Turbot-type, Plaice-type, and Sole-type (Table 1, Figs. 1.2-1.4). We dropped the fourth category proposed by Yazdani (1969), “*Ammotretis*-type” because it is easily represented by features of Sole-type feeders. We also expand Yazdani’s (1969) concept for Plaice-type feeders to include fast-moving benthic prey. To assign each of the 33 species included in our ancestral state reconstructions to one of the three functional feeding types, we combined diet data, our observations of morphology, and published accounts of feeding behavior (where available). Our feeding-type categorization allows us to distinguish among flatfish species that consume the same prey category using different behavioral and functional methods. For example, “Fish” prey can be large or small, and pelagic, demersal, or benthic; flatfishes can locate prey using vision, mechanoreception, or olfaction; flatfishes can ambush attack from a hiding position on the substrate, locate and pursue prey by swimming in the water column, or forage and pursue prey on the substrate by creeping, bounding, stalking, and lunging (Olla et al., 1972, Fox et al., 2018). Some species of flatfishes exhibit behavioral plasticity and complexity in feeding, while others have stereotyped behavior and a limited predatory repertoire (Homes and Gibson, 1983). Our three feeding categories are intended to reduce this complexity into groups that retain essential form-function characteristics.

Table 1.1 summarizes the three feeding types, together with prey types, predatory behaviors, and morphological features.

Feeding Type	Prey Type	Predatory Behavior	Morphology
<i>Turbot-type</i>	Active, motile prey, e.g. fishes, cephalopods.	Ambush predation from substrate and active pursuit in water column. Visual detection of prey.	Jaws: large and nearly symmetrical with long sharp teeth Caudal fin: large Abdominal cavity: large Eyes: large, moderately protuberant; migrate just past the dorsal midline of body
<i>Plaice-type</i>	Motile and sessile benthic prey e.g. crustaceans, benthic fishes, mollusks.	Active foraging on and above the benthos. Generally diurnal. Primarily visual detection of prey but can also use olfaction.	Jaws: medium sized somewhat asymmetrical Eyes: large, extremely protuberant Abdominal cavity: variable within the mid-range
<i>Sole-type</i>	Low-mobility infaunal and epifaunal prey e.g. polychaetes, worms, siphon tips of bivalves.	Crawls on substrate searching for food. Generally nocturnal. Mechanosensory and chemosensory detection of prey but can also use vision.	Jaws: small and highly asymmetrical, minute teeth Eyes: small and closely spaced Abdominal cavity: small

Table 1.1 Categorization of feeding types, prey types, predatory behavior, and morphology.

Psettodes erumei (Fig. 1.33) has the characteristically large, nearly symmetrical jaws and long sharp teeth of a Turbot-type feeder that eats fishes and squid in the water column. Its streamlined body and robust caudal fin allow active swimming pursuit of prey. The abdominal region can accommodate large prey. The relatively low number of vertebrae (9 abdominal and 14 caudal) and fin rays are associated with a stiffer, less flexible body. Large eyes used for visual detection of prey are moderately protuberant, with eye migration barely past the mid-line.

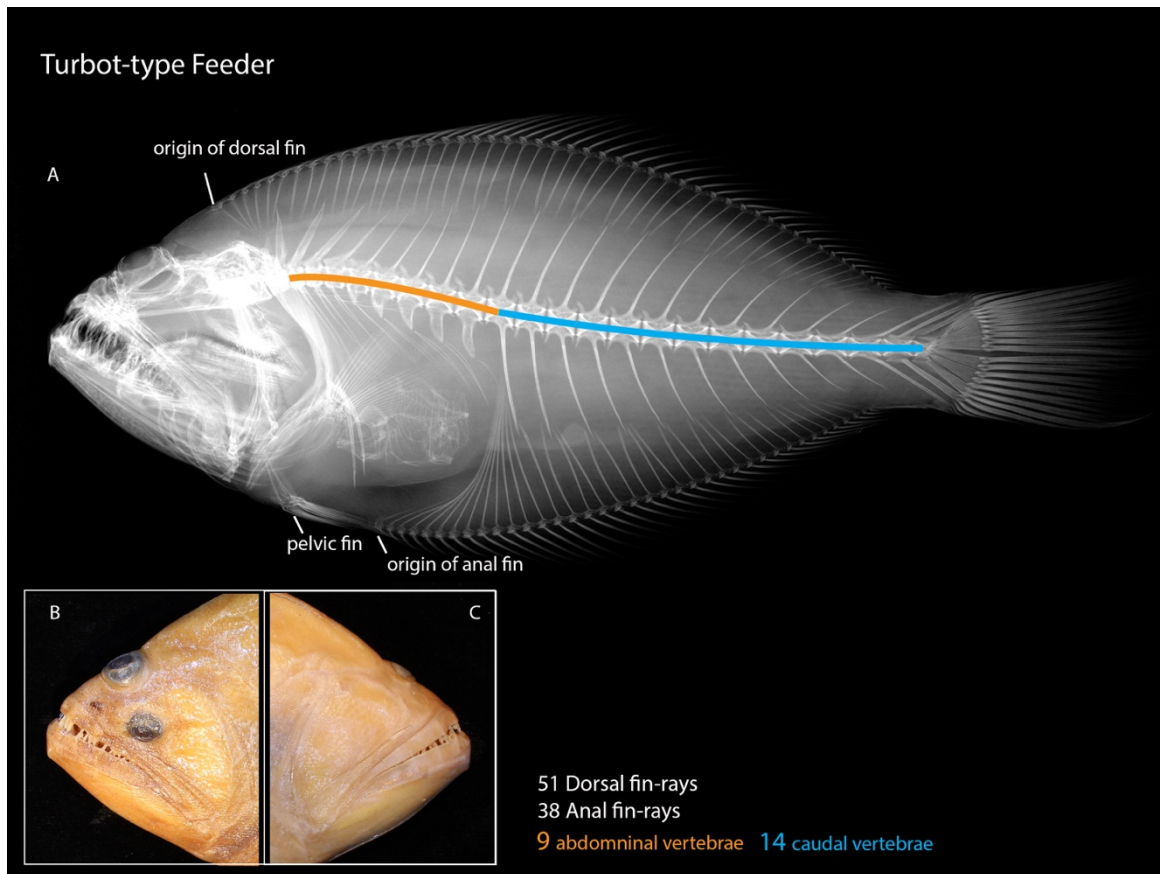


Figure 1.3. **Example of a Turbot-type feeder, *Psettodes erumei*.** Inset of eyed side (B) and blind side (C) of head showing large eyes and large symmetrical mouth. Note the fish prey item in the stomach.

The Plaice-type feeder *Bothus ocellatus* (Fig. 1.4) diurnally forages on small to medium benthic prey using vision. In comparison to a Turbot-type feeder, it has smaller, moderately asymmetrical jaws used to attack prey on the substrate. Large and extremely protuberant eyes allow visual detection and tracking of prey from a position on the substrate. Consumes generally small benthic prey. A higher number of caudal vertebrae (26) is associated with a more flexible body and an increased number of fin rays that can be used to walk on the substrate.

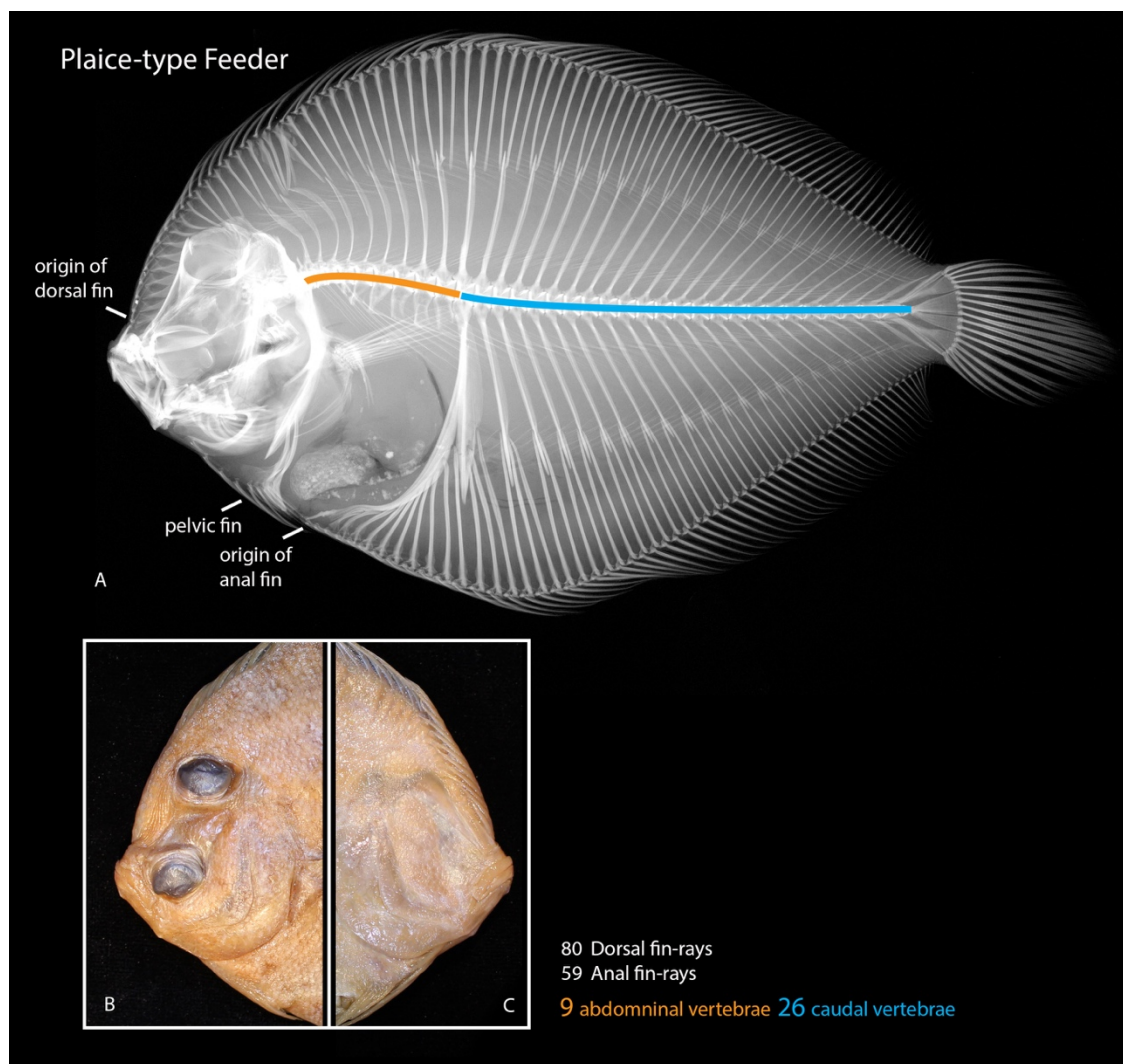


Figure 1.4. Example of a Plaice-type feeder, *Bothus ocellatus*. Inset of eyed side (B) and blind side (C) of head showing large eyes and medium-sized, slightly asymmetrical mouth.

Paraplagusia japonica (Fig. 1.5) is a Sole-type flatfish that eats small, low-mobility prey such as siphon tips of bivalves. The dorsal fin originates anterior to the head, and the caudal fin skeleton is absent, with the dorsal and anal fins confluent around the posterior tip of the body. In comparison with Turbot-type and Plaice-type feeders, Sole-type feeders have small, closely spaced eyes correlated with nocturnal feeding and reliance on other sensory systems for prey detection. The small, highly asymmetrical mouth opens on the blind side of the body; it enables feeding from the substrate on small infaunal and epibenthic prey. The abdomen is small, but the number of caudal vertebrae (43) is greater than in either the Turbot-type or Plaice-type feeders.

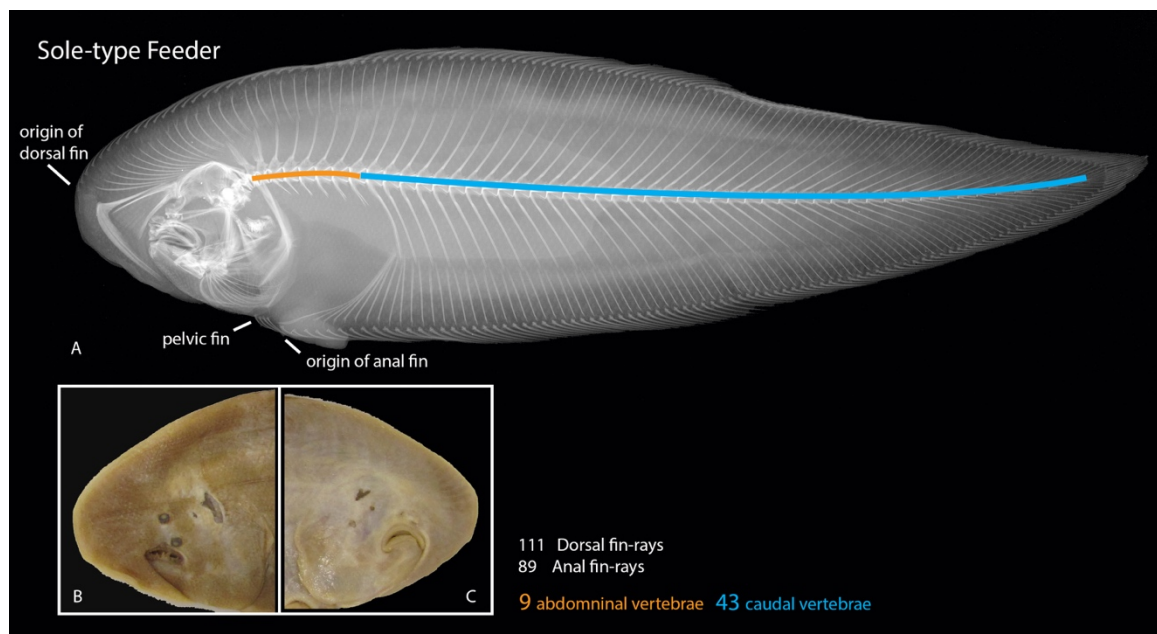


Figure 1.5. Example of a Sole-type feeder, *Paraplagusia japonica*. Inset of eyed side (B) and blind side (C) of head showing tiny, closely spaced eyes and small, highly asymmetrical mouth.

In all three feeding types, the abdomen has 9 vertebrae. Body elongation is indicated by the number of caudal vertebrae.

2.7. Ecological Character Mapping and Ancestral State Reconstruction

Only species that had both detailed diet data and were included in Rabosky et al. (2018) were used in ancestral state reconstruction of feeding type (N=27). We obtained substrate and depth data from FishBase ver. 12/2019 (Froese and Pauly, 2000) for N=47 species. If a single substrate-type (or no substrate type) was included in FishBase, we consulted additional sources. We combined mud substrate type with sand for our “Soft Bottom” substrate type because all species in our study listed in FishBase as residing on mud were, with further research, found to utilize sand bottoms in certain locations or to change preference in different life stages.

We used phylogenetic data from the Fish Tree of Life project (Rabosky et al., 2018) which combines sequences from the Euteleost TOL (Betancur-R. et al., 2013a), Rabosky set (Rabosky et al., 2018), and Near set (Near et al., 2012). Timetree files were downloaded from fishtreeoflife.org, imported into R, and pruned to our study species. An alternate tree based on data from Byrne et al. (2018) was used for comparison (see Supplemental Materials based on data downloaded from the Github repository https://github.com/sarisbro/Byrne_etal_SeqAlignments.tar.bz2).

We performed stochastic character mapping using the function `make.simmap` in `phytools` v. 0.7.47 (Revell, 2012) which modifies `ace` (Paradis et al., 2004) to compute likelihood using Felsenstein’s pruning algorithm. The routine `make.simmap` uses a continuous-time reversible Markov model for the evolution of discrete character traits, which is used along with tip states on the tree to simulate stochastic character histories. Stochastic character mapping using a probabilistic approach avoids the drawbacks of parsimony (Bollback, 2006). We used the default “empirical” method in `make.simmap`,

which maximizes the likelihood of the transition matrix and samples character histories for the discrete trait, from their posterior distribution. We created two ancestral state reconstructions for feeding mode: 1) with a prior imposed, setting the probability = 1 for “Turbot-type” at the root node, reflecting fossil evidence that this is the ancestral state for Pleuronectiformes; 2) using an “equal” prior distribution which produces a root node sampled from the conditional scaled likelihood distribution at the root (see Supplemental Materials for this Ancestor State Reconstruction).

3. Results

3.1. Feeding Types

The general classification of feeding types for 11 families studied is shown in Figure 1.6 along with estimates of total species diversity for each family. These are broad generalizations because all members of a family may not conform to one of the three feeding types. Families generally classified as Turbot-type are the least diverse (127 species); Plaice-type families have twice as many species (284), largely due to the diversity of Bothidae. Families generally classified as Sole-type are the most diverse, with 380 species. For details on classification see Supplemental Table 1.2 in Appendix.

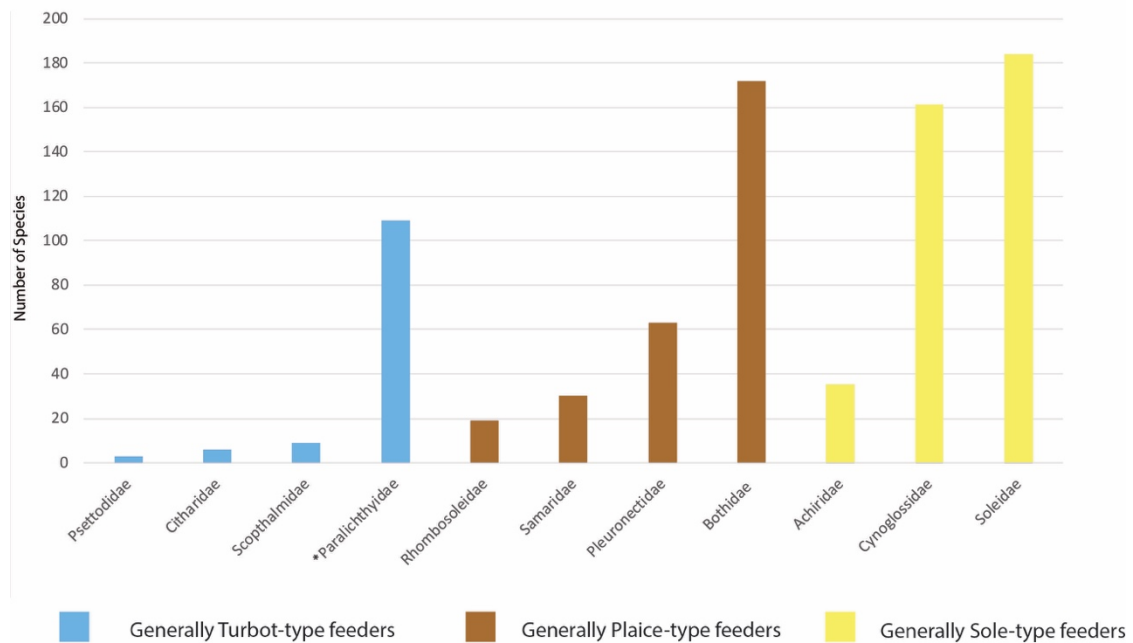


Figure 1.6. Number of valid species per family of Pleuronectiformes assigned to each of our feeding types, color coded. Species number is based on valid species listed in Eschmeyer's Catalog of Fishes. Paralichthyidae shows the highest species richness for turbot-type feeders. Bothidae are the most species-rich family of Plaice-type visual foragers on motile benthic prey species. Cynoglossidae and Soleidae show high diversity of Sole-type nocturnal foraging feeders. Species counts per family are as follows. Among turbot-type feeders: Psettodidae (3), Citharidae (6), Scophthalmidae (9), Paralichthyidae* (109). For Plaice-type feeders: Rhombosoleidae (19), Samaridae (30), Pleuronectidae (63), Bothidae (172). For Sole-type feeders: Achiridae (35), Cynoglossidae (161), Soleidae (184). Two families, Poecilopsettidae (21) and Achiropsettidae (4), were data deficient for diet and predation mode and were excluded. *Paralichthyidae is broadly considered paraphyletic.

3.2. PCA Morphospace

Principle component analysis of landmark-based shape variation in 67 species of flatfishes from 13 families found PC1 (46%) and PC2 (24%) explained 70% of the total variance (Figs. 1.7 and 1.8). PC2 and PC3 contributed 5.7% and 5.3%. PC1 primarily reflects variation in the primary and secondary body axes, i.e. increase in body length vs. width (because flatfishes undergo axial re-orientation during metamorphosis, body depth is functionally body width). Forms with wide bodies are on the high end of PC1 with

Scophthalmus maximus (Scophthalmidae) in the bottom right representing the positive extreme. The elongate body form of *Cynoglossus senegalensis* (Cynoglossidae) represents the negative extreme.

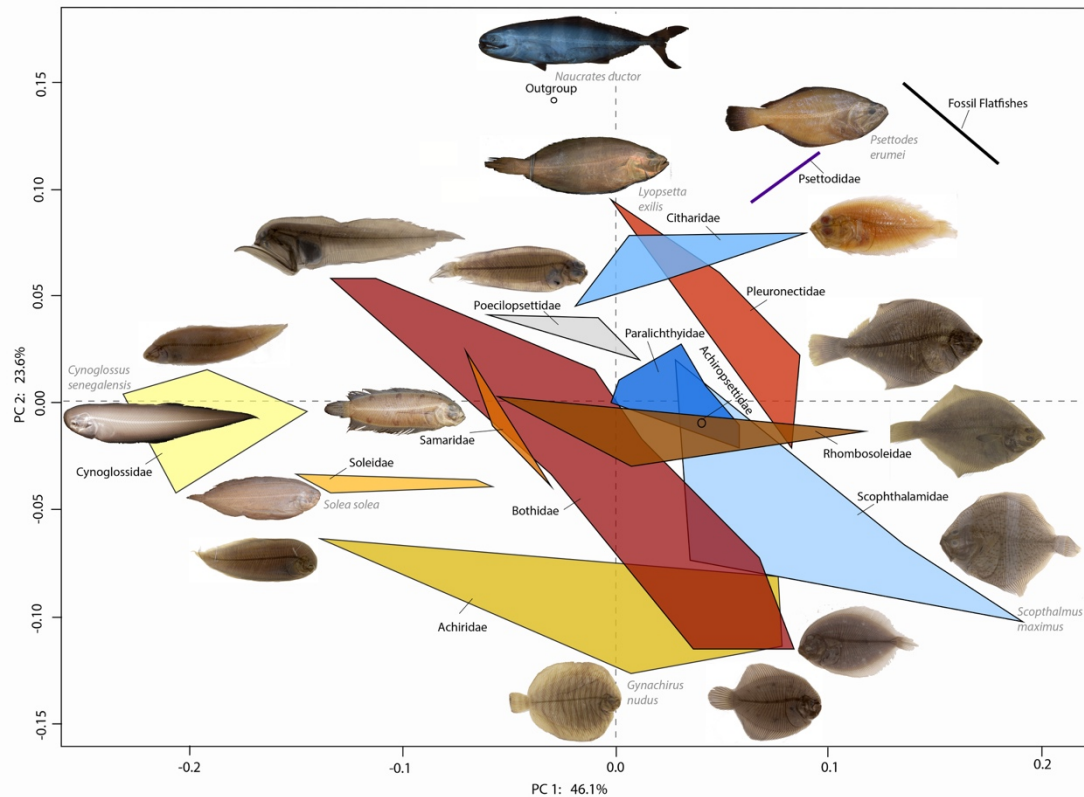


Figure 1.7. Landmark-based morphospace of Pleuronectiformes produced by PCA (N=67 species). Polygons represent the distribution of species examined within each family. Combined radiographs and photographs of species representing morphological extremes are placed adjacent to their point in the plot. The greatest variance within PC1 relates to body elongation in the upper left) and deep-bodied forms in the lower right) with additional influence from abdominal size (larger in the upper right), jaw size (smaller in lower left). PC2 reflects variance in origin of the dorsal, anal, and pelvic fins from a more ancestral position (top and upper right) vs. shifted far forward (bottom and lower left), and degree of eye migration from minimal (top) to extreme (bottom).

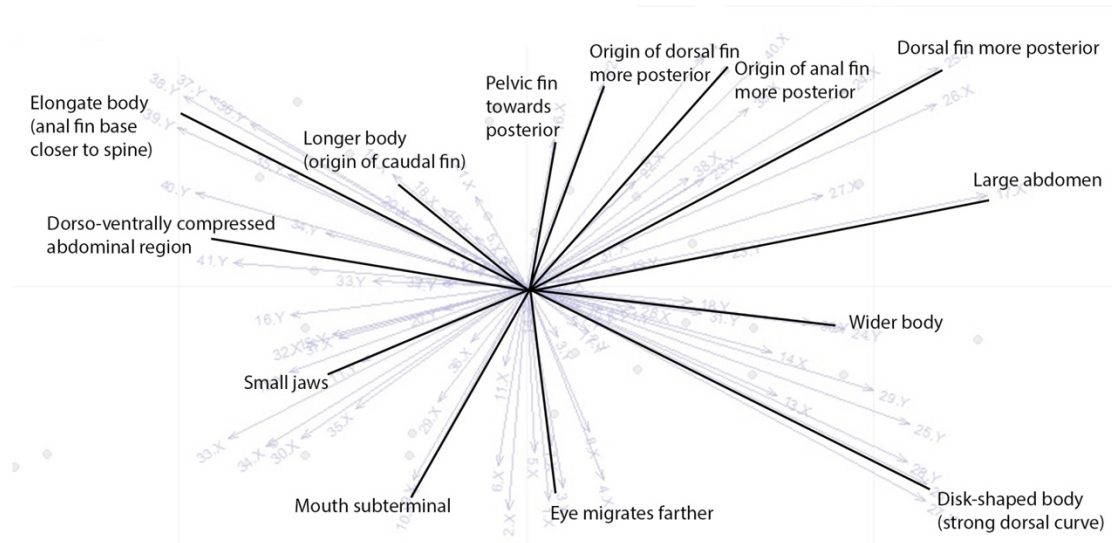


Figure 1.8. **Loadings plot for landmark-based PCA.** Variables that are far from the origin have the highest loadings on the principal components. PC1 is strongly influenced by variables on the far left (negative), e.g. elongate body form, and far right (positive), e.g. disk-shaped body. Variables that have the largest influence on PC2 are at the bottom of the plot (negative), e.g. extensive eye migration, at the top (positive), e.g. more posterior origin of dorsal, anal, and pelvic fins.

Jaw and abdominal sizes also contribute to PC1. Families in the lower left quadrant of Figure 1.7 have small jaws and small abdominal regions, typified by *Solea solea* (Soleidae), while those in the upper right have large jaws and large abdominal regions, e.g., fossil flatfishes and *Psettodes*.

PC2 reflects variation in fin and jaw position, and degree of eye migration. At the top of Figure 7, occupying the positive PC2 extreme, is the outgroup *Naucrates ductor* (Carangidae) along with *Psettodes spp.* and *Lyopsetta exilis* (Pleuronectidae); these taxa have dorsal, pelvic, and anal fins originating farther back on the body. Families with negative PC2 at the bottom of the plot, exemplified by *Gymnachirus sp.* (Achiridae), have dorsal and anal fins shifted far forward that wrap nearly all the way around the head and upper abdomen. Families with positive PC2 scores have superior or terminal mouths

while those at the negative extreme have small subterminal jaws. Lastly, variation in eye position contributes to PC2, with flatfishes at the top of the morphospace in Figure 7 having an eye that has barely migrated past the body mid-line, in contrast with those with negative PC2, which exhibit eye migration $\sim 180^\circ$ onto the opposite side of the body.

3.3. Meristic Morphospace

A PCA morphospace based on meristic data (Fig. 1.9) includes variation in vertebral number and aspects of dorsal and anal fin morphology assessed by fin-ray counts and measures of fin-ray lengths.

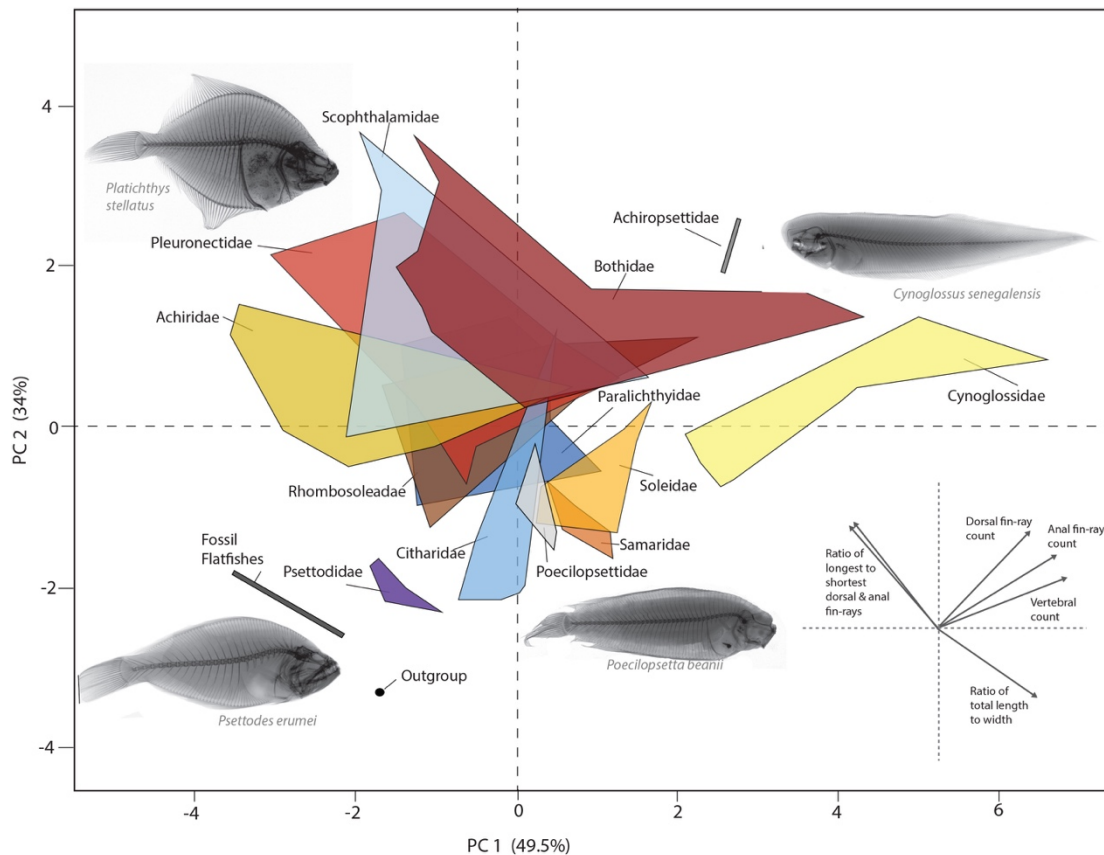


Figure 1.9. Morphospace of Pleuronectiformes produced by PCA based on vertebral and fin-ray counts and lengths of body axes and fin rays. Polygons represent the distribution of species within each family. Representatives of each quadrant are *Platichthys stellatus* (upper left) with a wide body and dorsal/anal fin rays that are lengthened into peaks in the mid-region of the body; *Cynoglossus senegalensis* (upper right) with high vertebral count and short dorsal and anal

fin rays; *Samariscus longimanus*, with an elongate body and a consistent length to fin rays; and *Psettodes erumei* in the lower left with low vertebral and fin-ray counts. (N=139 individuals, 67 species)

We found a large overlap in the positions of families within the meristic morphospace. PC1 (50%) and PC2 (34%) explained 84% of the total variance. Families with positive PC1 and PC2 on the upper right of the plot have high vertebral counts, which are in turn correlated with high median fin-ray counts, exemplified by tonguefishes such as *Cynoglossus senegalensis*, which has 61 vertebrae (9 abdominal; 52 caudal), 133 dorsal fin rays, and 106 anal fin rays. Taxa that are negative for PC1 have fewer than half as many vertebrae and fin rays, e.g., members of Achiridae have notably negative PC1 scores including *Trinectes maculatus* with 26 vertebrae (9 abdominal; 17 caudal), 51 dorsal fin rays and 37 anal fin rays. *Psettodes erumei* has 23 vertebrae (9 abdominal; 14 caudal), 50 dorsal fin rays and 38 anal fin rays. Vertebral and fin ray counts are even lower for the fossil flatfishes, †*Heteronectes* and †*Amphistium*. Families with positive PC1 and negative PC2 (lower right quadrant) have relatively elongate bodies and mid-range vertebral counts, e.g. *Solea solea* has 46 vertebrae (9 abdominal; 37 caudal), 77 dorsal fins-rays, and 63 anal fin rays; their fin rays are more consistent in length, i.e., the longest and shortest fin rays have a 1:1 or 1:2 ratio. Families in the upper left quadrant have wider bodies and dorsal and anal fins with a high ratio of shortest to longest fin rays, e.g. the longest fin ray is eight times as long as the shortest in *Platichthy stellatus* (Pleuronectidae).

3.4. Comparison of morphological measures relating to feeding type

To assess variation in morphological features within and between families related to foraging and predation, we compared means of four inter-landmark measures: eye size

and inter-ocular distance (Fig. 1.10); and jaw-length and abdominal size (Fig. 1.11). Measures were taken after Procrustes transformation in scale to account for size differences in specimens. Eye sizes and interocular distances were larger for families we identified as primarily visual predators (Turbot-type and Plaice-type, Fig. 1.6). Jaw length and abdominal size were largest for the two species of *Psettodes* and the fossil flatfishes †*Amphistium* and †*Heteronectes*. Other piscivorous flatfishes had large to intermediate jaws and abdominal size. Plaice-type feeders were consistently intermediate on jaw size, however they showed high variability in abdominal size, with Pleuronectidae on the large side and Samaridae on the small. Again Achiridae, Soleidae, and Cynoglossidae form a distinct cluster with small abdominal and jaw size.

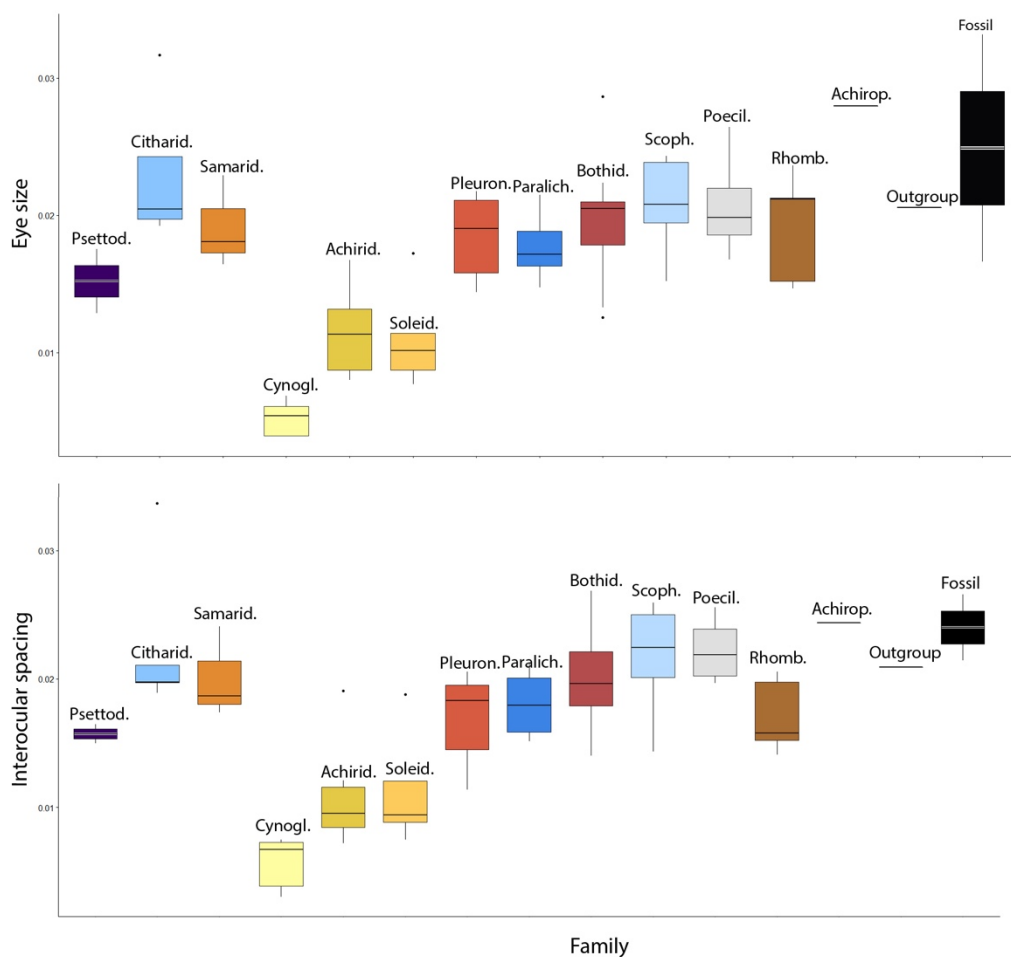


Figure 1.10. **Boxplot of relative eye diameter (top) and distance between the eyes (bottom).** Includes thirteen families of extant flatfishes, two transitional fossil species, and the outgroup, *Naucrates ductor*.

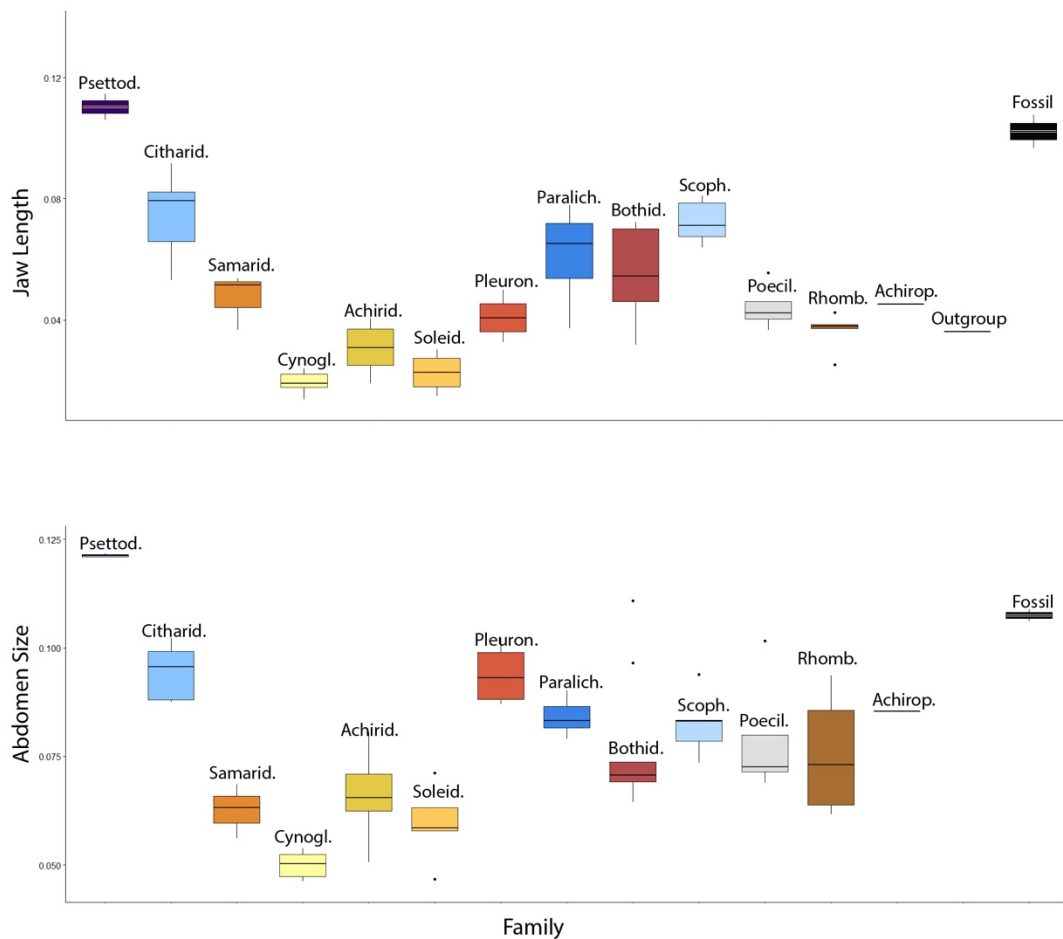


Figure 1.11. Boxplot of jaw length (top) and abdominal size (bottom) for thirteen families of extant flatfishes, two transitional fossil species, and an outgroup. Jaw length was measured from the anterior tip of dentary to the jaw joint; abdominal size was measured from the posterior of cranium to first caudal vertebrae.

3.5 Ancestral State Reconstruction

Ancestral state reconstructions for feeding (Fig. 1.12), substrate (Fig. 1.13), and water type (Fig. 1.14) infer a common ancestor for Pleuronectiformes that was a marine-dwelling ambush predator with a preference for soft-bottom substrates.

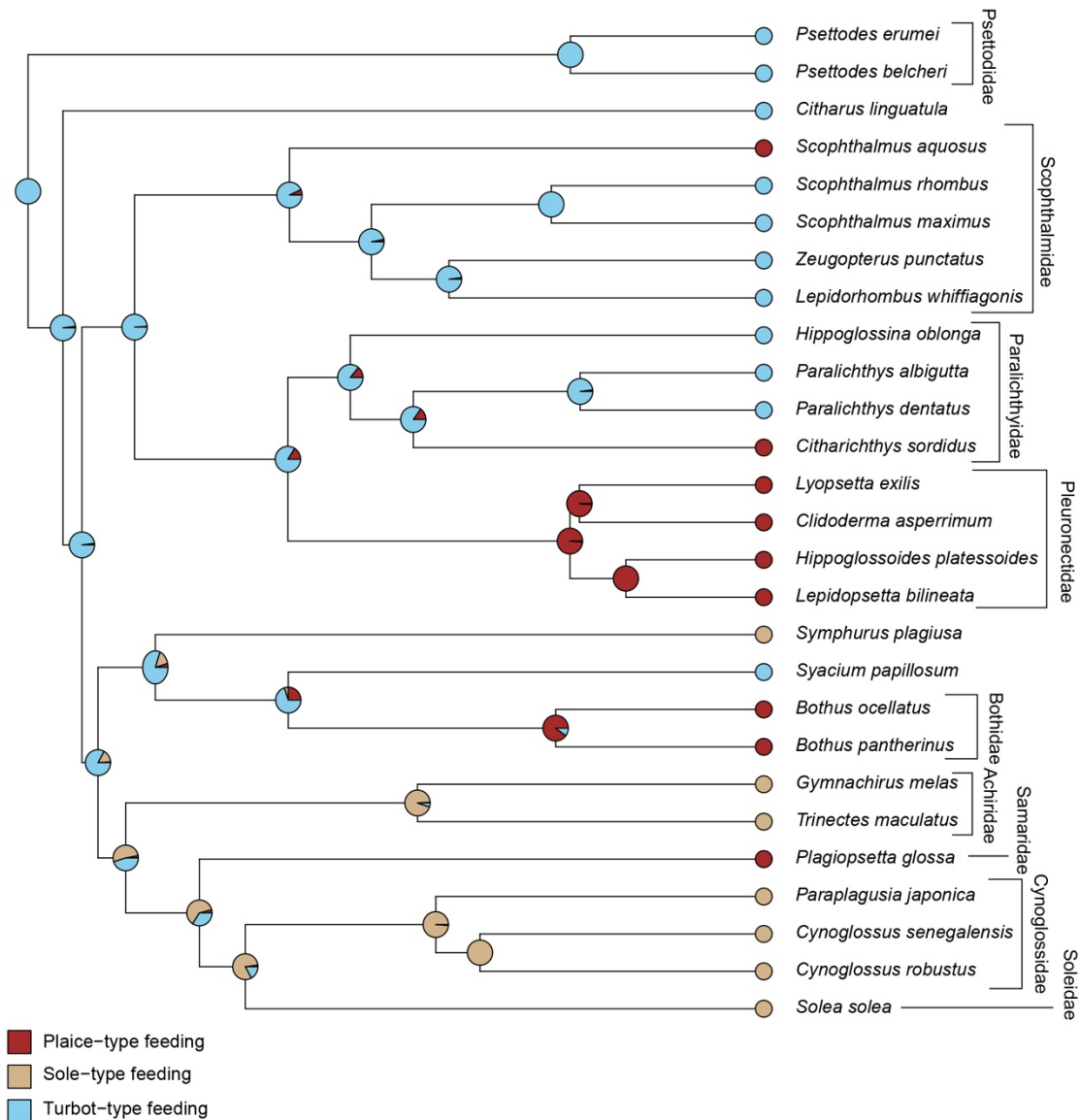


Figure 1.12. Ancestral state reconstruction of feeding type in flatfishes using stochastic character mapping methods infers multiple transitions from Turbot to Plaice feeding type, and a single transition from Turbot to Sole feeding type on the branch leading to Achiridae, Samaridae, Cynoglossidae, and Soleidae*. Feeding type categories are “Turbot-type” ambush and active pursuit predators on primarily fish prey; “Plaice-type” visual predators of benthic organisms; and “Sole-type” nocturnal predators on primarily epibenthic and infaunal prey. Root node prior was set to “Turbot type” based on fossil evidence of transitional forms and extant character state in the basal-most family. **Symphurus plagiusa* has been placed within Cynoglossidae by other molecular and morphological analyses.

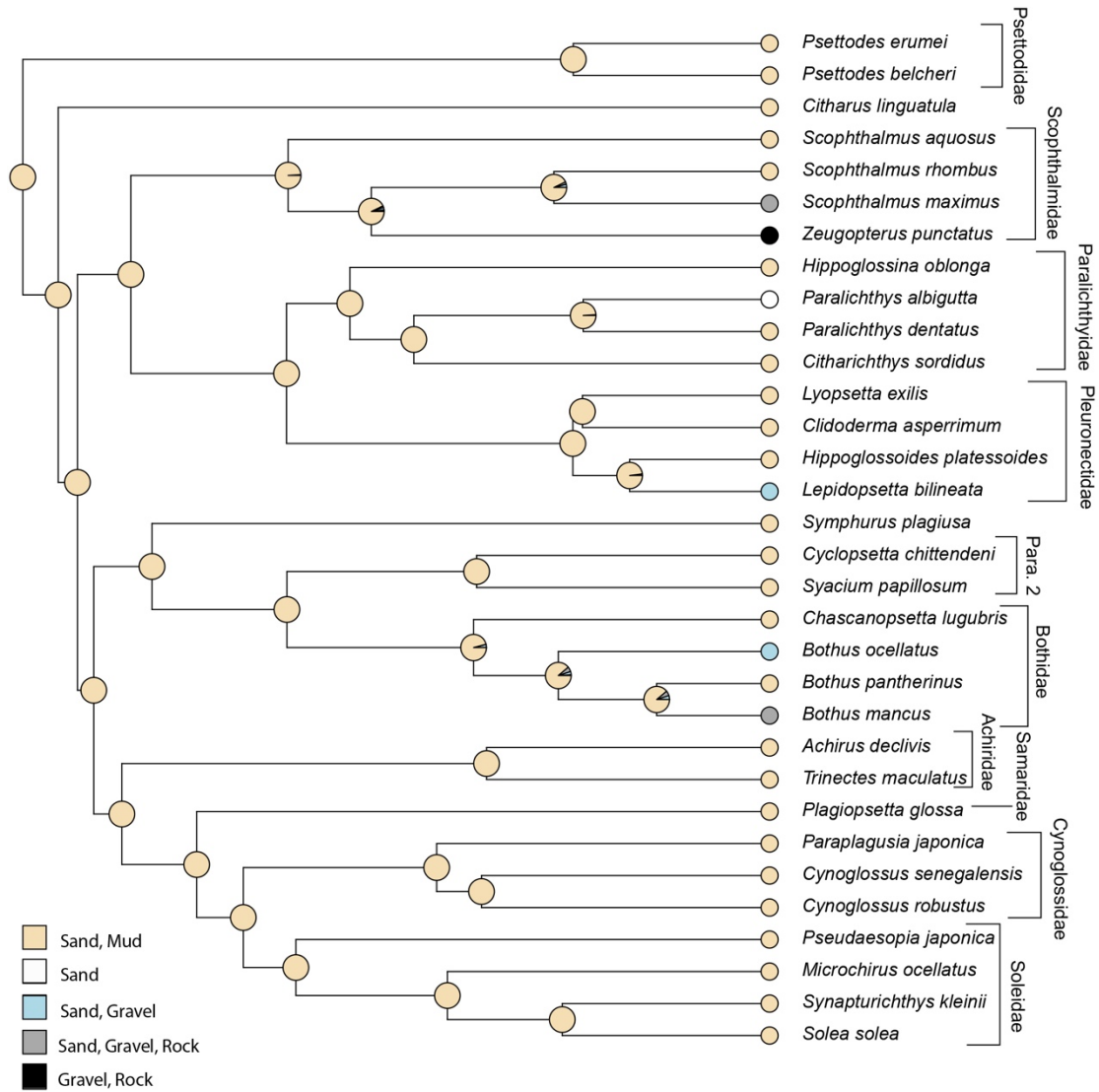


Figure 1.13. Ancestral state reconstruction of substrate type in Pleuronectiformes inferred a soft-bottom (sand/mud) at the root node with preferences for sand/gravel, sand/gravel/rock, and gravel/rock arising multiple times in distantly related families.

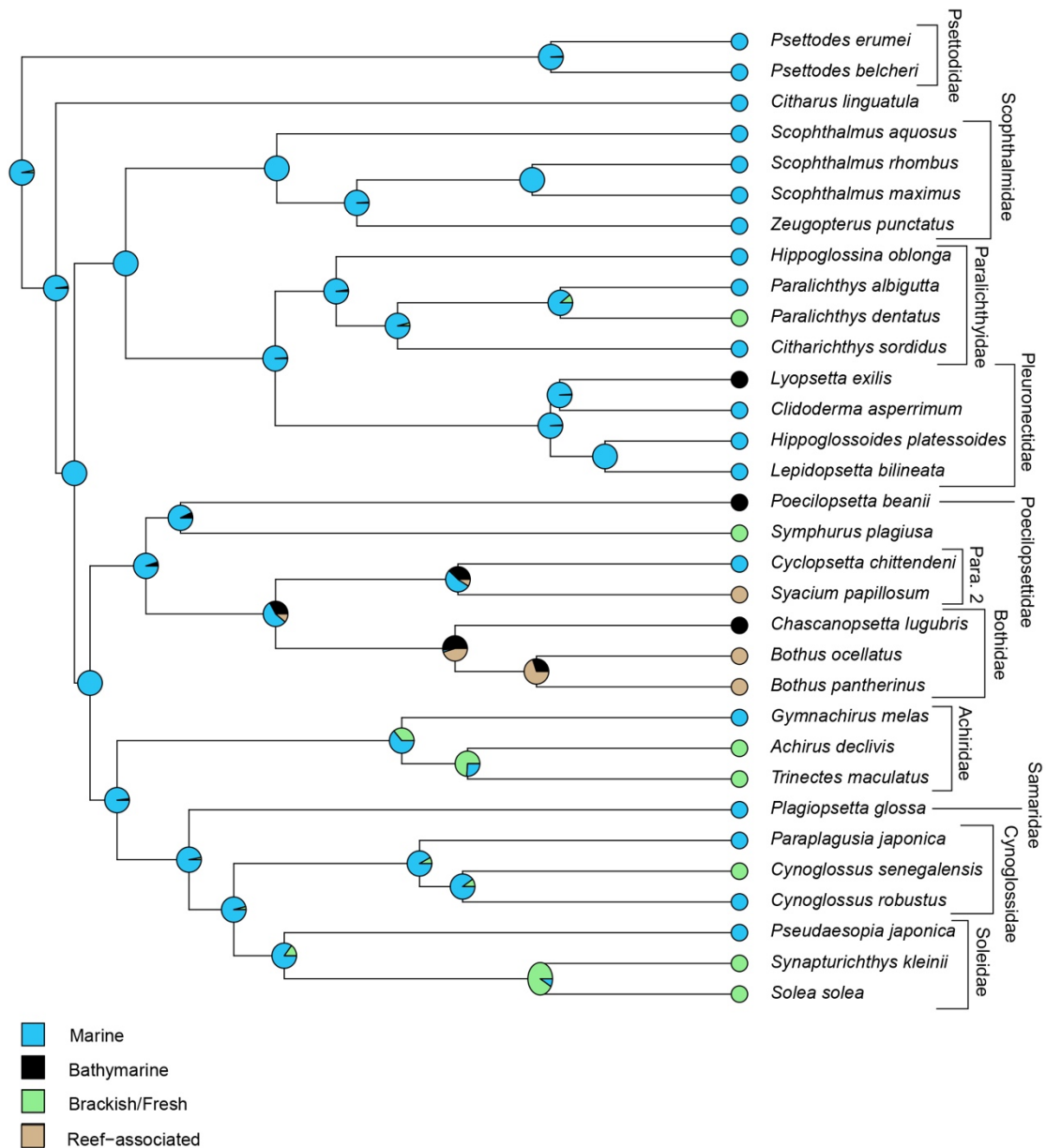


Figure 1.14. Ancestral state reconstruction of water type in Pleuronectiformes inferred a marine origin for the clade. Multiple independent originations of deep-water, reef-associated, and brackish/freshwater types are represented, with frequent transitions to brackish/freshwater inferred within Cynoglossidae and Soleidae.

Character mapping of feeding type (Fig. 1.12) showed Sole-type feeding arising once (excluding *Symphurus plagiusa*, which is generally placed in Cynoglossidae (Eschmeyer 2020) but was not placed near Cynoglossidae based on the phylogenetic data

set that we used to generate Fig. 1.12). Turbot-type feeding independently transitioned to Plaice-type several times; e.g., in Bothidae and Pleuronectidae (Fig. 1.12). Character mapping for substrate type (Fig. 1.13) showed an overwhelming preference for soft bottoms (sand and mud) with a few species living on other substrates such as sand, sand and gravel, mixtures of sand, gravel and rock, or gravel and rock. Ancestral water type (Fig. 1.14) was inferred to be shallow marine, with independent transitions to bathymarine, reef-associated, and brackish/fresh water. The turbot-type families Psettodidae, Scophthalmidae, and one branch of Paralichthyidae, are nearly exclusively marine, while the primarily Sole-type families Achiridae, Soleidae, and Cynoglossidae showed many transitions to brackish and freshwater.

4. Discussion

Although Pleuronectiformes share a suite of adaptations for hunting and hiding in benthic environments, this large and ecologically important group also displays incredible morphological variation that we explored using geometric morphometrics in a phylogenetic context. In summary: we found that the largest component of shape variation in flatfishes is body elongation, which is correlated with an increase in the number of caudal vertebrae, which is in turn correlated with an increasing number of dorsal and anal fin-rays. Some families have a highly conserved body shape while others display great variation. Specific measures such as jaw size and position, abdomen size, and eye size and position correlate to feeding type however body shape does not. Ancestral state reconstructions for feeding, substrate, and water type infer a common ancestor for Pleuronectiformes that was a marine-dwelling ambush predator with a preference for soft-bottom substrates. Multiple independent transitions to Plaice-type

feeding suggest convergent evolution on feeding and foraging on the bottom on a range of visually detected motile and sessile benthic prey.

Body elongation, the largest component of shape variation in flatfishes, is an axis of shape change that dominates diversification of fishes more broadly (Claverie and Wainwright, 2014; Price et al., 2019). We found that abdominal size and the origin and length of the dorsal and anal fins and their shape (curvature of the body and length of fin-rays) also contributed largely to shape variation. Jaw length, eye position, eye size, and body curvature emerged as additional important components of morphological variation.

Psettodidae, †*Amphistium*, †*Heteronectes*, and *Naucrates ductor* occupy opposite sides of the morphospace from Cynoglossidae and Soleidae (Fig. 1.7), with each of these groups distinctly clustered and non-overlapping.

Bothidae had the largest variance and overlapped with the greatest number of other families in both landmark-based and meristic morphospaces (Figs. 1.7 and 1.9). This overlap reflects both conservatism and convergence of traits. Within Bothidae extremes of body form were associated with transitions to bathymarine habitats, such as the gulper-type jaws, large gular pouch, and elongated body of *Chascanopsetta crumenalis* (reminiscent of the pelican eel, *Eurypharynx pelecanoides*), and to reef habitats, such as the wide-eyed and wide-bodied *Bothus ocellatus* (Fig. 1.1). Character mapping inferred a marine demersal, soft-bottom origin for Pleuronectiformes (Fig. 1.14); occupation of different aquatic environments and substrates may be driving morphological and species diversification. Members of the most speciose families (Bothidae, Cynoglossidae, and Soleidae) in our sample show more transitions to different

water types compared to the least speciose families (Psettodidae, Scopthalmidae, Citharidae).

According to both Rabosky et al. (2018) and Byrne et al. (2018), Samaridae is more closely related to Cynoglossidae and Soleidae yet in our landmark-based morphospace its body form overlaps entirely with Bothidae (Fig. 1.7). The large eyes and relatively symmetrical jaws of samarids as well as descriptions of active crepuscular foraging on the benthos, waving of the elongated pectoral fin (Kuiter and Tono-zuka, 2001) perhaps as a lure or as a sensory appendage, and documentation of small benthic fishes and invertebrates as prey, place samarids closer to a Plaice-type rather than Sole-type feeder. If the phylogenetic placement is correct, then this is an example of convergence in feeding type.

Elongation in vertebrates can be achieved by increasing the number or length of vertebrae (Ward and Brainerd, 2007; Wake, 2009); here we show that elongation in flatfishes is associated with an increase in the number of caudal vertebrae (Figs. 1.3-1.5, 1.9; Supplementary Table 1). Transitional fossil flatfishes and basal-most families such as Psettodidae have low vertebral counts (22-25), while members of the highly elongated and later-diverging family Cynoglossidae have more than twice as many vertebrae (45-61). There are several possible hypotheses for the functional value of increased vertebral number and elongation in flatfishes. Vertebral counts may increase flexibility, which in turn may lead to more effective C-start and escape maneuvering (Brainerd and Patek, 1998). Conformation to benthic surfaces might be aided by increasing vertebral count. Intriguing footage of highly elongated tonguefish crawling (Okeanos ROV mission, 2016, EX1605L1 CAPSTONE: ROV Exploration of the Marianas Trench MNM,

location Esmerelda Bank) document this flexibility, with the flatfish conforming its long body to dips and rises of large rocks on the seafloor, allowing its many fin rays to contact and push against the substrate (also see Fox et al. 2018). Axial flexibility and high vertebral counts may be a factor in burying, which flatfishes achieve by undulating the body rapidly in waves while in contact with the substrate. Flatfishes with a range of body and fin morphologies have been shown to bury in generally similar ways, however there may be functional specializations for different substrate types (McKee et al., 2016); flatfishes can exhibit strong preference for different substrates, which changes ontogenetically and has been shown to correlate to burying ability as well as food availability (Tanda, 1990; Phelan et al., 2001). Elongation may allow physical access to restricted spaces. Lastly, increased vertebral number in some groups may reflect a developmental response, rather than adaptation, to environmental conditions.

Achiropsettidae was centrally located in the landmark morphospace (Fig. 1.7) with an intermediate length to width ratio yet emerged as an outlier on the meristic morphospace (Fig. 1.9) due to its high number of vertebrae (51 vertebrae, 105 dorsal fin rays, 93 anal fin rays). “Jordan’s Rule,” in which there is an inverse relationship between water temperature and vertebral number, has been experimentally demonstrated to link to developmental conditions in some groups (Corral and Aguirre, 2019) and may play a role in the morphology of this exclusively circumpolar family.

Adaptive benefits of having a dorsal fin that is advanced onto the head – a synapomorphy for Pleuronectiformes (Chapleau 1993) – are unclear. Flatfishes show large variation in the position of the origin of the dorsal fin, from the ancestral condition far back on the head (Fig. 1.3) to its extreme forward location in Achiridae and

Cynoglossidae (Fig. 1.5). Fox et al. (2018) showed that the anterior portion of the dorsal fin is not used to push against the substrate during benthic walking in two species of Pleuronectidae; observations of the achirid *Trinectes maculatus* and the tonguefish *Symphurus plagiusa* confirm that fin rays on the head are not used for propulsion during walking Fox et al. (submitted). Fin rays on the head may have been co-opted for sensory purposes, particularly for species that hunt nocturnally or in deep-sea or turbid estuary environments. Pterygiophores supporting fin rays extend and enlarge the anterior margin of the head in members of Achiridae and Cynoglossidae (some of which have a rostral hook extending the anterior portion of their head). Species in these families show frequent evolutionary transitions to brackish and freshwater habitats (Fig. 13). Having a nearly continuous margin of fin rays may help these groups adhere to the substrate (like the thin edge of a suction cup) to avoid displacement by flowing tidal or river water. In handling *Trinectes maculatus* in locomotor studies Fox et al. (submitted) demonstrated firm attachment to the substrate and resistance to removal; spreading their dorsal and caudal fin rays broadly and arching their backs slightly created a tight seal with the potential for a negative pressure zone beneath. Anecdotal observations of other round-bodied species specializing in adhering to rock surfaces, e.g. the Topknot, *Zeugopterus* (Scophthalmidae) (Gosse, 1865) suggests that the round-body type with a fringe of fins surrounding is may be an adaptation for adherence.

The close phylogenetic relationship between Achiridae and Cynoglossidae + Soleidae raises interesting questions: our results show that the three families share a common ancestor and overlap in diet, feeding mode, and occupy similar environments,

yet have body shapes and vertebral and fin-ray counts on opposite extremes (Fig. 1.15 and Figs. 1.7 and 1.9).

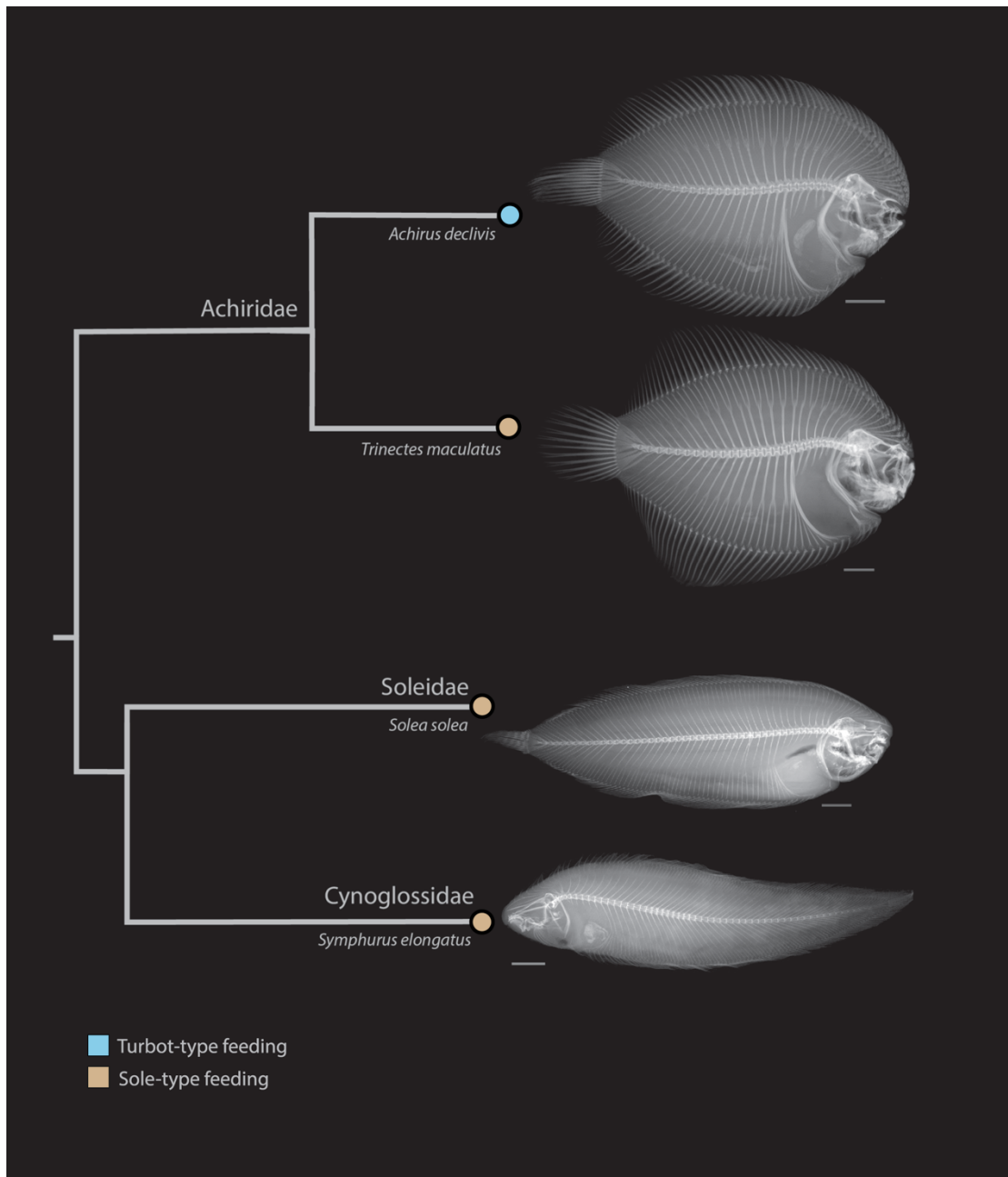


Figure 1.15. Body shape does not always correlate with feeding type. Species with similar body forms were found to have different feeding types, for example within Achiridae *Trinectes maculatus* is Sole-type and *Achirus declivis* is Turbot-type. Conversely, the same feeding type (Sole-type) was found in very different body forms, including the highly elongate members of Soleidae and Cynoglossidae, as well as in Achiridae, whose members are extremely wide bodied.

A wide body could help protect against gape limited predators (recall the colloquial name for *Trinectes maculatus*, “Hogchoker”); it could also increase maneuverability in the water column or contribute to ground effect efficiencies when swimming above the substrate (Fox et al., submitted). Our character state reconstruction (Fig. 1.12) shows a single origin for Sole-type feeding, suggesting that these families diverged onto different morphological paths to being successful nocturnal benthic predator of small infaunal and epifaunal prey.

In the diversity of natural systems there is not necessarily a one-to-one mapping of form to function (Collar and Wainwright, 2006), and flatfishes are no exception. Although eye and jaw size and position (Figs. 1.10 and 1.11) distinguish certain groups of flatfishes and correlate with feeding type, we also observed similarity in body forms between species with different diets and predation modes. This is evident from the overlapping of families with generally different feeding modes in the morphospace (Figs. 1.6 and 1.7) and a close examination of diet and behavior data. For example, *Achiris declivis* and *Trinectes maculatus* are closely aligned on the far positive end of PC1 in the family Achiridae, with nearly identical body shape, fin shape, jaw and eye size and position, as well as similar maximum body size (Fig. 1.15). *Trinectes maculatus* is a Sole-type feeder that forages nocturnally and primarily consumes polychaetes, clam siphons, and amphipods (Derrick and Kennedy, 1997; Curti, 2005; VIMS 2020) while *Achiris declivis* was identified by Duarte and Andreata (2003) as a Turbot-type feeder following the Yazdani 1969 classification, because in the locality studied it consumed mostly fishes (Gobiidae) and was thought to perform ambush predation. Complicating simple interpretations is the fact that many flatfish species are opportunistic predators that

vary their diet depending on their life stage, location, season, and prey availability. In a different study, *Achiris declivis* was found to consume primarily polychaetes and crustaceans in the Summer season with polychaetes reaching 100% of stomach contents in mid-size individuals and crustaceans reaching 80% of stomach contents in large-size individuals (Couto and Farias, 2001). *Synapturichthys kleinii* (Soleidae) has a typical sole body and fin shape and clusters in the morphospace alongside other soleids such as *Solea solea*, which is a well-studied nocturnal predator (Kruuk 1963) that uses chemoreception and mechanoreception (Applebaum 1983) to consume mainly polychaetes (Allen 2005). In contrast, *S. kleinii* been classified as a Turbot-type feeder by Dallaville and Chanet (2009); because the behavior of this species is known only from the single record of ambush-type predation on small fishes it is difficult to draw firm conclusions.

Our findings agree with the conclusion of Black and Berendzen (2020) that the largest component of body shape variation within flatfishes is body elongation, and we found similar patterns in family spread and clustering. Our landmark-based morphospace (Fig. 1.7) shows more differentiation among families than found by Black and Berendzen (2020; Fig. 1.2), likely because we included soft-tissue landmarks and made broader use of sliding semi-landmarks. Our study is the first to include fossils with intermediate body forms, as well as outgroups. Black and Berendzen (2020) concluded that more robust analyses are needed to determine the influence of ecological traits on body form. We agree and believe that current analyses are limited by data deficiencies for diet and unresolved phylogenetic relationships.

As the ecological roles, behavior, and interrelationships of the remarkable diversity of flatfishes become better known we look forward to a more nuanced and conclusive understanding of their evolution, form, and function.

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CHAPTER 2

BENTHIC WALKING, BOUNDING, AND MANEUVERING IN FLATFISHES (PLEURONECTIFORMES: PLEURONECTIDAE): NEW VERTIBRATE GAITS

¹ Claire H. Fox, Alice C. Gibb, Adam P. Summers, and William E. Bemis. (2018). *Zoology* 130:19-29.

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 Amiiiformes, 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, 2015).

There has been extensive research on the development (Schreiber, 2013) and evolutionary origin of cranial novelties in Pleuronectiformes (Friedman, 2008; Harrington et al., 2016). Specializations for benthic life include: modifications for feeding on or near the substrate (Gibb, 1995; Gibb, 1996; Gibb, 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., 2017). 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 midwater fishes with elongate median fins, and invertebrate

locomotor behaviors? Ultimately, we ask does benthic walking of flatfishes constitute an undescribed vertebrate gait?

2.0. *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 x 33 cm x 43 cm and 122 cm x 20.5 cm x 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 X 0.9 cm). Two Panasonic Lumix DMC - FZ200 video cameras (120 frames per second, fps, at a resolution of 1280 X 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.

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).

Table 2.1. 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)
<i>Psettichthys melanostictus</i>	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
<i>Parophrys vetulus</i>	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

Table 2.2. 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

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 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.

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. 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.

3.0. 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. 2.1).

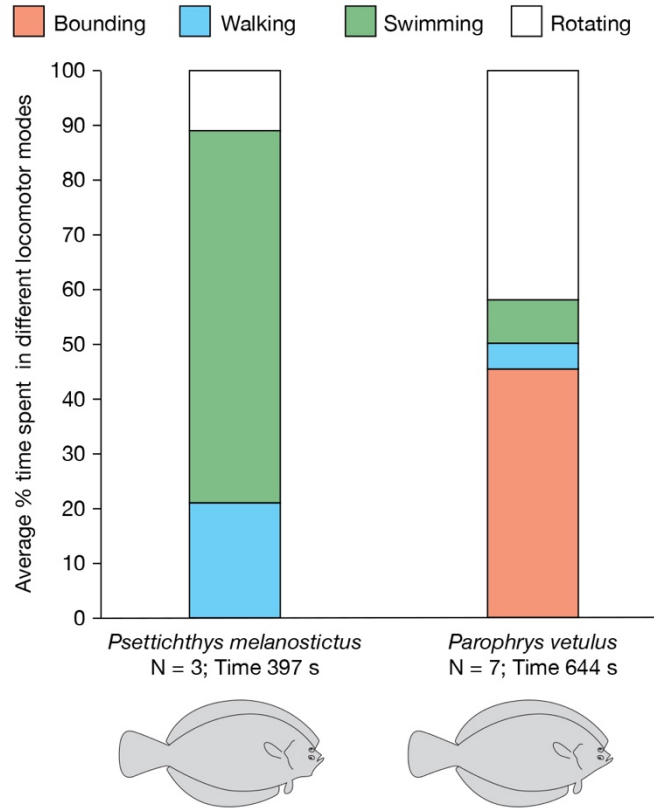


Figure 2.1. Percentage time spent walking, bounding, rotating, and swimming for two species of flatfishes, *Psettichthys melanostictus* (N=3) and *Parophrys vetulus* (N=3) during voluntary movements in seven-minute observation period.

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. 2.1). 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. 2.2 and 2.3).

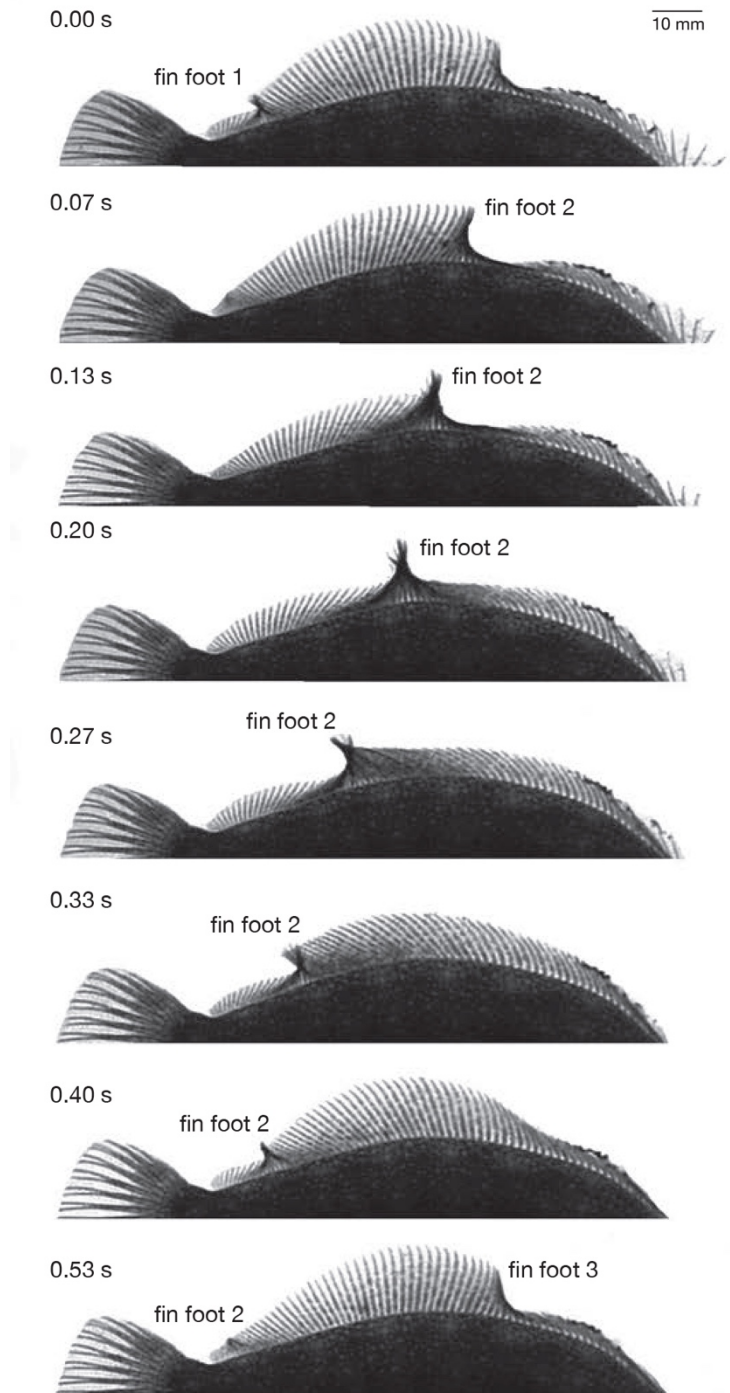


Figure 2.2. Frames from a high-speed video (120 fps) showing positions of dorsal fin rays during a benthic walking sequence of the Pacific Sand Sole, *Psettichthys melanostictus*. A single stride in a walk cycle is shown.

An exemplar forward walking cycle begins with an individual fin ray held approximately perpendicular (90°) to the body (Fig. 2.2). 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 2.2. 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. 2.3). 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).

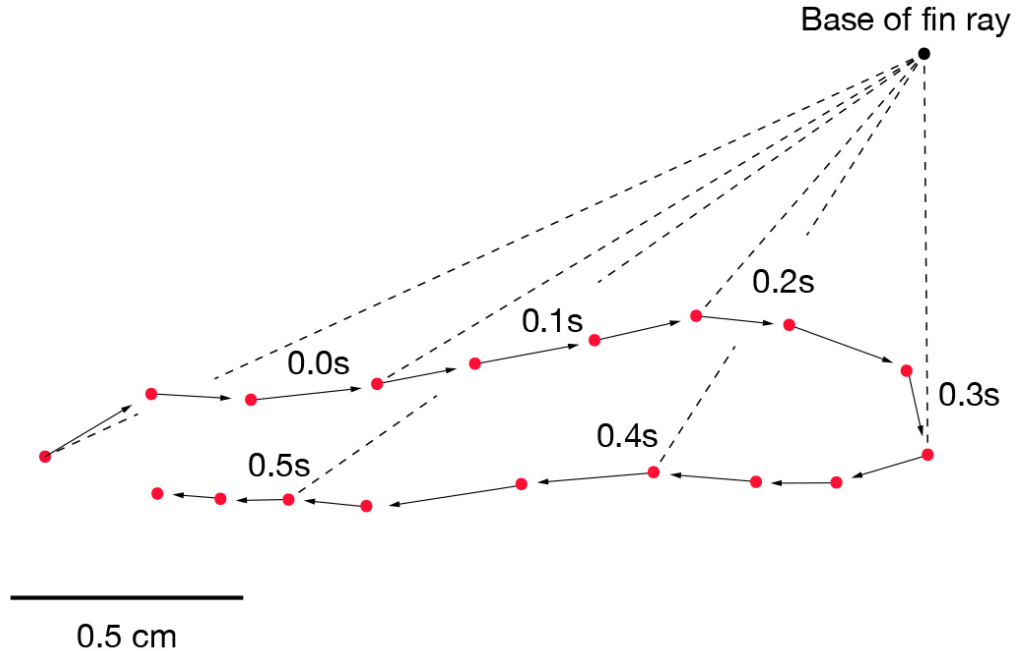


Figure 2.3. Tracing of the tip of a single fin ray from the middle of the anal fin of *Platichthys stellatus* in side view during one stride cycle (120 fps). Dotted lines connect the position of the fin ray tip (red dots) to its base (black dot). Fin rays angle down from the fin base to contact the substrate. 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.

Waves of fin-foot propulsion along the median fins are in phase and operate in parallel, producing a synchronous, symmetrical gait (Fig. 2.4). 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 $89.6 \pm 2.3^\circ$, at mid-wave $89.6 \pm 4.2^\circ$, and end-wave $88.9 \pm 4.1^\circ$. 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. 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.

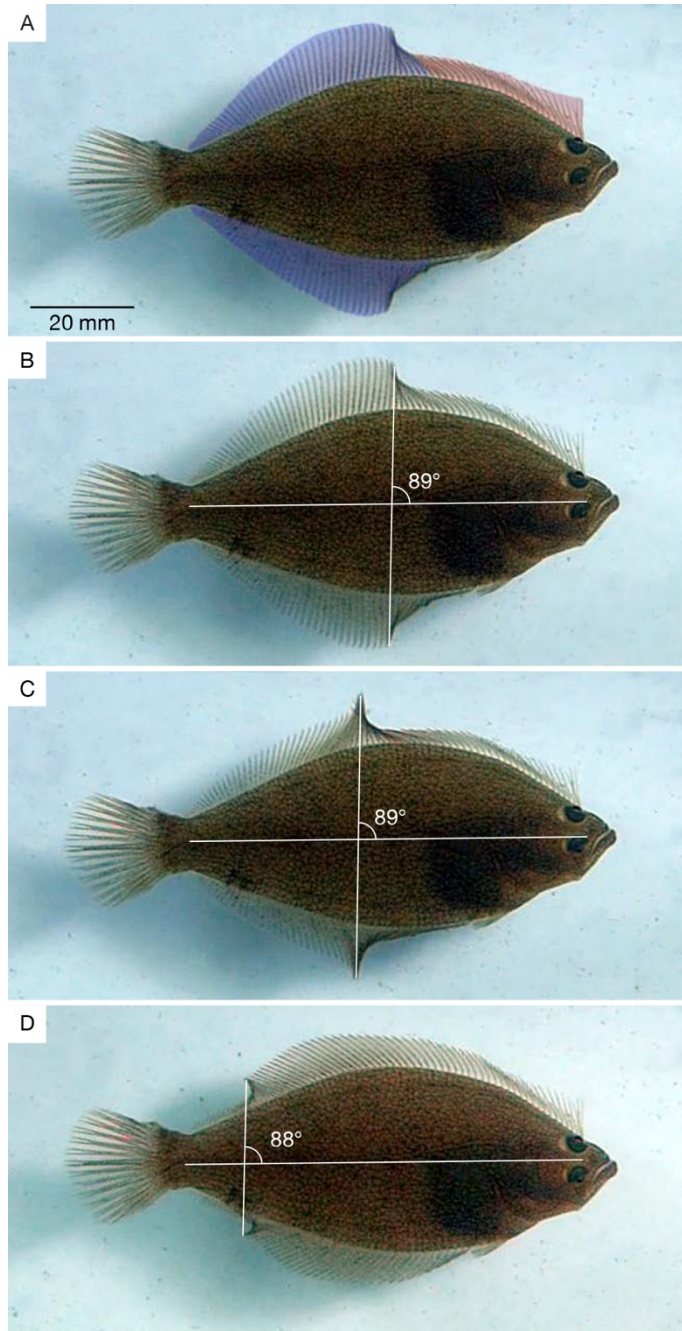


Figure 2.4. Fin rays in the dorsal and anal fin move symmetrically to produce steady forward locomotion. A. The anterior-most 35% of the dorsal fin of *Psettichthys melanostictus*, shaded in red, is not used in the propulsive wave. Fin regions active in the propulsive wave, shaded in purple, are equal in the dorsal and anal fins. B. Beginning of the wave mid-way down the body. C. Middle of the wave approximately $\frac{3}{4}$ of the way along the body. D. End of the wave at the posterior of the body.

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.

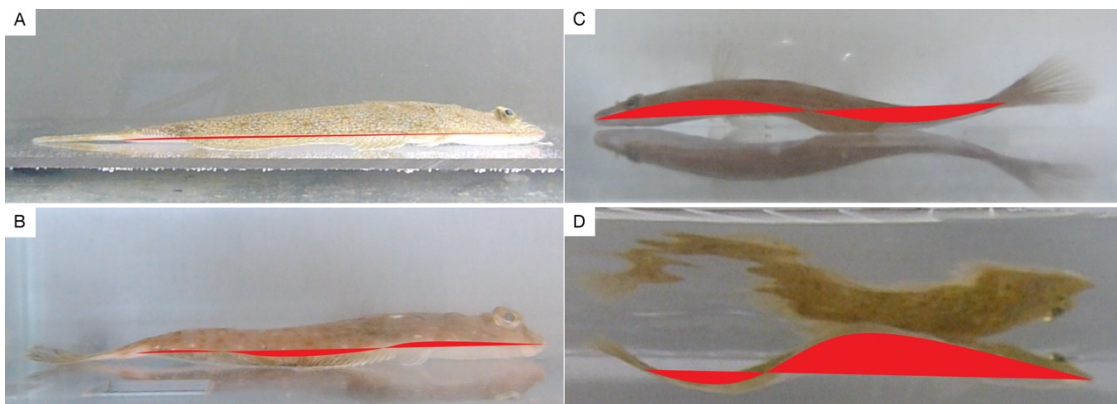


Figure 2.5. Undulations of the body during walking and swimming. Areas above and below the mid-sagittal line are shaded red. A. Video frame from a walking sequence of *Psettichthys melanostictus* showing no body undulation; 0.34 BL/s. B. Video frame from a walking sequence of *Parophrys vetulus* with 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.

Walking requires little to no undulation of the body (Fig. 2.5A - B; also see Supplemental Video 1) whereas larger amplitude undulations are used in swimming (Fig. 2.5C - 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. 2.6; 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. 2.6). 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.

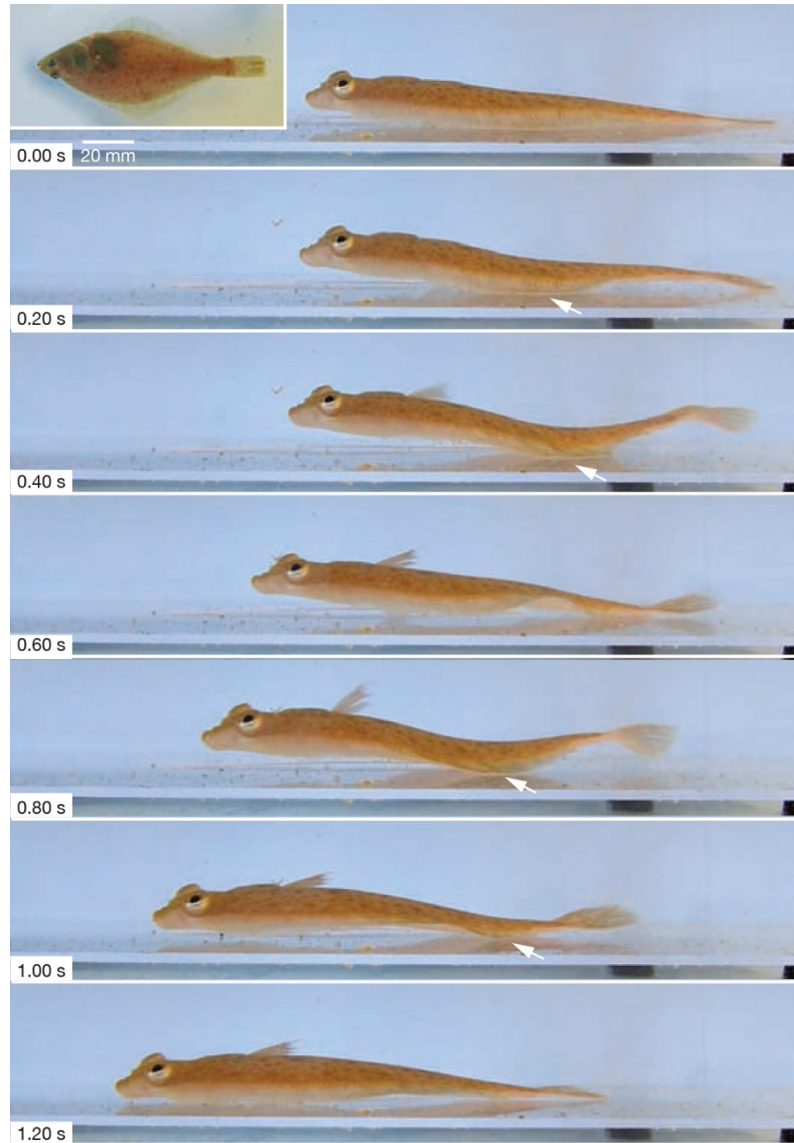


Figure 2.6. Frames from high-speed videos of *Parophrys vetulus* showing a bound. White arrows indicate areas of fin-ray contact with the substrate. Fin rays successively push against the substrate, lift the body, and propel it forward. An initial wave of propulsion against the substrate is followed 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.

Plots of locomotion in X-Y space highlight key differences between walking and bounding (Fig. 2.7). Walking is a continuous forward movement produced by successive waves of fin-feet (there are six waves in the sequence in Fig. 2.7A), each following immediately after the other. Bounding (Fig. 2.7B) 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 sequence shown in Figure 7B (also see Supplemental Video 3). Frequent changes in direction highlight the maneuverability typical of bounding behavior (Fig. 7B).

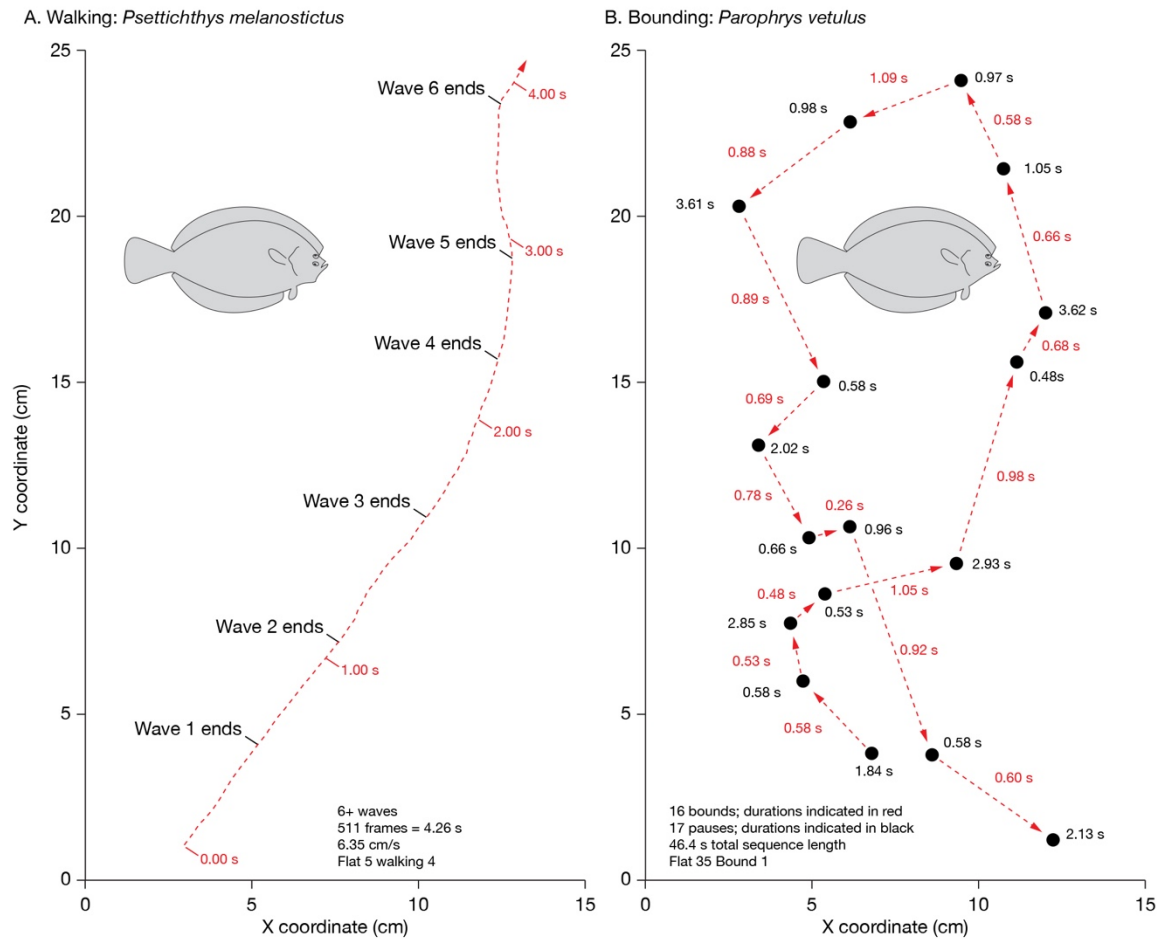


Figure 2.7. Movements of two species of flatfish, *Psettichthys melanostictus* and *Parophrys vetulus*, as seen from above in X-Y space. Movement paths indicated with red dashed lines and arrows. A. Walking sequence of *Psettichthys melanostictus* (4.2 s total) with six successive waves and times noted. 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.

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). Figure 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. 2.8).

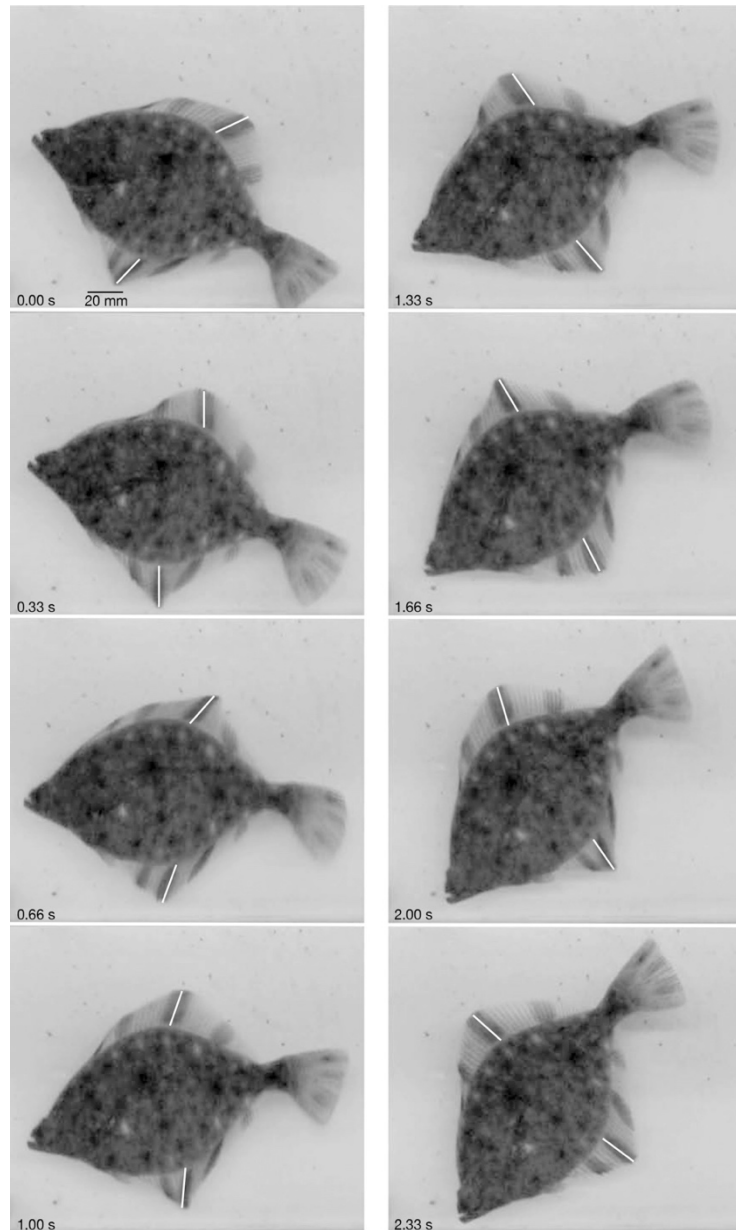


Figure 2.8. Frames from high-speed videos of *Platichthys stellatus* showing rotation in place. White lines highlight the fin-ray angles. The fin rays push against the substrate in opposite directions to produce rotation.

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

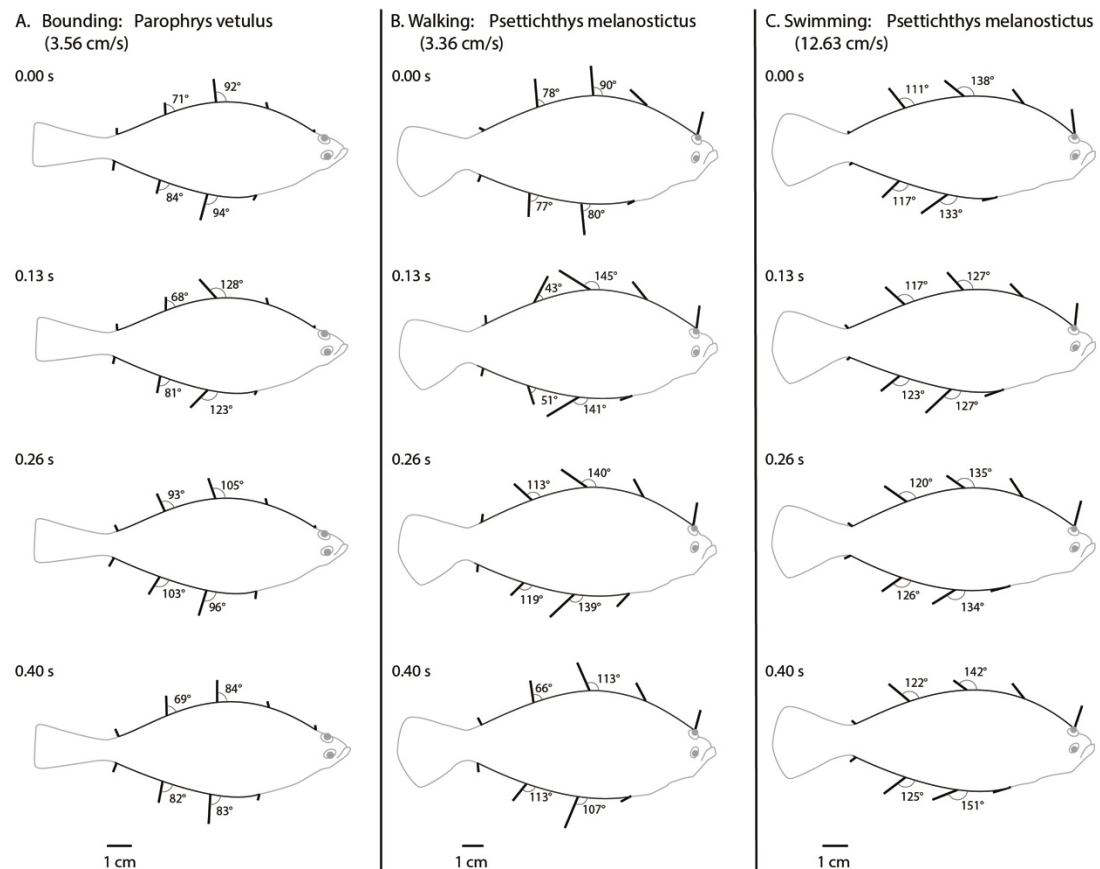


Figure 2.9. Comparison of fin ray angles for three locomotor behaviors at 0.4 s frame sampling. Heavy solid black lines indicate the five dorsal fin rays and four anal fin rays; angles are reported for two focal fin rays in the dorsal fin and two focal fin rays in the anal fin. A. Fin ray angles during 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.

The angles subtended by individual fin rays differ during three locomotor behaviors: bounding, walking, and swimming (Fig. 2.9). 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 = $74 \pm 14^{\circ}$ N=44; for bounding: mean = $37 \pm 9^{\circ}$ N=36; for swimming: mean = $20 \pm 7^{\circ}$ 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 2ure 9B 0.13 s, the convergence of dorsal fin rays is 102° (i.e., $145^{\circ} - 43^{\circ}$); this was the greatest fin ray convergence observed across all locomotor behaviors. Mean convergence angles were: walking $30 \pm 39^{\circ}$; bounding $-20 \pm 19^{\circ}$; and swimming $-15 \pm 9^{\circ}$.

Convergence during walking differed significantly from convergence during swimming (t-test; $p < 0.001$).

As in Figure 2.4, fin rays in the anterior portions of the dorsal fin do not contribute to the formation of fin-feet and show a limited range of motion in all four locomotor behaviors (Figs. 2.9 - 2.10). 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. 2.9 B - 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. 2.10).

Rotating: *Parophrys vetulus*

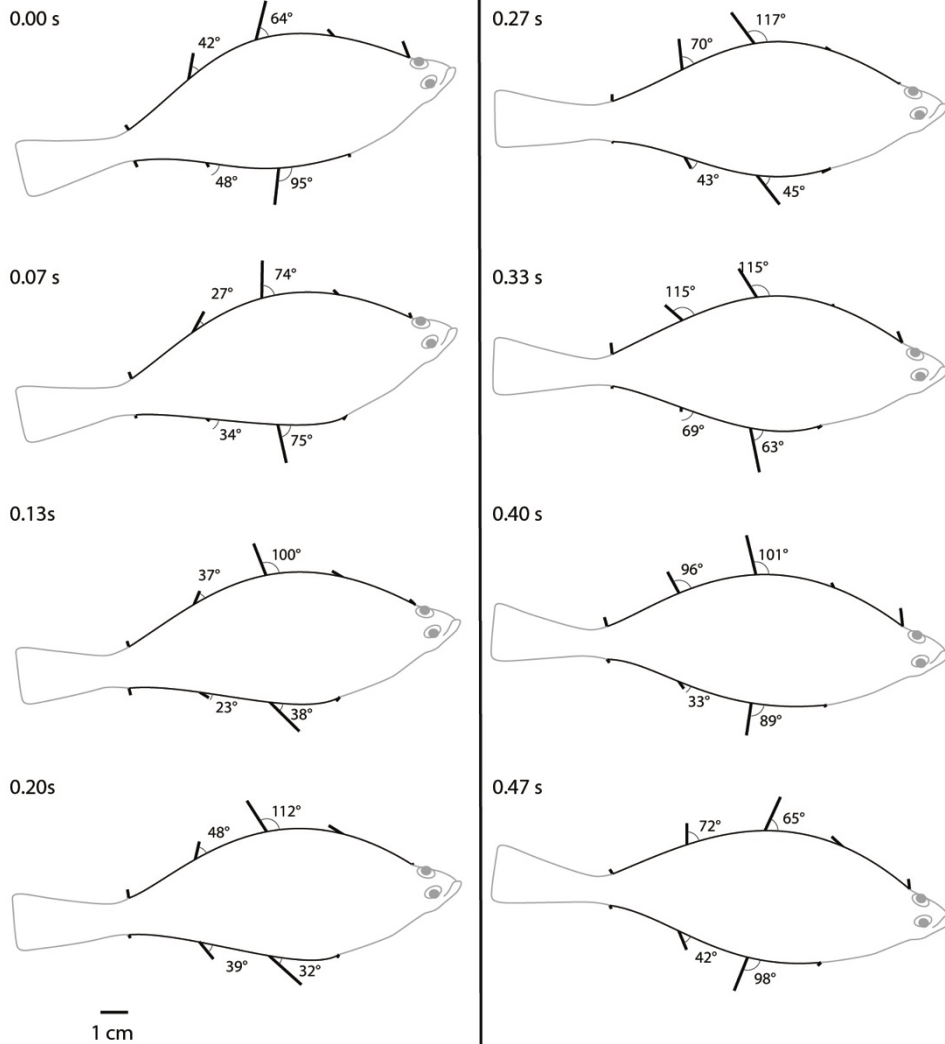


Figure 2.10. Fin ray angles during a rotation of *Parophrys vetulus* at 0.4 s frame sampling. Heavy solid black lines indicate the five dorsal fin rays and four anal fin rays; angles are reported for two focal fin rays in the dorsal fin and two focal fin rays in the anal fin.

The mean range of motion of the four focal fin rays during rotation was $65 \pm 18^\circ$ (N=36). The mid-body fin rays on dorsal and anal fins reach asymmetrical obtuse and acute angles at the beginning 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. 2.11; $F < 0.0001$).

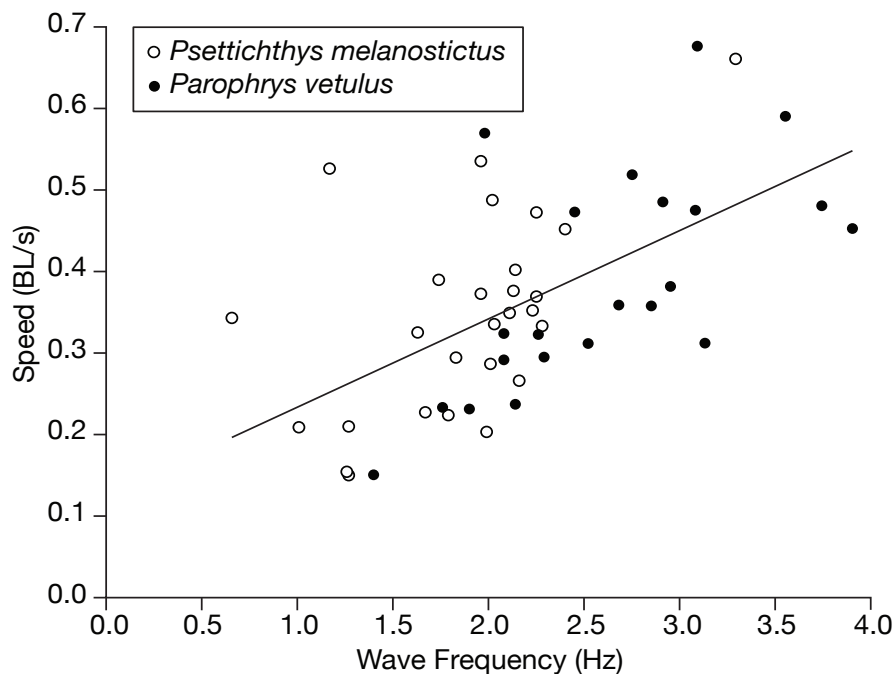


Figure 2.11. Speed in BL/s as a function of wave frequency for *Psettichthys melanostictus* (N = 27 sequences) and *Parophrys vetulus* (N = 22 sequences).

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. 2.12). 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. 2.13).

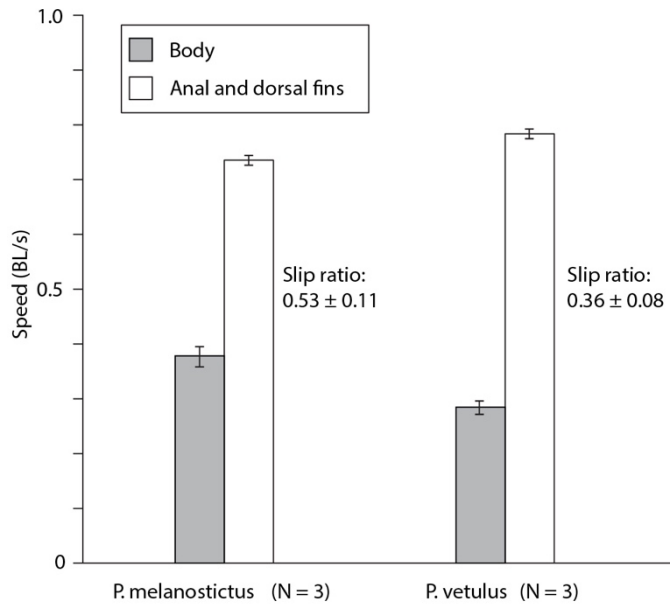


Figure 2.12. Average speed of body and median fins for *Psettichthys melanostictus* (N=3) and *Parophrys vetulus* (N=3). For each walking sequence analyzed 6 or 7 waves were included. Ratio between forward speed and fin speed is reported as Slip Ratio.

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*.

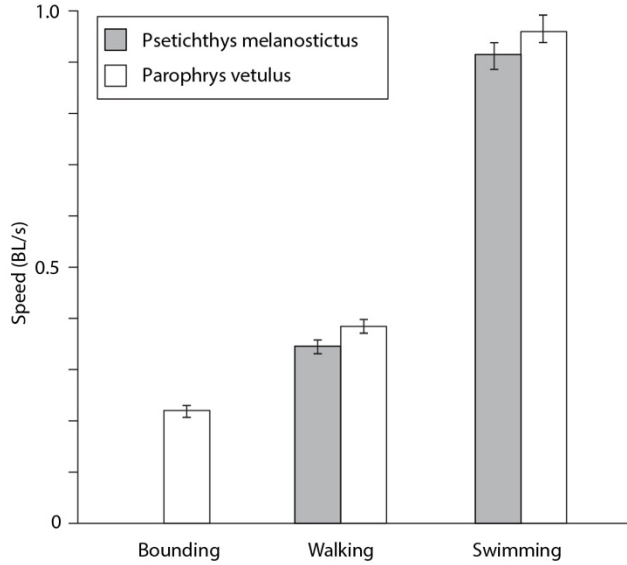


Figure 2.13. Comparisons of speeds of bounding, walking, and swimming in *Psettichthys melanostictus* and *Parophrys vetulus*. Note that none of 67 locomotor sequences captured for *P. melanostictus* included bounding. For *P. vetulus* bounding, 15 sequences from 6 individuals were analyzed; for *P. 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.

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.

4.0. 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 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 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.

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, 2014). Balistiform locomotion of triggerfishes similarly involves a rigid body and undulation of median fins; specializations of the fin skeleton and associated inclinators and declinators 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 (Achiiridae). Preliminary observations of benthic walking in tonguefishes (Cynoglossidae), which have elongate bodies, a diphyccercal 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.

5.0. *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.

Acknowledgements

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

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Supplemental Videos

Supplemental Video 1. Side view of benthic walking in *Psettichthys melanostictus*.

Supplemental Video 2. Top views of benthic walking sequences in six species of pleuronectid flatfishes.

Supplemental Video 3. Bounding of *Parophrys vetulus*.

Supplemental Video 4. Rotation of *Platichthys stellatus*.

CHAPTER 3

STEALTH LOCOMOTION: HYDRODYNAMIC CRYPSIS IN A FLATFISH, THE HOGCHOKER (*TRINECTES MACULATUS*)¹

¹Claire H. Fox, Sunghwan Jung, Seungho Kim, and William E. Bemis

Abstract

We found benthic walking in the Hogchoker, *Trinectes maculatus* (Pleuronectiformes: Achiridae) to be hydrodynamically cryptic – akin to “pressure camouflage” that enables the flatfish to move stealthily through its aquatic environment. Using Digital Particle Image Velocimetry methods we compared hydrodynamic flow in a 2-D longitudinal plane during swimming and walking sequences. Analyses of three flow regions around the fish (bow-wave, along the body, and in the wake) demonstrated that walking Hogchokers produce significantly lower mean velocity magnitude and vorticity in the surrounding water when walking than during swimming. Swimming Hogchokers produced thrust by undulating their body, generating counter-rotating proto-vortices on the upward and downward facing sides of their wide flat bodies that are shed into the wake and form a distinct and persistent hydrodynamic trail, visualized here in vorticity plots. Walking Hogchokers lie on their side on the bottom and use sequential portions of their dorsal and anal fins as “fin feet” to maneuver and move slowly along the bottom, with little to no body undulation. We found that fluid disturbance around the body and in the wake of a walking Hogchoker was comparatively small and left no persistent vortex signature. Vortex trails from swimming fishes are quickly identified and followed by many piscivorous predators. We interpret that hydrodynamic crypsis of the benthic-walking Hogchoker is advantageous because it does not leave a wake that could be detected by a predator. Hydrodynamic crypsis might also facilitate location and capture of epibenthic and infaunal prey.

Introduction

Visual crypsis is a familiar and well-explored concept, whereas hydrodynamic crypsis, of great importance in aquatic environments, is comparatively understudied (Ruxton, 2011). Swimming fishes produce turbulent hydrodynamic signals that can inform and alert potential predators and prey of their location, size, and direction of travel. The turbulent trail left behind a goldfish is detectable in the water up to five minutes after its passage (Hanke et al., 2000) and distance, speed, and size of the fish can be derived from the decay of vorticity, velocity gradient, and width of track, respectively. Different fishes (e.g., *Lepomis*, *Colomesus*, and *Thysochromis*) have distinct wake signatures that last from 30 s to >5 min producing water velocities detectable by piscivorous predators and at distances where vision and hearing often fail (Hanke and Bleckmann, 2004). Marine mammals and piscivorous fishes use these hydrodynamic signals to locate and evaluate potential prey. Harbor seals can detect vortex trails of swimming fishes and respiratory jets of flatfishes camouflaged on the substrate, rapidly locating the sources of these hydrodynamic stimuli without additional visual or chemical cues (Wieskotten et al., 2010; Niesterok et al., 2017). The specialized vibrissae of harbor seals can distinguish between hydrodynamic trails generated by objects of different sizes and shapes (Wieskotten et al., 2011). Fishes can sense even weak hydrodynamic changes using superficial neuromasts on their body surface and neuromasts in lateral line canals (Bleckmann and Zelik, 2009; Schwalbe et al., 2012). Goldfish discriminate between hydrodynamic signals that vary in speed, direction, size, and shape using their lateral line system (Vogel and Bleckmann, 2001). *Silurus glanis*, a nocturnal piscivorous catfish, detects and follows a series of past locations of prey fishes by tracking their wakes,

succeeding in prey capture using only their hydrodynamic sense (Pohlmann et al., 2004). Even fishes that are primarily visual predators integrate sensory information from both canal and superficial neuromast cells in localizing prey, resulting in more direct search paths and extending the area within which prey can be detected (Montgomery et al., 2002).

In addition to generating a turbulent wake that predators can track, swimming fishes also produce a bow-wave that can alert prey to their approach. Small fishes can respond to flow stimulus of a predator in less than 4 ms (Liu and Fetcho, 1999) compared to the 200 ms or longer that it takes them to respond to a visual stimulus (Burgess and Granato, 2007), and, using only hydrodynamic stimuli, respond with a fast start within 2 cm of the predator's approach (Stewart et al., 2014).

Flatfishes, the subject of our study, have been characterized as belonging to one of three major feeding groups (Yazdani, 1969; DeGroot, 1969, 1971): 1) Visual feeders that generally take free-swimming food, e.g., other fishes; 2) Visual feeders that mainly take bottom-living and slow-moving food, e.g., mollusks, polychaetes; 3) Nocturnal feeders that strictly take bottom-living food, e.g. polychaetes. There is evidence that stem flatfishes were piscivorous (Friedman, 2008), and species of *Psettodes*, the basal-most extant genus of Pleuronectiformes, belongs to feeding group 1 (they are ambush predators that eat other fishes, Kizhakudan et al., 2013). In contrast, the model flatfish chosen for our study, the Hogchoker (Pleuronectiformes; Achiridae: *Trinectes maculatus* shown in walking posture in Figure 2.1) is in feeding group 3, with derived morphological and behavioral specializations for nocturnal predation on infaunal and epifaunal organisms. Hogchokers exhibit both opportunistic and selective feeding patterns with primary prey

items being polychaetes, followed by siphons of tellinid clams, and amphipods (Derrick and Kennedy, 1997; Curti, 2005; VIMS Multispecies Research Group, 2020).

Flatfishes (Pleuronectiformes) have exemplary visual crypsis, achieving adaptive surface marking changes as rapidly as 2-8 s (Ramachandran et al., 1996) to match the luminance, color contrast, and spatial scales of a range of natural substrates (Akkaynak, 2017). Because flatfishes undergo a unique axial transformation during metamorphosis, the side-to-side body undulation of typical teleosts becomes oriented up-and-down. When inactive, flatfishes bury to hide in soft-bottom substrates (Fig. 2.2B) (Ellis et al., 1997). A unique walking gait along the substrate (Fox et al., 2018) entails little to no body undulation, which begs the question: is flatfish walking a form of hydrodynamic crypsis? We investigated this question using Digital Particle Image Velocimetry (DPIV) analyses of the hydrodynamics of the Hogchoker, *Trinectes maculatus* (Pleuronectiformes: Achiridae), a small, estuarine sole abundant along the Atlantic Coast of North America.

2.0. **Methods**

2.1. Animal Collection and Care

We chose *Trinectes maculatus* as a model flatfish for this study because of its relatively small adult size, availability, and tolerance of a wide range of salinity (a factor in water density). For DPIV analyses conducted at Cornell University we ordered six specimens of *Trinectes maculatus* from Gulf Marine Specimen Marine Lab, held them in recirculating tanks (15 – 18 °C, salinity 20 ppt) equipped with biological filtration units, a sand substrate, 12:12 day/night cycle, and fed them daily on bloodworms (Cornell University IACUC protocol 2017-0010). We video recorded individuals moving along the substrate and after the study was complete, adopted out the animals. Individuals spent

most of their time inactive and cryptic, buried under the sand, and instinctively perform burying movements even when substrate was not present.

For a locomotor studies of *Trinectes maculatus* conducted at the Virginia Institute of Marine Science (VIMS) in June 2017 specimens were collected via bottom-trawl during the monthly Chesapeake Bay survey conducted by VIMS. Fish were transported in aerated coolers using water collected from the capture site, held for five days in the VIMS Seawater Research Laboratory in recirculating tanks supplied with low-salinity water from the estuary and fed daily on bloodworms (VIMS protocol IACUC-2017-04-14-12050-ejhilton). After study, fish were released near their collection location.

2.2. Videography

Prior to videography, we photographed each fish from above with a scale-bar in the frame. We captured high-speed digital-video recordings of *Trinectes maculatus* walking and swimming. We video recorded sequences for locomotor analyses from the top and side using a GoPro Hero4 at 60 fps at a resolution 1280 X 720 pixels. DPIV videography was performed with a Photron Fastcam SA-Z camera (Photron USA, Inc., San Diego, CA, USA), Nikon 105mm 1:2.8 D lens with manual focus and f-stop 22, at a shutter speed of 1000 s^{-1} , record rate 500 fps, resolution 1024 X 1024 pixels. For illumination of particles during DPIV we used an array of three laser modules generating 150 mW green line (532 nm Diode-Pumped Solid State laser with integrated cylindrical lens optics and aperture angle of 90° manufactured by Apinex Inc., Durham, NC, USA). For particles we used hollow glass spheres with particle diameter = $10 \mu\text{m}$, s.g. = 1.05 (catalog # 1108952 Lavision Inc., Ypsilanti, MI, USA).

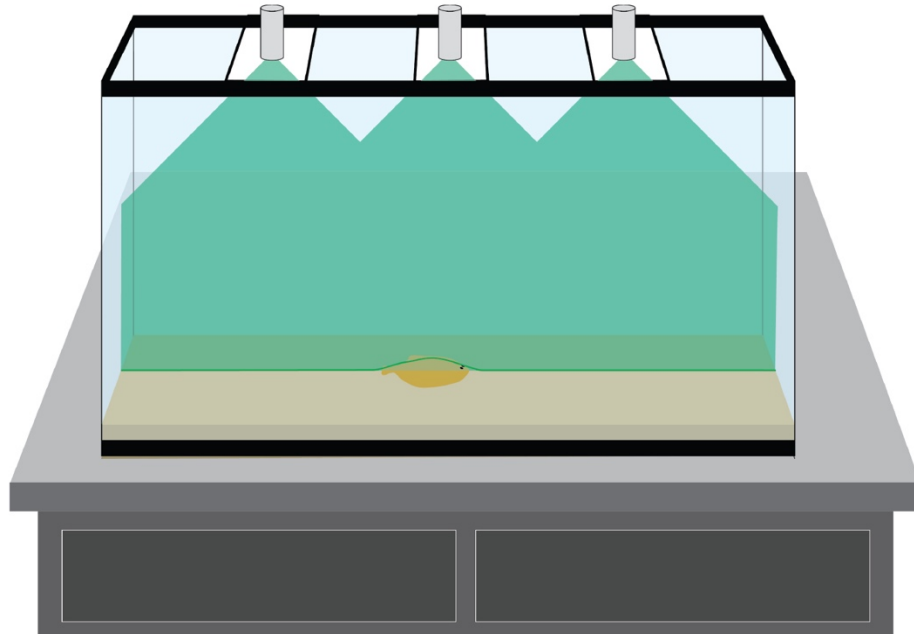


Figure 3.1 **DPIV experimental set-up.** Three green lasers with built-in light sheet optics were aligned with custom holders to point down into a still-water tank seeded with 10 μm hollow glass spheres, producing an illuminated plane that bisects the fish longitudinally when it moves left or right down the center of the long axis of the tank. The Photron Fastcam SA-Z camera is located in front of the tank with a 8.9cm X 8.9cm capture window positioned in the middle region, away from the tank side walls and just above the substrate. The substrate consists of fine sand embedded in a thin layer of aquarium-grade silicone adhered to an acrylic sheet.

During filming sessions lasting 10-30 min, individuals were transferred to a filming tank (76.2 cm x 30.5 cm x 45.7 cm) with non-circulating water of the same temperature and salinity as their holding tank. Still-water was used to permit visualization of weak flow structures (Hank and Bleckmann, 2004). A challenge in working with still water videography environments is dealing with residual water motion in the tank generated, for example, by previous swimming behaviors. As much as possible we selected video sequences with a minimum of residual water movement from prior locomotion and respiratory jets from gill ventilation. Wall interactions were minimized by using a tank over five times the width and eight times the length of the animal, situating the camera to capture movement in the center region of the tank, and centering

the laser line down the center of the long tank axis (Figs 3.1 and 3.2). The substrate was an acrylic plate with a smooth layer of aquarium silicon with fine sand embedded to provide traction, 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 flexible plastic or metal probe. Photographs for Figure 1 were taken using a Google Pixel 3 phone camera with 12.2 Mp, 1/2.55" dual-pixel sensor and f/1.8 aperture lens. We adjusted levels, contrast, and tone in Adobe Photoshop and digitally removed the background from Fig. 3.3A.

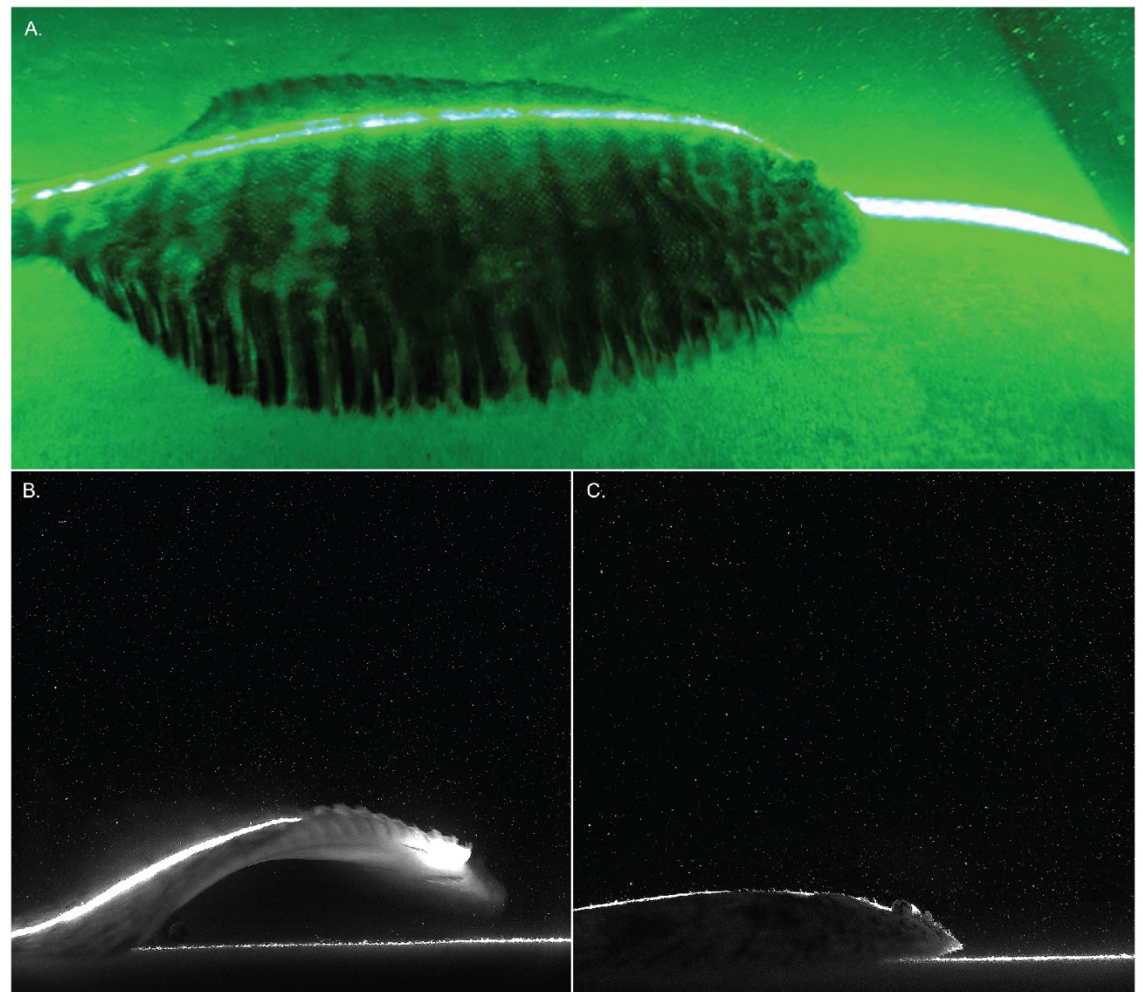


Figure 3.2. Orientation of *Trinectes maculatus* during DPIV experiments and example capture frames.

2.3. Locomotor and DPIV Analyses

We calculated body velocity during walking and swimming by auto tracking the eye using DLTdv5 digitizing tools for MATLAB (Hedrick, 2008). Each walking sequence used for kinematic analysis contained three or more successive fin-foot strides (Fox et al. 2018).

Digital Particle Image Velocimetry analyses were performed using PIVlab 2.31 and MATLAB R2018a. We pre-processed video frames in Tiff format with the “sharpen” filter in ImageJ.

In PIVlab, we used interrogation window sizes of 128 pixels in pass 1 and 64 pixels in pass 2 for full-frame analyses, and 64 pixels in pass 1 and 32 pixels in pass 2 for analysis of smaller flow region (i.e., bow-waves). We used an inter-frame temporal spacing of 2 ms – 4 ms to measure the velocity field, with swimming sequences requiring a higher frame sampling than walking sequences. Erroneous vectors (outliers) were eliminated, when necessary, by selecting velocity limits that excluded them and interpolating the missing vectors. We applied a low level of data smoothing. Vector fields and derivatives (magnitude, vorticity) were plotted in PIVlab to produce Figs. 3.4-3.7 and were exported in .csv format and imported into Excel to produce Table 1.

2.4. Statistics

We used Mann-Whitney U-Tests to compare velocity magnitude and absolute vorticity during swimming and walking for three body regions to estimate bow wave, flow regimes along the body, and flow regimes of the wake.

3.0. Results

3.1. Observations on Functional Morphology

Fin-feet form during walking when successive fin-rays converge together at the tip (Fig. 3.3A). In periods of inactivity the Hogchoker lies buried completely under the sand or mud with only a cluster of small features protruding above the substrate: two small eyes, an upward-facing nostril tube, and fringed lip edge (Fig. 3.3B). The family Achiridae is named for “achirine lines”, accessory branches of the lateral line that extend at right angles across the body surface toward the dorsal and anal fins, increasing the mechanosensory capacity of the eyed side (Fig. 3.3C). Fimbriae, fleshy projections of the epidermis, cover the blind side of the head from the operculum to the anterior margin, and the anterior dorsal and anal fin rays (Fig. 3.3D).

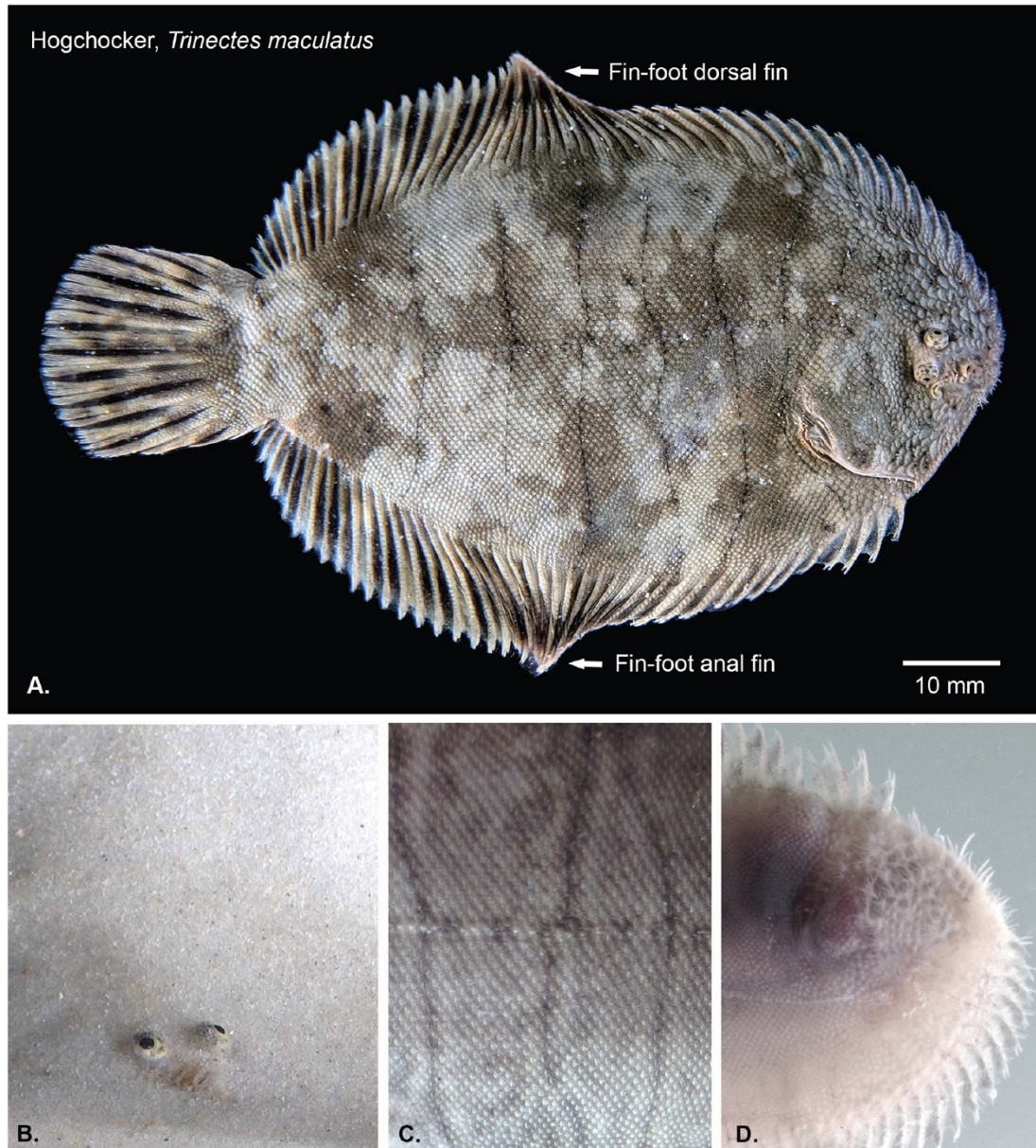


Figure 3.3. **Morphology features of *Trinectes maculatus* for walking on the bottom, avoiding detection by predators, and sensing prey.** (A). Eyed side during benthic walking. Dorsal and anal fin rays converge into symmetrical fin-feet that push against the substrate. Note camouflaged body. (B). Cryptic appearance when buried in soft bottom sediments with only the eyes and mouth protruding. C. Accessory branches of the lateral line extend the mechanosensory system. (D). Blind side showing sensory papillae near naris and mouth.

3.2. Kinematics of Walking and Swimming

During swimming, the entire body surface of the flatfish, from the tip of the rostrum to the tip of the tail, flexes in an undulating wave. Hogchokers lack pectoral fins and the small pelvic fins are fused with the anal fin. Thrust generation from paired fins can thus be discounted. The dorsal and anal fins have soft, flexible fin-rays and no bony spines. During steady swimming, the caudal, dorsal, and anal fins undulated along with the body. Together, the body and fins form essentially a flat, flexible, thrust-producing surface that increases in flexibility along its margins and toward the tail. Moving the fins out of line with the body during swimming creates drag used during steering and braking maneuvers. When swimming close to the substrate, the flow on the eyed side (the right side of the body in *Trinectes maculatus*) is impacted differently than the bottom facing, or blind (left) side.

During walking there is little to no body undulation. Forward propulsion results from sequential bunches of dorsal and anal fin-rays on the margins of the body (Fig. 3.3A) cycling forward and back in contact with the substrate to push the body forward (Supplemental Video 1). The fin rays are small appendages that lift only slightly off the substrate during the recovery phase of each stride.

3.3. Swimming and walking speeds

Swimming and walking differed significantly in mean speed (Mann-Whitney $U=0.5$, $p < 0.05$ two-tailed). For 24 sequences from 7 specimens of *Trinectes maculatus*, mean swimming speed was $12.46 \pm 2.12 \text{ cm s}^{-1}$ ($1.20 \pm 0.26 \text{ BL s}^{-1}$) and mean walking speed was $2.79 \pm 2.49 \text{ cm s}^{-1}$ ($0.26 \pm 0.21 \text{ BL s}^{-1}$).

3.4. Velocity magnitude of flow during swimming vs. walking

Figure 3.4 shows the magnitude of 2D velocity fields around either swimming or walking flatfish. As a Hogchoker swims or walks, it stirs fluid around. Walking causes small disturbances to the fluid surrounding the body. However, a swimming Hogchoker creates a strong fluid flow trailing the body. As the strong flow is formed, the surrounding fluids swirl around. To compare the amount of water disturbance and turbulence produced by walking and swimming we found the mean velocity magnitude and vorticity for three regions: 1) bow wave surrounding the head; 2) flow above and under the body; and 3) wake behind the body (30 video sequences, 17 trials, N=4). Results are summarized in Table 3.1.

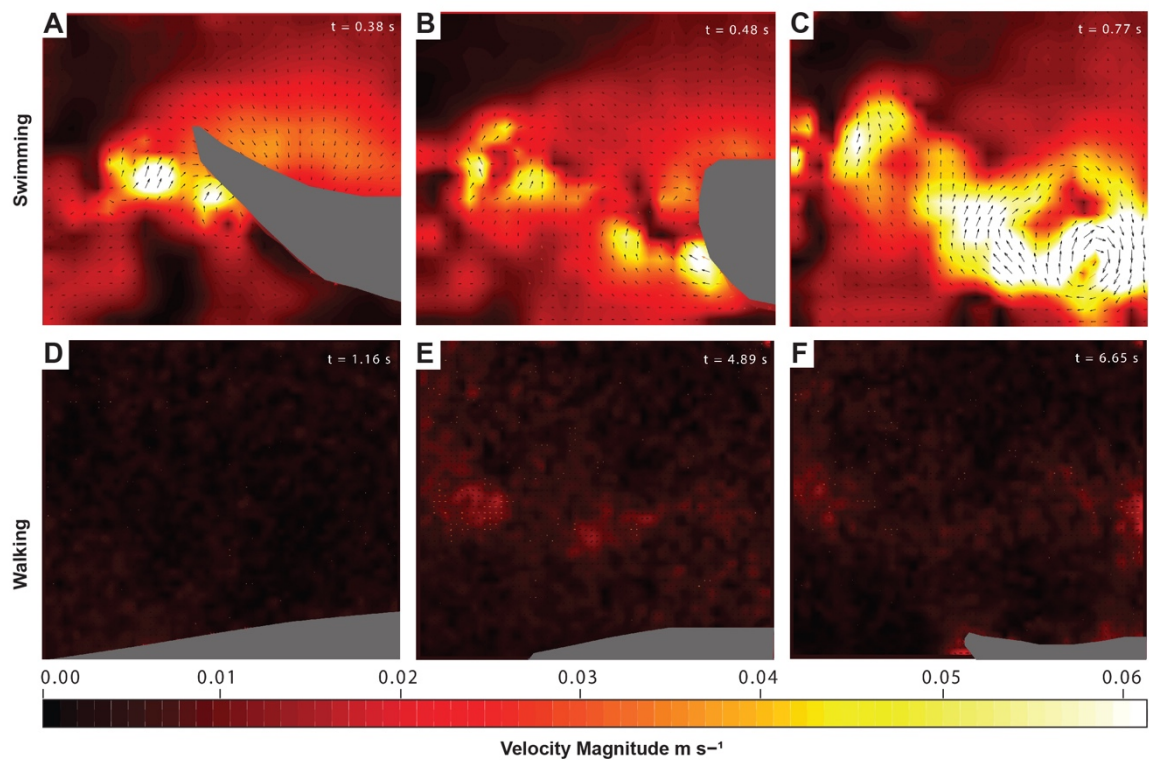


Figure 3.4. High velocity and strong turbulent flow in the wake of a swimming Hogchoker contrasts with lower velocity and minimal water disturbance during walking. Velocity magnitude of fluid in the wake of swimming (A-C) and walking (D-F) *Trinectes maculatus*. High velocity flow regions are white and yellow, mid-velocity flow regions are orange and red, low-velocity regions are dark red to black. Flatfish are shaded in grey, and direction of travel is left to right.

	No. of Trials	Velocity Magnitude cm/s ± SD	Vorticity 1/s ± SD
Swim Bow Wave	3	2.86 ± 0.75	4.05 ± 1.44
Walk Bow Wave	3	1.15 ± 0.27	0.53 ± 0.14
Swim Body	6	3.60 ± 0.67	2.75 ± 0.70
Walk Body	6	0.74 ± 0.17	0.65 ± 0.09
Swim Wake	6	2.96 ± 0.59	3.93 ± 0.95
Walk Wake	6	0.25 ± 0.09	0.33 ± 0.08

Table 3.1. Average velocity magnitude and vorticity in three flow regions (bow wave, body, and wake) for swimming and walking *Trinectes*. (30 sequences from 17 videos; N = 4)

The mean velocity magnitude of flow around and behind the flatfish differed significantly between swimming and walking, with an average 15 times higher velocity magnitude in the wake during swimming compared to walking. Mean velocity magnitude of flow around the body in walking and swimming was 3.6 cm s^{-1} and 0.7 cm s^{-1} ; the distributions in the two groups differed significantly (Mann-Whitney $U=0.5$, $p < 0.05$ two-tailed). Mean velocity magnitude of the wake in walking and swimming was 3.0 cm s^{-1} and 0.2 cm s^{-1} ; the distributions in the two groups differed significantly (Mann-Whitney $U=0$, $p < 0.05$ two-tailed).

Mean vorticity of the fluid around and behind the flatfish was significantly higher during swimming and averaged 12 times higher in the wake. Mean vorticity in the fluid around the body in walking and swimming was 2.8 s^{-1} and 0.6 s^{-1} ; the distributions in the two groups differed significantly (Mann-Whitney $U=1.5$, $p < 0.05$ two-tailed). Mean vorticity in the wake during walking and swimming was 3.9 s^{-1} and 0.3 s^{-1} ; the

distributions in the two groups differed significantly (Mann-Whitney $U=0$, $p < 0.05$ two-tailed).

The mean velocity magnitude of the bow-wave was three times higher for swimming (2.9 cm s^{-1} for swimming and 1.1 cm s^{-1} for walking), and vorticity was eight times higher (4.0 s^{-1} for swimming and 0.53 s^{-1} for walking). Due to the small number of video sequences that included the flow region in the bow wave front of the head, we did not apply a statistical measure of significance.

3.5. Vorticity and wake structure during swimming vs. walking

When the Hogchoker swims forward, a series of reversed von Karman vortices form in the wake. In our experiments, we did not observe clear alternating von Karman vortices, but still observed strong vortex underneath the trajectory of the tail fin (Fig. 3.5A-C). This vortex structure benefits the fish swimming forward by creating pressure variation around the body and fins. In contrast, when the flatfish walks in contact with the surface, we did not observe any clear vortex or swirling flow structure behind the body.

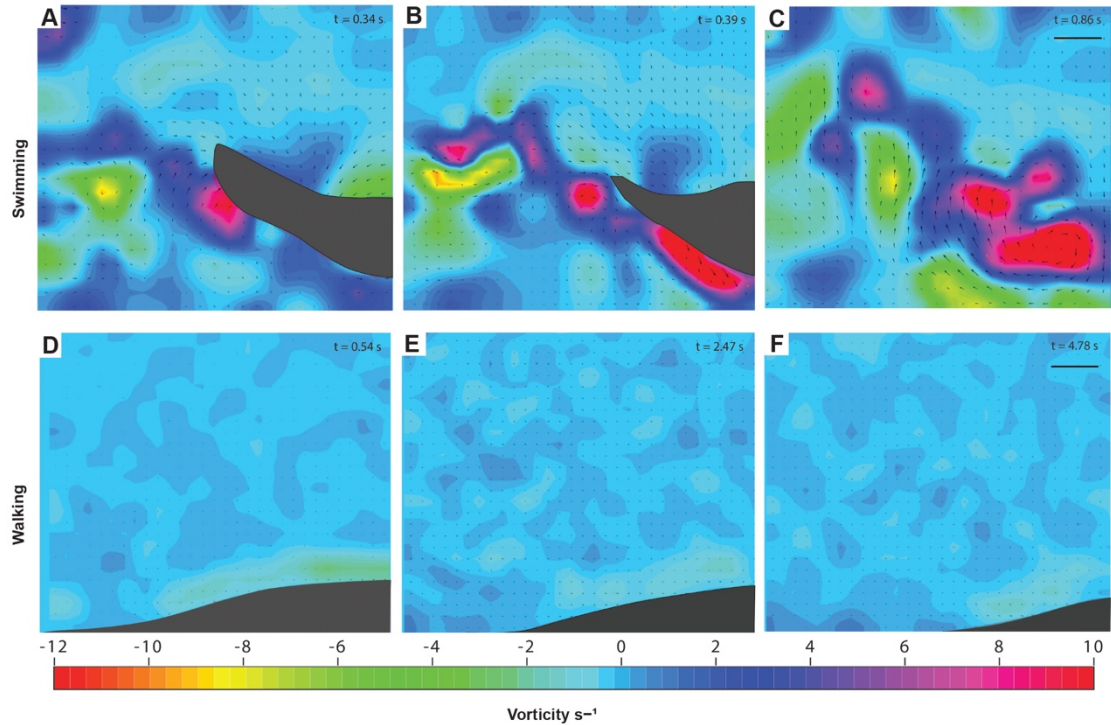


Figure 3.5. Vorticity plots of the flow in the wake of swimming (A-C) and walking (D-F) *Trinectes maculatus* show a persistent hydrodynamic trail of vortices left behind after swimming that is absent after walking. Dark blue colors represent positive vorticity (clockwise rotation) and yellow-green colors represents negative vorticity (counter-clockwise). Flatfish are shaded in grey, and direction of travel is left to right.

Interpreting complex flow accurately is difficult with only 2-D data, thus we can offer limited observations based on velocity fields in what is functionally sagittal plane (Supplemental Video 2). An undulatory swimming sequence begins with the Hogchoker flexing the body upwards. As the fish raises the head and moves forward into the

surrounding fluid, the snout pushes fluid upwards and upstream (Fig. 3.6A; Fig. 3.7B).

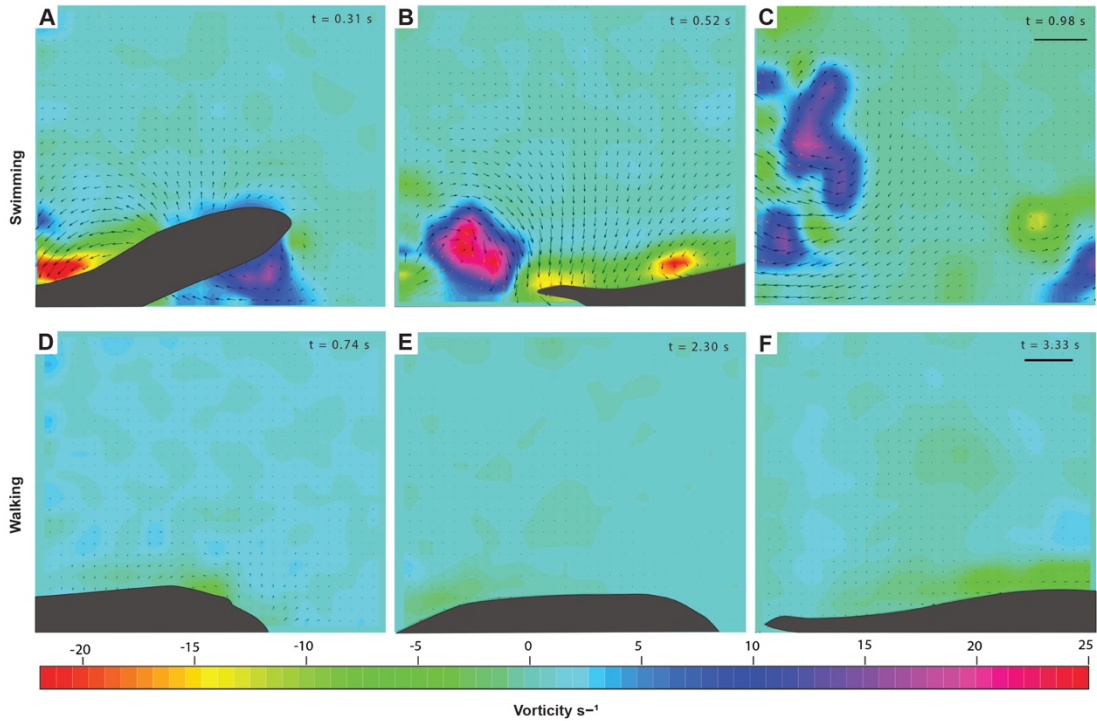


Figure 3.6. Fluid vorticity of flow around swimming (A-C) and walking (D-F) *Trinectes maculatus* shows high vorticity around the head, along the body, and in the wake during swimming, and low vorticity during walking. Counter-rotating flow is indicated in blue (clockwise) and green (counter-clockwise). Flatfish are shaded in grey, and direction of travel is left to right.

Negative pressure zones form mid-way down the concave back and under the head that pull fluid backwards and inwards to form proto-vortices, or “regions of elevated vorticity adjacent to the body” (from Müller et al., 2001). The flow rotates in opposite directions on either side of the body as the proto-vortices travel from high to low pressure zones formed by the crests and troughs of the body wave, towards the posterior (Fig. 7B-D). As the undulation progresses down the body the tail lifts upwards, with the top side of the tail pushing water up and forward into the negative pressure zone formed by the concave top side of the body; on the underside, the undulating body pushes a bolus of reverse-rotating fluid underneath the fish towards the posterior (Fig. 7E-F). The flatfish swims

very close to the substrate and likely exploits benefits of ground effect. The underside of the body and the substrate form a narrow channel or a lubricating layer for fluid flow, which allows the flatfish to slide over it. As the proto-vortex underneath the body reaches the posterior it joins the upstream-directed water formed by the upward movement of the tail. When the tail changes direction and moves downwards a reverse vortex is shed into the wake of the fish (Fig. 3.6B; Fig. 3.7I). Counter-rotating vortices are shed off the top surface of the tail (Fig. 3.7H-I) or form secondarily in the surrounding fluid, in response to the movement of the primary vortices shed from the bottom surface of the flatfish (Fig. 3.5A-C). Such strong vortices with lower pressure shed off from the tail will suck and lift sediment particles upward, increasing the turbidity of the trailing fluid. The vortices form a distinctive hydrodynamic trail behind the fish that persists long after the fish has moved on (Fig. 3.6C; Fig. 3.7L).

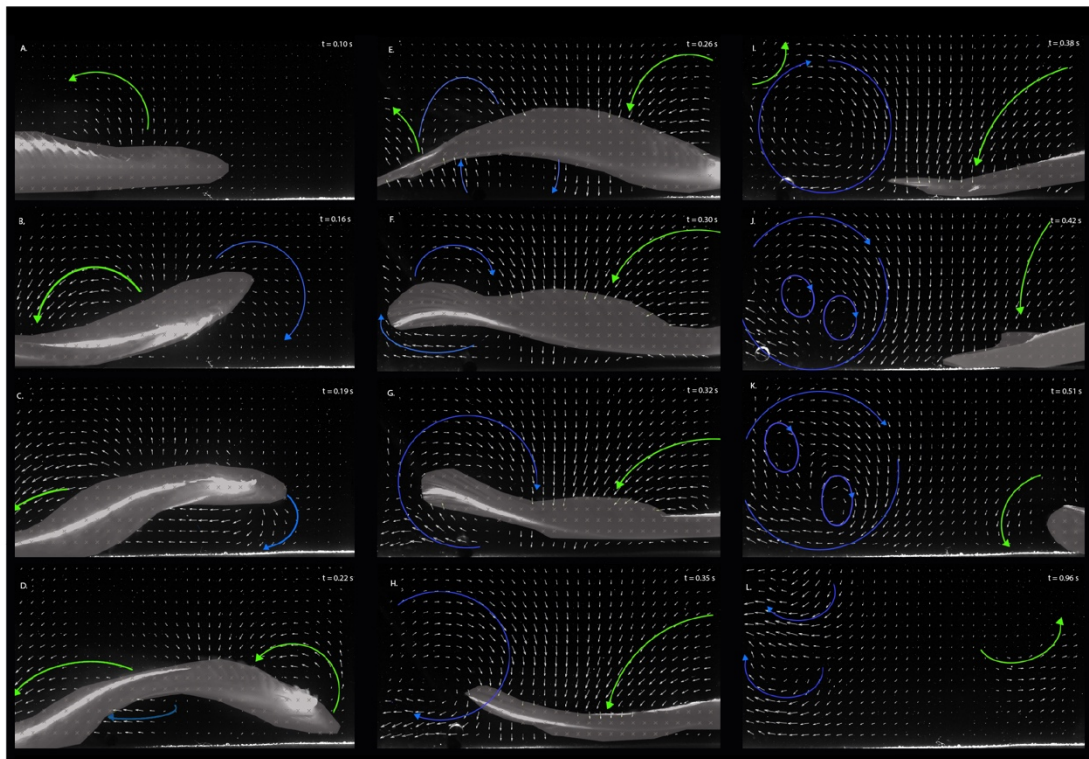


Figure 3.7. **Sequence of vector fields surrounding swimming *Trinectes maculatus* show proto-vortices forming in regions of high and low pressure along the undulating body and vortex shedding in the wake.** White arrows visualize magnitude and direction of fluid movement. Semi-circles indicating proto-vortices and circles indicating vortices are drawn in blue for clockwise flow and red for counter-clockwise flow.

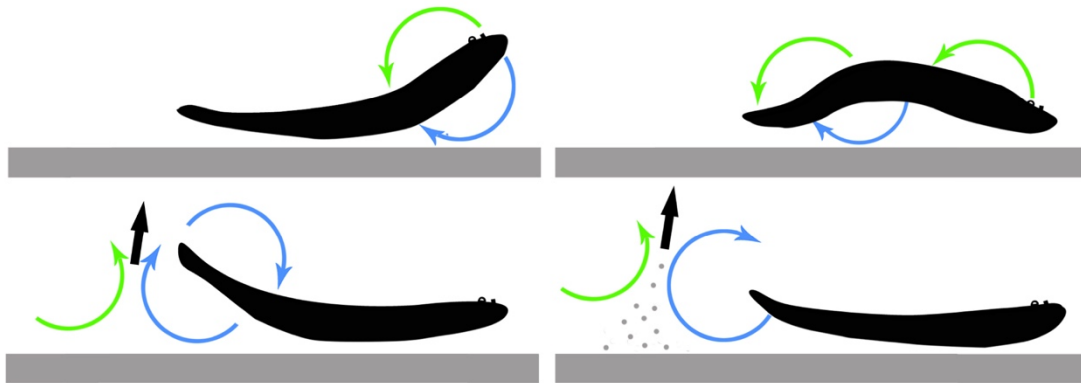


Figure 3.8. **Schematic illustration of flow around *Trinectes maculatus* swimming close to the substrate.** Opposite-rotating proto-vortices form above and below the undulating body and are shed as vortices in the wake. The turbulent flow lifts sediment up from the substrate and increases turbidity. Semi-circles indicating proto-vortices and circles indicating vortices are drawn in blue for clockwise flow and red for counter-clockwise flow.

Walking Hogchokers, in contrast, disturb the fluid very little (Fig. 4D-F; Fig. 5D-F; Fig 6D-F). To initiate a bout of walking the flatfish raises the head slightly off the substrate—likely to reduce adhesion—and steps forward, forming a small bow-wave. Because the flatfish is moving at slow speed the momentum imparted to the water is minimal. The head does not make large up and down movements during walking and there is low velocity magnitude and low vorticity in the bow-wave region (Fig. 3.6D), as well as along the body and in the wake (Table 1; Fig. 3.5D-F; Fig. 3.6E-F). A thin region of shear flow forms in the boundary layer between the body and surrounding fluid (Fig. 63.E-F). Walking flatfish have little to no body undulation and proto-vortices are not apparent as they were during swimming. The tail lifts slightly off of the substrate during some stride cycles (Fig. 3.4F; Fig 6F) and stays in contact with the substrate during

others (Fig. 3.3.5F); in both cases the fluid behind the fish is minimally disturbed and there are no clear vortices or persistent trail left in the wake.

4.0. **Discussion**

4.1. Benthic walking in flatfishes is hydrodynamically cryptic

We found benthic walking in Hogchokers, *Trinectes maculatus*, produces minimal hydrodynamic signals, a phenomenon termed hydrodynamic crypsis. Hydrodynamic crypsis enables the fish to move stealthily through its aquatic environment. Swimming produced a distinct hydrodynamic foot-print of vortices behind the fish that was entirely absent during walking (Figs. 3.5 and 3.6). Measures of water disturbance (mean velocity magnitude) in three flow regions – the bow-wave in front of the fish, along the body, and in the wake – differed significantly (Table 1). The greatest difference was found in the wake, where a strong backwards flow (Fig. 3.4) and trail of vortices (Figs. 3.5 and 3.6) was produced by flatfish during swimming, and not during walking.

4.2. Characteristics of the hydrodynamic flow during walking and swimming

Flatfishes can initiate swimming using opercular jetting to reduce adhesion to the substrate, particularly in escape responses (Brainerd et al., 1997). Once they are swimming (schematic Fig. 3.8) they undulate their body producing regions of high and low pressure. Resulting flow forms proto-vortices, or regions of increased vorticity, that are posteriorly-directed in opposite-rotating flow along the top and the bottom surfaces of the body. Negative or clockwise-rotating vortices were shed from the trailing edge once per tail-beat and were joined by counter-rotating vortices shed from the top surface of the body or induced in the surrounding flow. Hogchokers voluntarily swam close enough to

the substrate to impact the flow on the bottom side of the fish, likely benefitting from ground-effect. Comparing the kinematics and hydrodynamics of flatfishes to carangiform and anguilliform swimmers is an interesting challenge because flatfishes have characteristics of both. The mode of swimming used by flatfishes, with specific amplitudes that increase continuously from nose to trailing edge, is a pattern characteristic of a more anguilliform species (Webb, 2002). However, unlike eels, flatfishes have a flat, wide, plate-like body form that allows them to “surf” above the substrate. Like many other flatfishes, Hogchokers have a distinct caudal peduncle characteristic of carangiform swimmers (e.g., Bluegill Sunfish, *Lepomis macrochirus*; Drucker and Lauder, 1999). However, swimming flatfishes do not produce thrust by oscillating the caudal fin separately from the body and lack pectoral fins; in that way, they are more similar to anguilliform swimmers, which produce thrust continuously with their body (Müller et al., 2001). Anguilliform swimmers produce a more complex wake, with each tail beat shedding at least two pairs of vortices and no significant downstream flow, compared to carangiform swimmers, which have two staggered vortices per tail beat and a strong jet (Drucker and Lauder, 1999, 2002; Tytell et al., 2004; 2010). Hogchokers appear to leave a wake more similar to the staggered vortices model of carangiform swimmers because we observed single vortices being shed with each half-tailbeat and a strong backwards flow behind the fish. Additional study with 3-D DPIV methods would help to resolve this comparison.

4.3. Relevance to predator detection

Piscivorous fishes including Northern Stargazer, Southern Stingray, Clearnose Skate, Spiny Dogfish (VIMS Multispecies Research Group, 2020) and piscivorous birds

such as the Little Blue Heron (Rodgers, 1982) prey on Hogchokers. Flatfishes provide up to 30-33% of seal diets (Hammond et al., 1994; Lance and Jeffries, 2009) and 79% of cormorant diets (Leopold et al., 1998). Burying behavior and skin camouflage are adaptations that help flatfishes evade visual predators. Predatory fishes and seals use their hydrodynamic sense to detect and localize swimming prey fishes by tracking hydrodynamic trails left behind when they swim; benthic walking, which is hydrodynamically cryptic, may be another class of anti-predator adaptation. Future work on responses of different potential predators to benthic walking and swimming flatfishes will be needed to confirm hydrodynamic crypsis.

4.4. Relevance to prey capture

Benthic walking in Hogchokers is a relatively slow locomotor mode as it is in other flatfishes (Wirtz and Davenport, 2017, Fox et al., 2018) and walking produces a bow-wave with reduced velocity magnitude compared to swimming. A slow-ram strike speed reduces bow-wave induced flows and may contribute to the ability of predatory fishes to surprise hydrodynamically sensitive prey (Holzman and Wainwright, 2009). The polychaete *Nereis (Hediste) diversicolor* responds rapidly to mechanical and visual stimuli (Evans, 1969) as well as chemical cues from fishes dispersed in the water (Schaum, 2013) by withdrawing into its burrow and reducing emergence. Reflexive withdrawal of clam siphons, extended during filter feeding, is controlled by fast-acting nerve pathways (Mellon 1965). Amphipods decrease their movement when exposed to chemical cues of macerated conspecifics (Sehr and Gall, 2015). Minimizing water disturbance by benthic walking rather than swimming may slow the dispersal of prey-alarming scent cues generated by the Hogchokers and its feeding activity. Compared to

swimming, benthic walking allows a Hogchoker to approach benthic prey with a reduced visual profile (flattened against the substrate) and a reduced magnitude bow-wave, which, given the reaction speed and sensitivity of its prey to pressure and chemical cues, is advantageous.

Benthic walking facilitates pause-travel predation in flatfishes. Pause-travel predation (Andersson, 1981) is characterized by low speed, frequent turning, and short move and attack lengths, movement patterns documented in *Parophrys vetulus* (Fox et al., 2018) and juvenile *Pleuronectes platessa* as they locate and nip the siphons from buried clams (Ansel, 1995; Hill et al., 2000) or capture worms and amphipods (Holmes and Gibson, 1983). *Pleuronectes platessa* search for prey using a “shuffle” up to 10 cm s⁻¹ and approach prey using a slow “creep” 1 cm s⁻¹ or less (Holmes and Gibson, 1983). Feeding behavior of the Common Sole, *Solea solea*, a nocturnal predator of sessile benthic prey with morphological and behavioral similarities to the Hogchoker, has been well studied. Sensory papillae on the snout and underside of the head of the Common Sole, that resemble those of the Hogchoker (Fig. 3.1C) – once thought erroneously to be chemosensory – appear to protect a protrusion of superficial neuromasts clustered around the naris (Appelbaum and Schemmel, 1983). Foraging behavior of the Common Sole is induced by the onset of darkness (Kruuk, 1963), and they locate prey using a combination of mechanoreception and olfaction (De Groot, 1969, Appelbaum and Schemmel, 1983). In the presence of live prey, Common Sole cease swimming, reduce their activity level, and forage by moving their head side to side, pivoting on the substrate (Batty and Hoyt, 1995), and tapping their head against the surface (Maia et al., 2009), bringing their head in close sensory contact with their prey (Holmes and Gibson, 1983). Benthic walking

allows flatfishes that specialize on epibenthic and infaunal prey to approach slowly, with less water disturbance to alert prey, and use their mechanosensory and olfactory senses in close proximity to their prey.

4.5. Additional potential advantages of benthic walking

Diverse fishes independently evolved the ability to walk along the bottom, from lungfishes (King et al., 2011) to frogfishes (Dickson and Pierce, 2019). In addition to providing hydrodynamic crypsis, benthic walking may be energetically advantageous. Theoretically the metabolic costs associated with fish swimming should be lowest at intermediate cruising speeds and follow a U-shaped curve (Di Santo et al., 2017). The aerobic costs of swimming by *Pleuronectes platessa* are highest at 0-5 BL s⁻¹ (Duthie, 1982), the locomotor speed range within which benthic walking has been observed in other species of pleuronectid flatfishes (Fox et al., 2018). In kinematic tests, *P. platessa* held station on the bottom and only began to swim at current speeds > 25 cms⁻¹ (Webb, 2002); Arnold and Weihs (1978) described *P. platessa* assuming a “clamped-down posture” to adhere to the substrate at low current speeds, and as the current increased, gradually switching to “posterior-fin beating response” with successive movements of fin rays on the dorsal and anal fins (Arnold and Weihs, 1978: p. 148), a locomotor mode that we equate with benthic walking (Fox et al. 2018). Analyses of energetic costs of swimming – including anaerobic metabolism – in the Clearnose Skate *Raja eglanteria*, a negatively buoyant flat-bodied benthic fish, substantiated the U shaped curve for metabolic cost, with higher costs at lower speeds attributed to more energy needed for postural equilibrium and to counteract induced drag (Di Santo et al., 2017). Although it appears that flatfishes benefit from ground effect when swimming at slow speeds close to

the substrate (Webb, 2002) energetic studies to date suggest that swimming at low speeds is still relatively costly for flatfishes. Neutrally buoyant fishes must expend energy simply to maintain position in the water column (Lauder and Madden, 2007). The ability of flatfishes to rest immobile on the substrate and use an alternate locomotor mode – walking – at lower speeds is likely to be energetically advantageous. Future work on the energetic costs of benthic locomotion in fishes is needed to evaluate this potential benefit.

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CHAPTER 4

IMPACT OF A MOBILE SCIENCE PROGRAM ON PHYSICS ATTITUDES AND ENGAGEMENT ¹

¹ Claire H. Fox and Bruce V. Lewenstein

Abstract

A mobile physics program serving primarily small cities, towns, and villages in New York State (USA) was evaluated for its impact on students aged 8-13. In countries where student interest in STEM careers is declining and equity and diversity in science has made slow gains, informal science programs have the potential to engage underserved audiences and change attitudes; however, claims of positive outcomes need to be tested. We investigated student attitudes and responses to the “Physics Bus” program using surveys and focus group interviews. The 120 students in our study indicated low levels of awareness of Physics as a subject before the program. After the program we found increased positivity and enthusiasm for Physics, increased interest in discovering how things work, as well as confidence in doing this type of science themselves. Although there was little evidence of specific knowledge gain, we did find evidence that the program activated wonder, curiosity, and interest in learning more. An emergent theme was the appeal of the “Do It Yourself” style of the interactive science exhibits, in which inexpensive and everyday materials assembled with duct-tape and hot-glue are used to explore physics phenomena in surprising ways. Informal science programs like this one, where Physics is perceived as relevant and fun, can help shift the perception of science as difficult, boring, and “not for me”.

Introduction

Mobile Science Labs are uniquely positioned to provide active, hands-on science to under-resourced rural and urban youth and communities (Franzblau et al., 2011; Jones & Stapleton, 2017), providing equitable access to the types of positive early childhood experiences that spark interest in science careers (Maltese et al., 2010, 2014). Attracting more students to STEM fields is critical to maintaining a globally competitive economy - in the past decade growth in STEM jobs in the United States (24.4%) was much faster than non-STEM occupations (4%), a trend that is predicted to continue (Noonan, 2017). Increasing equity and effectiveness of STEM education can help address gaps in workforce demands, create a more scientifically literate populace, and increase the representation of women, Blacks, and Hispanics in STEM jobs (Morrell & Parker, 2015). Although much emphasis has been placed on improving STEM Education in schools (National Research Council, 2011) there is growing acknowledgement that out-of-school experiences play a significant role in developing interest in science and are well suited to broadening participation in STEM (Bell et al., 2009; National Research Council, 2015). Evaluation of informal STEM experiences is an inherently challenging (Allen & Peterman, 2019) but necessary endeavor, to test claims of efficacy and impact (Banerjee, 2017) and understand the mechanisms behind any positive outcomes.

Informal science experiences outside of the school classroom have been shown to have a strong association with interest in, and pursuit of, STEM careers (Dabney, 2012; Simpkins et al., 2006). How does interest start, and how is it sustained? Motivational research posits that novel, surprising, and intense situations can trigger interest in individuals who have no previous exposure or prior personal interest; according to

expectancy-value theory if a subject is perceived as personally relevant, useful, and meaningful, over time short-term situational interest is more likely to advance to long-term individual interest (Hidi & Renninger, 2006); and that interest development process can be supported (Renninger & Hidi, 2002). Interest and identity formation in science is a complex variable that is shaped by an interplay of factors (Regan & DeWitt, 2014) including parental beliefs (Perera, 2014), culture (Coburn & Aikenhead, 1997), media (Steinke, 2017), informal science experiences (Dabney et al, 2012), classroom curricula (Häussler & Hoffman, 2002), science teachers and the ways they teach and treat students (Xu et al., 2012). Attitudes towards science are complex: interest in specific science subjects varies, and attitudes towards science in general are often different from attitudes towards ‘school science’ (Osborne et al., 2003). A drop-off in personal interest in science and science careers is broadly observed starting between ages 10 and 14 in developed countries (Jenkins & Nelson, 2005; DeWitt & Archer, 2015). Student interest may be “switched off” by school science (Barmby et al., 2008), in contrast to developing countries where interest in science careers remain high (Sjøberg 2005; Schreiner & Sjøberg 2007).

Of particular concern for personal interest is Physics. Physics has a reputation for being obscure, difficult, and boring. Fascination and curiosity about physical phenomena in early years may be undermined by student’s later classroom experiences, as exemplified by this excerpt from large longitudinal study, from a girl who deeply loved physics in primary school but found that in high school ‘physics got more complicated...It’s too dusty ... too one-tracked, maybe ... Sometimes I think that physics can be seen as very cold’ (Krogh & Thomsen, 2005, p. 296). Krogh and Thomsen’s

metaphor of (cultural) border crossing investigates how student attitudes towards physics reflect the degree of congruency between their home and peer values and ways of being, and the culture of their classroom science – similar to Costa’s conceptualization of science as ‘another world’ (Costa, 1995). The majority of students reported a negative reputation for physics as strange, difficult, or boring; along with physics self-concept and teacher’s treatment of students, the reputation of physics explained 51% of the variation in student’s attitudes towards physics (Krogh & Thomsen, 2005). Many students perceive physics as isolated facts that are disconnected from the real world (Milner-Bolotin et al., 2011). The rigid discourse around physics needs reconfiguration for a wider range of student gender and ethnic identities or it will continue to be rejected as “horrible, difficult, and nasty” by individuals who are not compatible with the currently available dominant scientist identities (Hughes, 2001).

Costa (1993) calls for science courses to be more compelling and relevant, organized around the question ‘How does chemistry (or biology or physics) impact my personal life and society?’ Perceiving the utility of science and the relevance of science to everyday life is strongly and positively associated with the desire to study and work in science (Sheldrake, 2017). Researchers have shown experimentally that when under-performing students with low expectations of success are encouraged to connect science topics with their daily life, their interest and performance increases substantially (Hulleman & Harackiewicz, 2009). These connections may be especially important for traditionally under-represented communities (Dawson, 2018). There may be a limit, however, to what curricular reform can accomplish in terms of encouraging authentic, intrinsically motivated learning experiences and positive science identities within the

context of a school environment that is focused on tests and achievement (Carlone, 2004). The U.S. Next Generation Science Standards (NGSS), released in 2013, are a reform effort designed to increase equity, student engagement and conceptual learning in STEM. A 2019 study of Hispanic/Latino and Black middle school students' self-efficacy in STEM found lower confidence, self-efficacy, and career interest in physical sciences compared to life sciences; no improvement was found in students from NGSS aligned classes (McCormick, 2019).

Informal science experiences can be learner-centered and interest-driven in ways that can be challenging to provide in a classroom environment. Motivation and identity are key elements of the strands of science learning described in the National Research Council's report on *Learning Science in Informal Environments* (Bell et al., 2009). The first strand states that learners will “experience excitement, interest, and motivation to learn about phenomena in the natural and physical world.” The sixth and final strand says that learners will “think about themselves as science learners and develop an identity as someone who knows about, uses, and sometimes contributes to science.” This paper explores whether a particular instance of informal science education – a mobile physics lab – can make the connections between physics and daily life that research suggests are

useful for generating the motivation and identity that lead to longer-term engagement with science.

Program Description

Background and Philosophy:

The Physics Bus is an affordable, accessible, innovative public science program with the mission to re-establish physics as something anyone can do with the stuff around them; whether for fun, utilitarian purposes, or for the beauty in it.

The Ithaca, NY (USA), based Physics Bus program was founded in 2013 by Erik Herman and a small team of coworkers and community volunteers. The impetus for the Physics Bus program grew out of Herman's personal struggles and dissatisfaction with classroom education both as a student and as a teacher – and the joy and accomplishment he felt when tinkering, inventing in the garage, and sharing the wonder of physics phenomena. Herman was inspired to start a science outreach program by his experience traveling with the Arizona Mathematics Roadshow and Physics Factory demo shows. He wanted to combine the reach and charisma of the mobile bus (e.g., Barbagallo, 1997; Fox, 2015) with a program model that allowed visitors a self-directed, hands-on experience. The program was inexpensive to start - the Ithaca-based program began with the gift of a free vehicle, a small vintage schoolbus that had been used as a party bus by a local fraternity, and was subsequently filled with physics exhibits built by volunteers and high school students out of junk. Additional funding from a local Family Foundation supported an upgrade to a larger schoolbus in better condition (2001 International 3800 body, 10 windows on each side, approximately 10.7 meters in length). The idea of using cheap everyday materials was influenced by Brian Jones' Little Shop of Physics

(Colorado State University, 2020), Arvind Gupta's Toys from Trash (Gupta, 2020), Science Toymaker (Harrison, 2020), and the Global Alliance of Community Science Workshops (GACSW, 2020). Similar efforts have been tested elsewhere (García-Guerrero & Lewenstein, 2020). The Physics Bus program received some support from Cornell University during the years 2014-2019 while Herman was employed as a science engagement specialist at the Cornell Laboratory for Accelerator based Sciences and Education. However, the Physics Bus operated during the study period as a separate non-governmental organization (in the United States, usually called a nonprofit organization or 501(c)3 organization after the relevant tax code) and considered itself to be a grassroots, community-based program. The program model has experienced national-level growth. As of May 2020 there are five Physics Buses operating out of Ithaca NY, Tucson AZ, St. Louis MO, Gainesville FL, and Watsonville, CA; the programs operate under a common nonprofit organization and while each one has its own style, they share

similar methods and motivations. This study only evaluated aspects of the Ithaca-based Physics Bus program; hereafter referred to for brevity as “the Physics Bus”.

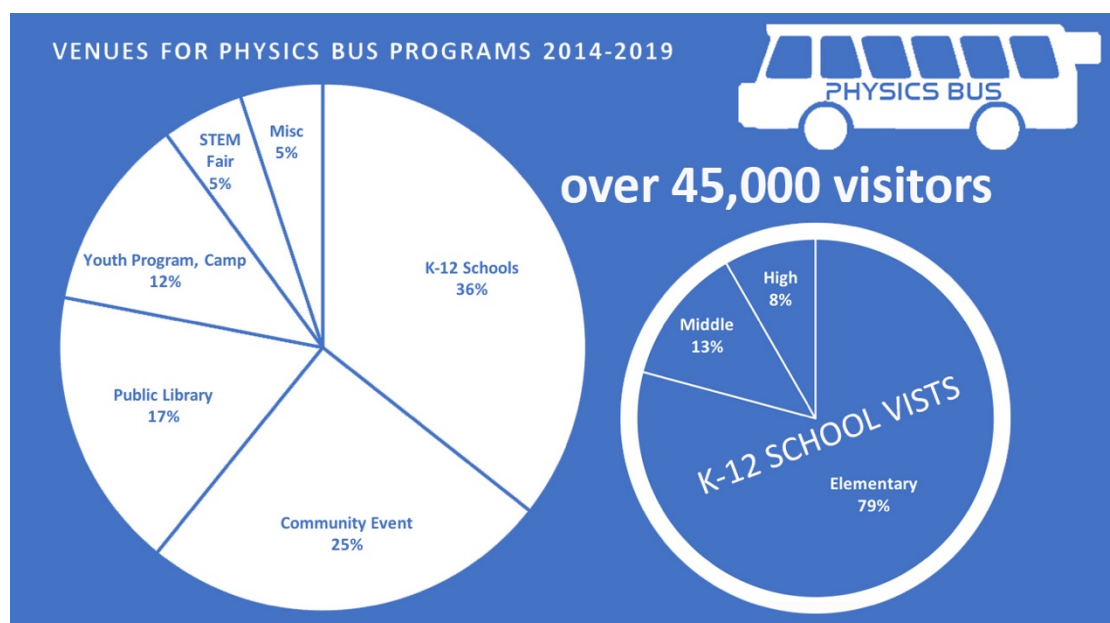


Figure 4.1. Venues hosting the Physics Bus (Ithaca-based program) from 2014-2019.

Population served:

Between 2014 and 2019 the Physics Bus reached approximately 45,000 visitors through a variety of venues (Fig. 4.1). The largest demand for the Physics Bus program came from K-12 Schools (36%) predominantly at the Elementary level, followed by Community Events (25%) encompassing a wide range of seasonal/regional festivals, music events, and block parties. The Physics Bus is a popular request for Public Libraries (17%). A small but significant percentage of programs took place at STEM themed fairs (5%) and the remaining miscellaneous venues (5%) include visits to churches, country clubs, academic conferences, and private events. Most programs were in the Upstate NY region with occasional daytrips and overnight trips, and one or two 7-14 day tours each year in

other parts of the country. The Physics Bus disproportionately serves towns, villages, and small cities with populations under 50,000.

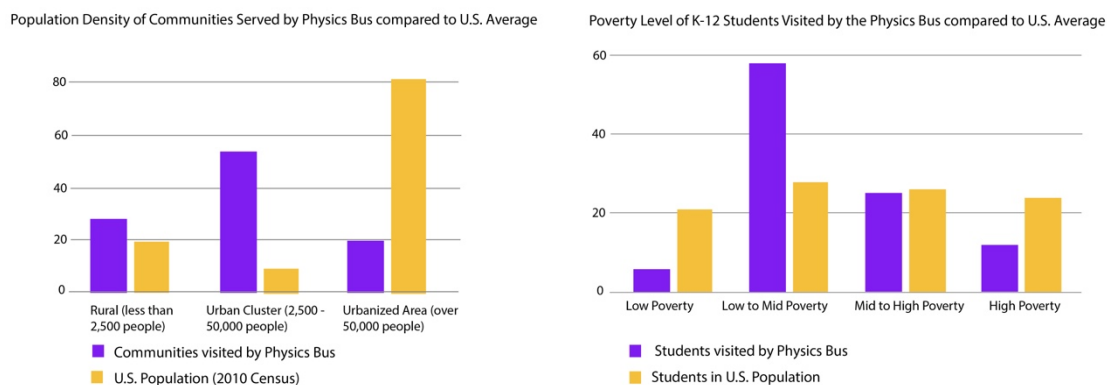


Figure 4.2. Populations served by the Physics Bus program in 2014-2019 compared to the U.S. population. The program disproportionately visited rural towns and small cities. Within school-age populations the program reached more low to mid poverty students than average, and fewer students on the extremes of high or low poverty.

Typical visit experience:

The bus parks in a pre-arranged location and one or two folding tables of exhibits and interactive features are put outside, under a crank-out awning if the weather is inclement (Fig. 4.3).



Figure 4.3. The Physics bus is a decommissioned school-bus covered in aluminum foil, with garbage-can “rocket thrusters” on the back and functioning solar panels on the roof. (1) Tables of exhibits and large interactive physics experiences are placed outside the bus. (2) Inside most of the passenger seats have been removed and the remaining seats form a bench and café table with exhibits. (3) Two built-in display tables run down either side of the bus. They have storage underneath and a metal countertop which allows the exhibits, with strong magnets embedded in the base, to stay in place during transit.

The front and back door of the bus are open with a set of metal steps out the back. The program consists of around two dozen colorful, homemade physics exhibits on two long counters down either side of the bus interior and at a couple of outdoor stations. The

counters are surfaced with metal and the exhibits are attached with magnets allowing transport with the exhibits in place. Solar panels on the roof of the bus and a large array of batteries provide power to exhibits and interior LED light strips. If it is an event with scheduled groups such as a school visit, a program representative (usually doubling as the driver) greets the group, introduces themselves, and may choose to give a short 1 minute introduction. They oversee the visit for safety, repair and refill exhibits as needed, and field any questions. In the early years of the program 2-5 trained volunteers (generally teens from a charter school or college students) accompanied an adult staff member, but recently the program has used a single facilitator/driver and requests that the venue provide helpers. The visits in this study had one staff member and one or two trained volunteers. The interior space, behind the entry stairs and driver's seat, is approximately 8 meters in length. Twenty small children or 15 teens/adults fit comfortably onboard at once and at large public events the bus fills shoulder-to-shoulder. Visitors move at their own pace around the interior encountering exhibits, interacting with them by poking buttons, singing, swinging, dropping, drawing, cranking, with simple signs (that are often ignored) providing basic instructions like "push down here." It is a noisy, active environment - there is a lot of laughter, shrieks of excitement, people often call their friends or family over to what they just experienced to share, laugh, and wonder. Outside

the bus visitors try out more exhibits, make giant bubbles, launch water rockets, get bottles stuck on the roof, and run around and play.

Expected outcomes

Expected outcomes based on extended conversations with the program's founder and director over a period of years (and confirmed for this study) are that visitors will:

1. Experience joy, wonder, and excitement in association with physics
2. Feel increased curiosity and an intrinsic desire to learn more
3. Gain an intuitive understanding of a variety of physics phenomena
4. Become aware of the relevancy of physics in their daily lives
5. Be inspired to tinker with electronics and mechanics and explore science at home
6. Believe that science is something they can engage in – that it can happen outside of academia and be done by everyday people with free materials.

Although this list of expected outcomes was developed inductively, we note that many of them map onto the “strands of learning” from Bell et al., (2009).

Methods

For this study we obtained Institutional Review Board approval for human participant research from Cornell University's Office of Research Integrity and Assurance, protocol #1903008709.

Recruitment

In Fall 2018 and Winter 2019 we contacted Elementary and Middle Schools within a 2 hour radius from Ithaca, NY that receive school-wide Title 1 funding (a federal aid program awarded to public schools in the United States with high percentages of low-

income students). Schools were offered a free program in exchange for participation in the study. Previous visit logs were consulted to ensure that the schools and communities considered for inclusion had never received a Physics Bus visit prior to the study. One Elementary School and one Middle School agreed to participate. The Elementary School is in a city with a population under 50,000; it is classified as a high-poverty school and performance on state standardized tests are far below average. The Middle School is in a village with a population under 2,500; it is classified as a mid-high poverty school and performance on state standardized tests are close to average. Parent consent forms were sent home with students prior to the visit. Students who turned in parent consent forms also provided verbal assent prior to participating in the paper survey or focus group. A total of 120 students (102 3rd and 5th graders and 18 6th and 7th graders) took the survey, and 12 students participated in the focus group interviews, in two groups of 6. Approximately 70% of visiting students in 3rd and 5th grade participated in the survey. Schools were asked to randomly select 6 student participants, from the sub-set with parental permission, to take part in the focus group interviews. We do not know what specific methods the teachers used to select students, thus the sample was neither representative nor random; rather, the focus-groups allowed the researchers to explore questions raised by the study in more depth. Students who participated in the focus groups reported a range of school science achievement.

Demographics

Student eligibility for free or reduced-price lunch (FRPL) was used as a proxy measure for the concentration of low-income students in a school, following the categorization of the U.S. National Center for Educational Statistics: High-poverty schools are defined as

public schools where more than 75.0 percent of the students are eligible for FRPL; mid-high poverty schools are those where 50.1 to 75.0 percent of the students are eligible for FRPL; mid-low poverty schools are those where 25.1 to 50.0 percent of the students are eligible for FRPL; and low-poverty schools are those where 25.0 percent or less of the students are eligible for FRPL.

We applied U.S. Census Bureau data to categorize program locations: ‘Rural’ encompasses all population, housing, and territory not included within an urban area. ‘Urban’ is subdivided into Urban Clusters (UCs) of at least 2,5000 and less than 50,000 people, and Urbanized Areas (UAs) of 50,000 or more people.

Instruments and Analyses

We used a mixed-methods approach utilizing surveys, focus-group interviews, and observation.

Survey: All students with permission (both parental consent and child assent) were given a one-page paper pre-survey immediately prior to the program, and a one-page paper post-survey immediately after the program. The survey was developed specifically for this study. The pre-program portion consisted of two short answer questions and six 5-point Likert scale questions. The post-survey portion consisted of three short answer questions, one yes/no/maybe question, and four 5-point Likert scale questions. The Likert scale questions had verbal prompts and a smiley face visual analogue scale. The survey was kept short in order to minimize disruption to classroom teaching time, and to reduce survey fatigue especially for the younger Elementary students. Short-answer questions were included to allow students unlimited expression in their understandings and feelings about Physics, what they liked most and why, and the thoughts and questions raised by

their experience. Short-answer responses were transcribed as written (preserving spelling and grammatical errors, which were at times extensive enough to require interpretation) and went through two rounds of coding. Emergent categories were developed during the first round, which were then split, merged, reconsidered and renamed as needed during the second round. Some short answer questions included here were edited for spelling.

Interview: The focus group interviews were 45min in length and were conducted at the end of each visit day. The interviews were semi-structured, with questions exploring the student's attitudes towards school science, future science careers, and science in general, their thoughts and reactions to the Physics Bus program, what they liked and disliked about the experience, and whether they gained any new understanding of physics phenomena. The focus group interviews were audio-recorded at the time and later transcribed without names or identifying data, and the audio files were then deleted.

Observations: One author of the study, CHF, observed K-12 student and public interaction with the program in her role as employee and volunteer for the Physics Bus from 2014-2018. These observations were combined with the survey and focus group results to identify the primary factors responsible for increased science interest and enthusiasm.

Results

Background levels of Interest

To provide a background level of interest before the program, students reported their interest in a range of activities on a five-point Likert scale: Make arts or crafts, Explore how things work, Play a sport, or Read a story. Third and fifth graders loved Making arts and crafts more than any other activity (66%), followed by Sports (60%) and Exploring

how things worked (51%). Reading was by a large margin the least-loved activity (34%) as well as the most hated, with 16% of students reporting that they either disliked (7%) or hated (9%) reading. Sixth and Seventh grade students loved Sports (75%) most of all, followed distantly by Making art or crafts (31%). Only 17% loved Exploring how things work. Again, Reading came in last with only 7% of students loving the activity, and nearly 47% hating it.

Focus group participants were interviewed about what kind of science they liked, whether they liked science in school, or were interested in becoming scientists. Reflecting national and international data and as expected based on the literature review (Jenkins and Nelson, 2005; DeWitt and Archer, 2015) the Elementary students were generally positive about science. Every student was able to quickly name a science topic that they enjoyed, e.g. learning about animals ('I like doing research on animals, that's what we're doing in science, and I really like it'), engineering and making things, and learning about the universe (because it is 'so mysterious'). Some of their school science experiences were positive, others were negative – they all vigorously agreed that learning about the weather 'was so boring... because we would read the same thing over and over again. It was just memorizing.' Two students who felt that they would not be able to be scientists when they grew up explained why: one said they were 'bad at memorizing' and the other lamented 'I have a lot of ideas... but I don't think I would be very good at making them a reality... because when someone tries teaching me something, like my brother, when they're not showing me, and they're just talking to me, I really don't get it.' They reflected a belief that science is a transmissive subject where achievement is dependent

on factual knowledge and ability to learn from lecture. In the terms of the Bell et al. (2009) strands of learning, these students did not identify as science learners.

Middle school students in the focus group expressed mixed interest in science. None participated in science outside of school, and none had met a scientist before. When asked if they liked science in school, they replied with a range of responses from ‘not really’, ‘for the most part’, and ‘depends’. One student said it ‘can be very boring’ and another said that they ‘look forward to the hands-on parts.’ One student felt that she could become a scientist because she had very good grades in science ‘it takes a lot of stuff though, like needing to study in school, a lot of effort.’ The others were not interested in science careers, because they were ‘not good at it’ or because it took ‘too much work.’

Awareness of Physics as a Science subject

The majority of 3rd and 5th grade students (51%) and 6th and 7th grade students (54%) surveyed had no idea when asked ‘What is Physics’, with answers such as ‘IDK’ and ‘I don’t know what Physics are?’ Of the Elementary students who had an idea of what Physics was, 26% indicated that it had something to do with science e.g. ‘it is some kind of science’, and 16% identified one or more correct Physics topic e.g. gravity, motion, energy, or how things work. Survey results from middle school students indicated that 40% knew that it was some kind of science; 13% thought it had something to do with movement e.g. ‘physical stuff that you do every day’ suggesting a possible confusion with Physical Education, exercise, or using your physical body.

Enthusiasm and positivity towards Physics

After students participated in the Physics Bus program, we found increased enthusiasm and positivity towards Physics as a subject, with the greatest gains observed in the

Elementary age group. Positivity was defined as the voluntary use of positive words (e.g., awesome, amazing, fun, cool, like it, or excited) when writing in their own words what they thought of Physics. For 3rd and 5th grade 30.5% used positive words to describe what they thought of physics before the program; Post-visit this increased to 78%. Post-visit 29 elementary students specifically used the word ‘Fun’ to describe Physics, an increase of 21 students. Only one student used the word ‘Awesome’ to describe Physics pre-program; 11% of students described Physics as ‘Awesome’ after the program. Middle schoolers in the focus group, when asked to describe their Physics Bus experience to a friend, all expressed a positive impression, e.g. ‘fun and interesting’, ‘I’d say it was cool’, and ‘a fun experience and I did a lot of learning...about what Physics was and how we could use it in our daily lives and how we can use it with different scraps’.

Favorite exhibits and why they were engaging

Students favored physics exhibits that multiple people could explore at once, and that had surprising results or complex interaction (Figs. 4.4 & 4.5).



Figure 4.4. Favorite exhibits in order of popularity: 1) Water bottle rockets 2) Giant Bubbles 3) String Loop 4) Mini-plasma Cutter 5) Van de Graaff 6) Magnet TV 7) Fresnel Lens Helmet 8) Laser writer

When asked which exhibit was their favorite, two were picked 3-4 times more frequently than any others: Water Bottle Rockets and Giant Bubbles. Other popular exhibits were a mini-plasma cutter used to cut aluminum foil, a vacuum cleaner that blew a string loop, and a Van de Graaf machine that built up a charge and shocked people. Also mentioned were a Fresnel lens mounted on a recycling bin that is worn like a helmet, a magnet dangling in front of a Cathode Ray Tube color TV, a ‘spy screen’ made from an LCD monitor with the outer polarizing filter removed, a tornado produced by water vapor

pulled upward by an old computer fan, a laser writer made from a blue laser pointer and glow-in-the-dark paper, an electromagnet ring jump machine, and a mini-vortex ring shooter made out of a cool mist humidifier covered by a Frisbee with a hole.

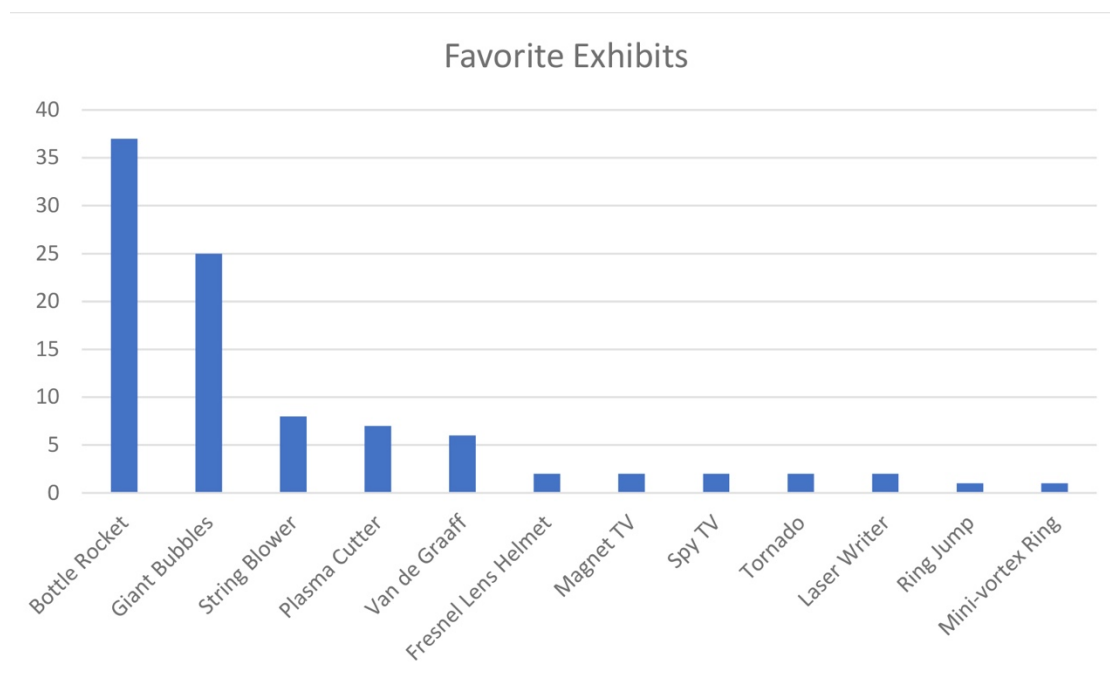


Figure 4.5. Twelve most popular exhibits and the number of students who chose them as their favorite

Students gave a range of answers for what they liked about their favorite exhibit. Aside from saying that they liked ‘everything’ about it, they most often noted the impressive height or size (21%), e.g. ‘I liked them because the rocket amazed me on how high it went.’ ‘I had to hold the bottle and it went soooo high.’ They also liked that it was fun or funny (17%), e.g. ‘it was awesome and really fun to do’, ‘I liked that it was very fun and we asked really funny questions.’ Students liked that it was made of junk (9%; see section in results on “Everyday materials and DIY aesthetic). Some liked the memorable or intense physical sensation ‘getting electrocuted and hurt that’s why’, ‘it went through my body.’ Other students liked it because it was an interactive (8%) or social (7%)

experience ‘when people came by you shot them with string,’ ‘I like it because if you touch someone they get electric,’ or ‘playing around with friends with the bubbles.’ Some students appreciated the beauty of some of the physics phenomena ‘I liked all of the cool color.’ Other students liked the unexpected ‘I like that everything surprised you.’ Several liked the variety ‘that there are a lot of things to do.’ Lastly, the students appreciated having freedom ‘I liked that you could write anything on it’ and ‘we got to go off by are self’s.’ Students liked the idea of science that is self-initiated and experimental. As one focus group participant explained, ‘I am not up to the science that people are like...all the things they know I know, like this is how you do slime...I like science where you just come up with it in your mind and you’re like, ‘this might be science, why not do it?’

Student interest and confidence

After participation in the program, students made large gains in interest and confidence. Asked on a five point scale how interested they were in discovering how things work, after the visit 80% of elementary students said that they were ‘definitely’ interested in discovering how things worked, a 30% increase from before the program. After the program nearly half of the elementary school students (48%) indicated that they ‘definitely’ could make a science gadget, a 17% increase. The percentage of middle school students who felt that they ‘definitely’ could make a science gadget jumped from 8% before the program to 42% after, and those saying they ‘probably’ could increased from 21% to 50%. As a student in the focus group explained, ‘I never made one before, but I would, if I had like all the supplies and stuff and had all the old and new stuff to like make it.’ Students in both focus groups easily identified what exhibit they would make, and spontaneously shared different ways they’d like to use the exhibits in non-scientific

ways, such as using the lie-detector circuit as a musical instrument to DJ, using the Fresnel lens helmet in a comedy routine, or using the swirling colors of the magnet TV to increase focus and calm feelings.

Student curiosity, wonder, and desire to learn more

Student curiosity, wonder, and interest in learning was activated by the Physics Bus program. When asked whether they ‘would like to learn more about’ their favorite exhibit 87% of elementary students said yes. When asked ‘what did you wonder about when using the exhibit’ there were only two student who didn’t wonder at all. One offered the explanation ‘Nothing...I was so distracted by how cool it was.’ What students wondered about largely fell into two categories: how it was made (39%), and how it worked (34%). Typical responses were ‘how they made it, what junk they used’, ‘that I could make it at home’, and ‘I wonder what materials did they need.’ Students were curious about the specific physics phenomena they observed, such as ‘how the foil burned’, ‘how did the magnet go so smooth thru,’ ‘how when you twist the handle the lit come on,’ ‘how do tornados form,’ and larger questions such as ‘how we get electricity.’ Other students wanted to push the limits of the exhibits and try new things, and wondered what would happen (9%), e.g. ‘how much air could go into the bottle’, ‘how high it could go’ and ‘if we shocked the bubble what would happen.’ Some students wondered about how the program was run: ‘how long it takes to set up,’ ‘how they replace the magnets’ and where the ideas for the exhibits came from,’ ‘how people found out how to make these things’, and ‘how people thought about this stuff.’

Content knowledge

We found that students were applying their previous experiences and reasoning abilities to make sense of the physics phenomena, however we found little evidence that new knowledge or intuition was gained from the program experience. Although the purpose of this study was not to assess student content learning, we did ask a couple of questions of focus group participants to see if they had gained intuition about underlying physics phenomena (one of the expected outcomes of the program experience). Here we report on Elementary student's post-visit understandings of two physics topics that are explored in multiple exhibits on the bus (Fig. 4.6).

1) Three exhibits on the bus visualize the wave nature of sound: an oscilloscope made out of an old cathode ray TV with a microphone attached, a guitar with a spinning strobe disk under the strings, and a transparent tube attached to a speaker with Styrofoam beads that stand up in waves when the sound is adjusted to certain frequencies. After the program, when asked 'If you could see sound, what do you think it would look like?' none of the Elementary students referred to exhibits in their answers. One of the students said immediately 'I think it would just be air waves' which likely reflects pre-existing knowledge, as the waves in the program exhibits were visualized in physical materials. Another said that it would 'look like how it sounds', i.e. 'if you hear a clap you would see someone clap.' A third student recalled what sound waves looked like in a Magic School Bus video (a popular television and online media edutainment show produced by Scholastic Inc.), i.e. little green circles for small noises and large red circles for loud noises. When the researcher prompted the students by asking them what they saw when they plucked the guitar exhibit, one student said, 'I just plucked it, I didn't see anything', another said, 'I saw things moving over and over,' and another reported that when they

‘plucked it more hard it [the strobe drum] would spin faster.’ None of the students in the focus group mentioned wiggly or curvy lines or waves.



Figure 4.6. Physics Bus exhibits provide surprising sensory experience with a range of physics phenomena. Exhibits 1-5 visualize the wave nature of sound. (1-2) Modified acoustic guitar with strobe belt behind the strings. (3-4) Oscilloscope made from an old cathode ray tube TV hooked up to a microphone. (5) Clear tube with small styrofoam beads showing standing waves, connected to a speaker and adjustable tone generator. Exhibits 6-7 feature electromagnetism. (6) Large wire coil connected to A/C power and push button, surrounding a transparent bowl with permanent magnet inside. (7) Classic “Ring Jump” demonstration.

2) At least three exhibits on the bus are based on electromagnets, including a popular exhibit consisting of large transparent plastic bowl with a button in the middle, which controls AC electrical current in a large coil of wire around the outside of the bowl. The

coil of wire is partly obscured by patterned duct-tape holding the coil onto the bowl.

When the button is pushed a magnet wrapped in tape vibrates and bounces around in the bowl. When asked how they thought it worked, most of the focus group students knew that the object bouncing was a magnet and several used words like ‘repel’ and ‘interact.’

Answers that went into more detail were incorrect: ‘there was something in it, like hitting on the top’, ‘the bowl that it was in would make the magnet spin, because it would like tap on one spot and would keep going around.’ One of the students thought ‘maybe it was kind of alive, maybe it wanted to get away from whatever that was.’ None of the Elementary or Middle school students mentioned the coil of wire, or electricity – observations that would be necessary to make an intuitive leap to understanding an electromagnet.

When asked the broad question ‘did you discover anything or learn anything?’ on the bus, a student in the focus group shared that he ‘learned that you can make plasma’ because the ‘guy said that the rod and tinfoil make plasma.’ He had heard of plasma before the program and thought that it was ‘a kind of energy’, but he didn’t realize that the arc on the aluminum cutter exhibit was actually plasma, or that you could ‘just make it,’ until the teenage volunteer on the Physics Bus engaged with him. This suggests that program staff or trained volunteers have the potential to aid the process of knowledge acquisition from the Physics Bus exhibits.

Everyday materials and DIY aesthetic

The use of inexpensive, common materials and homemade nature of the exhibits (Fig. 4.7) made a strong positive impression on students. When asked why they liked their favorite exhibit, some students specifically noted that it was because it was made of

everyday and reused materials: ‘I liked how they used junk and made it so cool,’ ‘It was cool that you could burn stuff with simple objects’, ‘That you can make new stuff out of trash,’ ‘I liked that most of it was made out of trash then it was turned into science.’

When asked how the science on the Physics bus was different than science in their school a student explained ‘I think it’s different because you don’t need to buy the things you need to make a project. You already have the things you need you can just re-use them. You already bought it in the past and you can just save it and use it for something else.’

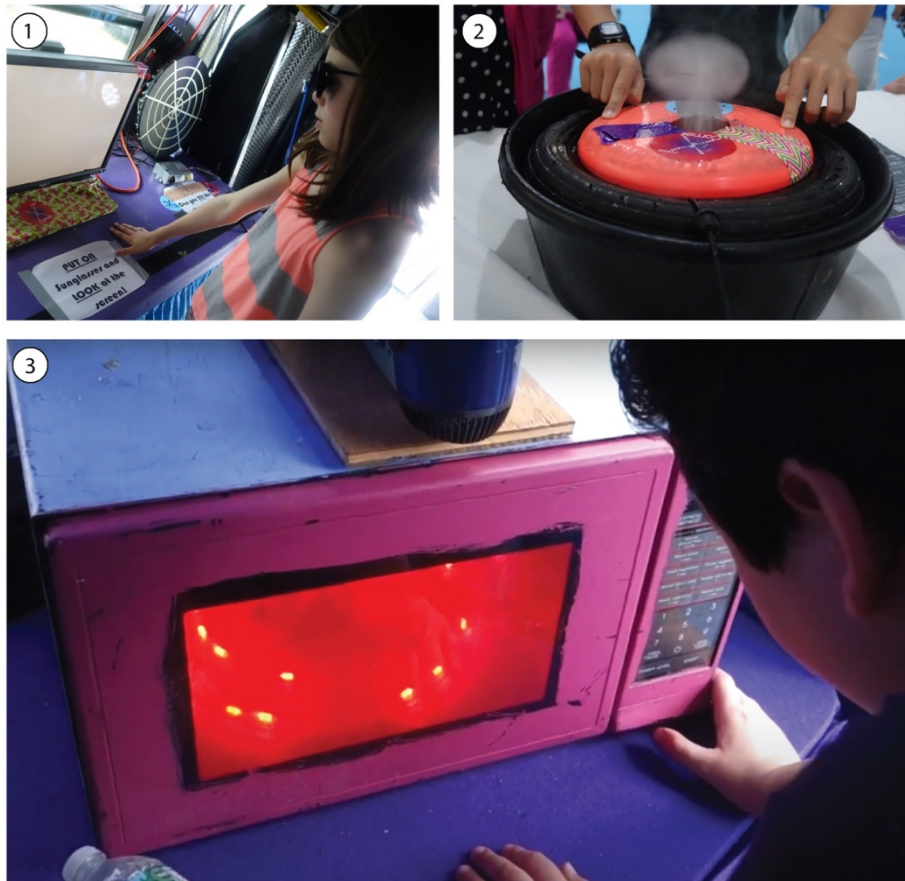


Figure 4.7. Familiar, everyday materials are repurposed to produce physics exhibits. (1) Home-made “spy screen” LCD monitor with polarizing filter removed and polarized sunglasses (2) Vortex ring exhibit made from a Frisbee with a hole in the center over a basin with cool mist humidifier components (3) Microwave oven with small neon bulbs lying on the glass tray

Students wanted more science like this, ‘I want science to be more fun, especially science, which is like something that can be really fun, and the teachers usually want to do science where you go out and buy stuff, where it seems like they really don’t have to, they could just... the stuff they’re buying isn’t really the best, you can get other things just for free.’

Physics Bus compared favorably to school science and other informal science opportunities

The Physics Bus program was exceptionally well rated by students relative to other science experiences. On a five point scale 97% of 3rd and 5th graders, and 92% of 6th and 7th graders indicated that the Physics Bus was either ‘better’ or ‘the best’ compared to other science that they have done.

When focus group participants were asked to share what they thought of the Physics Bus experience, and how it was different from science in school, the Elementary students felt that they learned more because it was fun, and they were able to directly experience science. They were also impressed by the re-use and the simplicity of the materials:

S1: It’s very different because on the Physics Bus you learn more.

S2: Because it’s like science in Ms.X’s class... it’s like ‘eh’... it feels like work, but when you’re actually learning stuff, it’s fun.

S3: It’s different because, like S2 said, we just do working and reading and writing, and on the Physics Bus you actually get to do it, instead of like writing about it.

S4: They are COMPLETELY different because in school you have to be [indistinguishable] the safest thing you possibly can do. And you really don’t do anything because you are usually working in a group. But when you are on the bus you do something and you don’t have to wait.

S5: So its like also more fun, and it's not expensive....you can just find stuff in a dumpster, you can just dumpster dive. So you can, just like he said with the trashcan thing, I was like it was simple to make, you don't need a ton of money.

S6: Something so simple can just be so amazing.

The middle school students appreciated the hands-on nature and freedom to self-guide:

S1: I loved it better...because it had more hands-on experience.

S2: And we had more freedom - instead of telling us we needed to focus on one topic, and one thing, and we had to do all the experiments by ourselves, we could explore the different science topics we learned about for our whole school career.

S3: Yeah, and you get to figure it out for yourself without someone like telling you.

Middle schoolers also recognized the relevance of physics to their daily lives:

R: Did anyone else discover or learn anything?

S1: I learned how important physics is in your everyday life and how much we use them even if we don't notice

R: Did you not know that before?

S1: Because I didn't really think about it. Because everyday we use different things that we are used to using every single day, that we don't think about how there is so much science behind it and how physics is used.

R: So you never realized that.

S2: [another student] I'm usually too busy using the thing, to think about it

R: Does this make you curious about anything that's in your daily life, like how it works?

At this point, students explained how the delivery method (outside the classroom, onboard and around a modified schoolbus) was a critical part of the successful engagement:

S2: I feel like it was like better, because it was outside of like our normal environment, like outside of the classroom.

R: So you liked it because it was outside of your normal classroom? Like if we'd taken all of the exhibits and we'd put them in your classroom, and it wasn't in a bus...

S2: I'd still be attracted to them, but like I'd just be like 'um ok, whatever'

R: So you think the bus makes a difference? What do you all think?

[chorus of agreement]: OUTSIDE.

R: The fact that it's outside and you got to run around? OK

S3:...I feel like if we were in a classroom I'd probably get 20 minutes and then I'd be bored instead it was like an hour

S4: Yes and it's because we've been in the same areas for more than 100 days. Like in the classroom over and over and over again. Even if we were just on the bus, I still feel like since we're in a different area than we are used it would be more fun.

Discussion

The Physics Bus program generated strong interest and enthusiasm in Elementary and Middle School students, with 97% of third and fifth graders rating it “better” or “the best” compared to other science they have experienced. Participants left the program feeling animated, excited, and happy, and described ‘what they thought of Physics’ afterwards as fun, awesome and cool. These responses highlight the importance of including “excitement, interest, and motivation” in the definition of science learning (Bell et al. 2009). Positive informal science experiences like those offered on the Physics Bus counteract the reputation that physics is boring, detached from daily life, and difficult. The experiences develop an alternative narrative that young children pick up on quickly, in only one visit: physics is exciting and fascinating, it is part of our everyday life, and exploring it is intuitive and fun. The quotes above also highlight the degree of self-reflection allowed by Physics Bus experiences (“I’m usually too busy using the thing, to think about it”), as suggested by the fourth strand of science learning: being able to “reflect on...their own process of learning about phenomena.”

Students in our study had low awareness of Physics as a subject prior to the program, suggesting the potential for informal science programs to fill an important gap

in STEM ecosystems. Early childhood is a key time period for sparking interest--65% of physical scientists report that their interest in science began before middle school (Maltese et al., 2014). We found that most students (51-53%) did not have any idea what 'Physics' was before going onboard the Physics Bus, and only 16% showed awareness of what topics physics covers aside from being generally "science" related. As noted above, those numbers increased substantially after visiting the Physics Bus. Lack of awareness of physics in primary school students may reflect their parents and teacher's limited familiarity or interest. Parent interest and attitudes towards science have a significant impact on their children's interest in science (Perera, 2014, Burt & Johnson, 2018), and many parents never studied physics – some from lack of interest, others for lack of opportunity. Nationwide only 63% of High Schools offer physics (U.S. Department of Education, 2014). The percentages of U.S. high school graduates who completed coursework in physics increased from 21% in 1990 to 36% in 2009. The increase is good news, as Physics is required for many STEM careers, but the percentage of high school students taking Physics lags far behind Chemistry (70%) and Biology, which increased from 91% to 96% in the same time period (U.S. Department of Education, 2014). Primary school teachers are an important source for encouragement and inspiration in early childhood, but they broadly lack confidence teaching science, particularly Physics and Engineering. Although 77% feel very well prepared to teach Reading/Language arts and 73% Mathematics, only 31% feel very well prepared to teach Science; this breaks down further with only 13% feel very well prepared to teach grade-level Physical Science and 3% feel prepared to teach Engineering. (Banilower et al., 2018). This is a widespread problem – in the majority of 21 European countries 4th grade teachers have pronounced

lower confidence teaching Physical Science compared to Life Science and Earth Science (Klepaker & Almendingen, 2017). Many of us can recall an elementary teacher happily bringing in tadpoles, identifying birds, sprouting seeds, and similar life-science experiences; how many joyfully took apart electronics to see how they worked, hooked up a hobby motor, or generated a high-voltage arc? When typical home and school environments are not providing encouragement and positive early childhood impressions of Physics, mobile programs offer a way to access those experiences. Though specific physics knowledge was not produced by the experience on the bus, the experience did contribute to greater understanding of the general field and way of approaching the world. These areas fall under Strands 2 and 3 of the National Research Council's (Bell et al., 2009) definition of learning in informal environments (Strand 2: "Come to generate, understand, remember, and use concepts, explanations, arguments, models, and facts related to science"; Strand 3: "Manipulate, test, explore, predict, question, observe, and make sense of the natural and physical world").

Mobile science programs reach audiences that might otherwise be underserved. The Physics Bus program disproportionately served rural audiences – 28% of programs took place in towns and villages of less than 2,500 people, and 81% of programs took place in communities of under 50,000 people, where easy access to science museums, zoos, aquariums, and other informal science institutions is often limited. Recent work in the United Kingdom has highlighted the importance of moving beyond institutions, to address the sense of disconnectedness that underserved audiences feel (Dawson, 2018). By appearing at public schools and free community events the Physics Bus reaches a wide swath of the populace, not just those that are already interested in science. People

attending a Reggae festival or chili cook-off probably do not leave home expecting to have a science experience, but when they spot the Physics Bus they are drawn in by curiosity, peer pressure, or both, and end up having a great time. Organizations sponsoring science events at music festivals have made similar observations (Bisbee et al., 2020). We believe that offering engagement opportunities broadly and for free is an important step towards increasing equity in science. Research has shown that parents offer science opportunities to girls only if they express interest, whereas boys receive science opportunities regardless of their interest (Alexander et al., 2012). How is anyone to know that they like physics if they aren't given opportunities to have fun with it? Ultimately, issues of equity serve the sixth strand of science learning: the ability of learners to "think about themselves as science learners and develop an identity as someone who knows about, uses, and sometimes contributes to science" (Bell et al. 2009).

From our data we identified four primary factors in the Physics Bus's approach to STEM outreach/enrichment that we believe are responsible for the high levels of science interest and enthusiasm reported by students, and observed by the researchers:

- 1) The experience is self-directed. Self-determination theory posits that honoring natural curiosity and giving people the freedom to explore, experiment, and take initiative results in positive gains in intrinsic motivation and well-being (Ryan and Deci, 2000). Similarly, the concept of free-choice learning highlights the importance of individual agency (Falk et al., 2001). Visitors to the Physics Bus have a rich and stimulating environment with many opportunities. They can spend time with whatever they like the most and exploration is self-paced. There is no didactic signage explaining what you

should learn, no required content, no instructors, and no rules. Visitors generate their own questions and apply their own ideas and prior experiences to make sense of what they experience. They are filled with excitement and wonder. After the program many visitors find that they want to learn more and have a keener interest in discovering how things work.

2) Novelty and intensity of the fun. Climbing onboard a transformed schoolbus is, in and of itself, a memorable experience. Once inside, people are amazed and surprised by what they encounter. As one 3rd grader put it, “you are basically in an unknown territory - you don’t know what’s going to happen!” The bright lights, rapidly moving parts, and cacophony of sound produced simultaneously by many physics devices in a small space—most of which are only held together with hot glue and duct-tape—could be chaotic and overstimulating, but nearly all visitors find the experience to be very fun and exciting. Reaching out to get shocked by a friend’s fingertip, feeling a cold mist vortex ring on your face, marveling at the swirling of rainbow colors, feeling and hearing the explosion of pressure from a water rocket. These are the types of novel, surprising, intense experiences that have high potential to spark interest (Hidi & Renninger, 2006).

3) Relevancy. Expectancy-value theory holds that interest is reinforced when a topic is perceived as personally relevant, useful, and meaningful (Hidi & Renninger, 2006; Hulleman & Harackiewicz, 2009). Students were generally very interested in making arts & crafts and playing sports and were less interested (or actively resistant to) figuring out how things worked or reading. The creative, physical experience of science onboard the Physics Bus appealed to them and related to their pre-existing interests. They also were impressed with how it used everyday household materials such as a microwave

oven, pinata, toy keyboard, vacuum, speaker, humidifier, treadmill motor, slinky, foil-covered salad bowl, and hand-held mixer. Scientific equipment comes from “another world” but these items come from children’s home culture, and their relevance is immediately apparent.

4) Authenticity. The unpolished, homemade nature of the exhibits further connects visitors to the world they already know. This authenticity encourages people to believe that they too are capable of scientific tinkering. After the Physics Bus program many more students felt that they would be able to create a science gadget than before the program. When people believe that they can be successful in a given field (in this case, applied physics and engineering) they are more likely to maintain interest and persist (Pajares, 2005; Wang et al., 2013).

These factors suggest that the Physics Bus provides a “third space” or hybrid space where students and community members can apply funds of knowledge (González et al., 2005) and discourse from their home lives. Third-space or hybrid space learning environments support individuals who are traditionally marginalized in formal learning settings (Moje et al., 2004, Barton & Tan, 2008), facilitating the types of cultural border crossing (Krogh & Thomsen, 2005) needed to develop positive science attitudes and self-concept. Providing a space where individuals’ rich funds of everyday knowledge experiences are drawn upon and valued helps to “legitimize multiple ways of participating within the science learning community” (Barton & Tan, 2008).

The importance of these third spaces is highlighted by evidence that anti-science beliefs in the U.S. stem not from a lack of science literacy, but rather from attitudinal and cultural differences (Morgan et al., 2018; Kahan, 2012). Although confidence in science

leaders is relatively high in the U.S. and science as generally perceived as important and beneficial, concerns remain about cooperation from the public during health crises, continued funding for basic research and climate-related R&D, and maintenance for environmental protections (Funk et al., 2019; Besley & Hill, 2020; Scheufele et al., 2020). Instead of focusing on raising test scores, programs like the Physics Bus focus on changing perceptions and attitudes towards science.

Limitations of this Study

This study was based on student responses from eight 3rd and 5th grade classes from one Elementary School and seven 6th and 7th grade classrooms in one Middle school in a rural region of upstate New York, U.S.A. Student recruitment for the study in the Middle School was low resulting in a small sample size for this age group; however, informal program assessment with a larger group of middle school students (data that could not be used for this study) yielded very similar results. Elementary student responses used in the study were consistent with a pilot study conducted the year before with 65 3rd and 5th grade students from a different rural upstate NY school.

Future Directions

Most directly, we would like to see this study extended longitudinally, to find out what students remember about the experience a few years later, how it fits into their developing attitudes towards science and physics. We would also like to hear from children and adults who encounter the program at community events. In some

communities, simple postcards sent home with school students may allow data to be gathered several years after an original experience (Trautmann & Lewenstein, 2009).

In addition to research implications, the value demonstrated by this study also has implications for practice: With grant funding and private support, programs like the Physics Bus could supplement their fee-for-service business model and positively impact more underserved regions and high-poverty schools (Fig. 4.2). One-time interventions can initiate interest but because beliefs are only moderately stable across time, continuous promotion is needed for maintenance (Simpkins et al., 2006). The Physics Bus program could increase long-term impact by pointing interested visitors towards ways to extend their experience such as activities for the home and classroom, afterschool programs, or media.

Ultimately, we would like to see more consideration of recreational and community-based science engagement. Research and program assessment have been heavily focused on the end-goals of school achievement and the professional STEM pipeline. When the learning of lay people are considered, it is often in the contexts of museums (Bell et al., 2009) or in Citizen Science, in which ordinary people contribute data to institutionally based science research projects or participate in community-based research (Pandya & Dibner 2018). We are interested in the ways that non-scientists can (and already do) engage in science and technology related pursuits voluntarily and on their own terms by gardening, hunting, fixing things, tinkering in the garage, and sharing their knowledge and enjoyment with other community members (Avery, 2013; Lewenstein, 2013; Liu & Falk 2013). The founders of the Physics Bus envision a world

in which people of all ages dabble and play with science for the fun and joy of it. In the process, they will pick up skills and understandings that are useful in daily life.

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Competing Interests

The authors of this publication have no financial or competing interests. CHF was a volunteer co-founder and employee of the Physics Bus program from 2014-2016 and volunteered occasionally from 2017-2018. CHF and the Physics Bus directors Erik Herman and Chris Bell served together on the Board of the Free Science Inc. from 2017-2019, a program which received fiscal sponsorship from the parent nonprofit of the Physics Bus, the Physics Factory Inc, from 2016-2018. Data for the study was collected in 2019.

Author contributions

CHF conceived and led design of the study, conducted data collection, analyzed the data, and led writing of the manuscript. BVL contributed to study design and theoretical framing and helped in drafting the manuscript.

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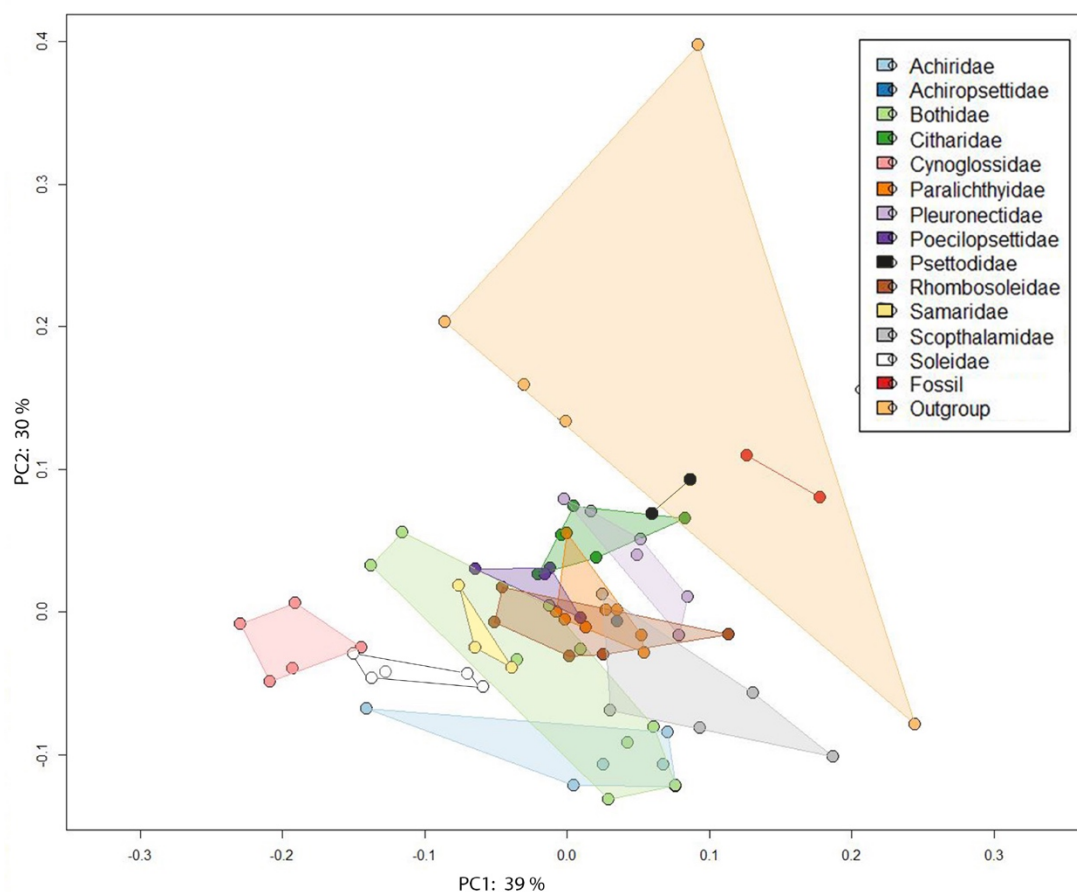
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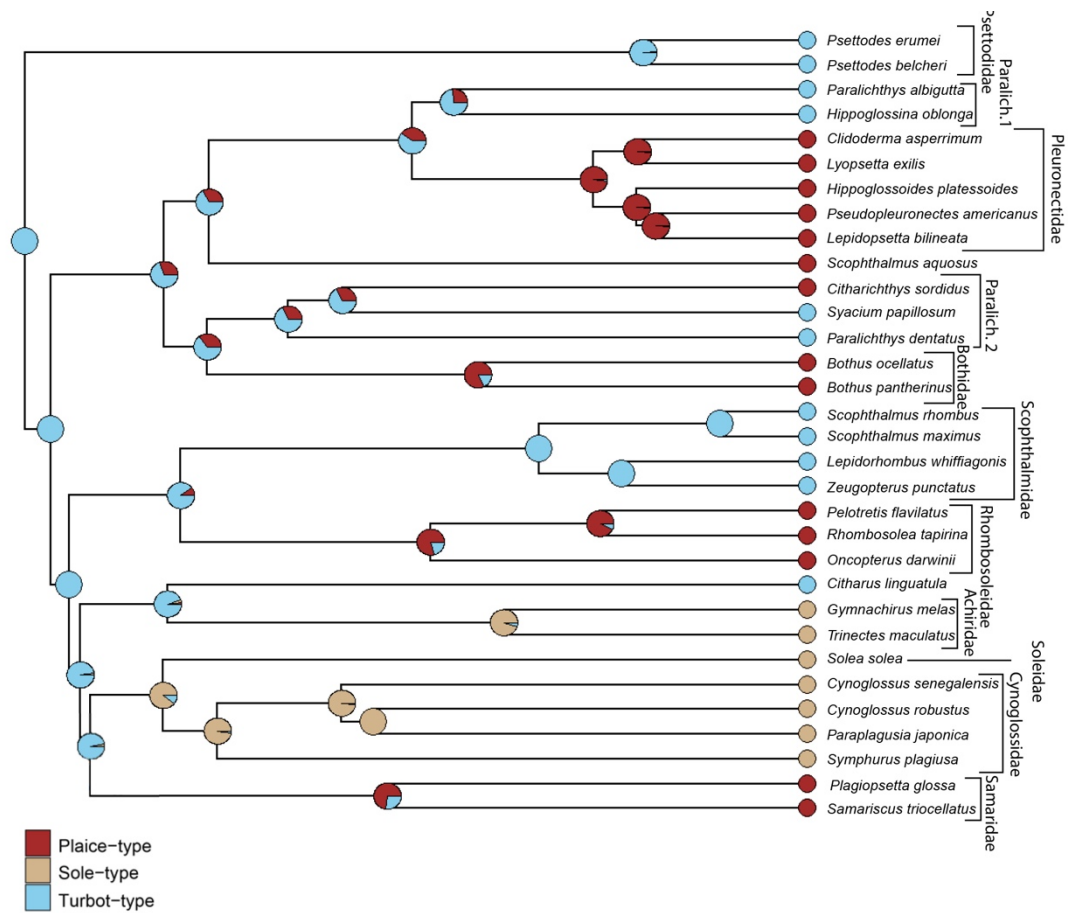
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APPENDIX

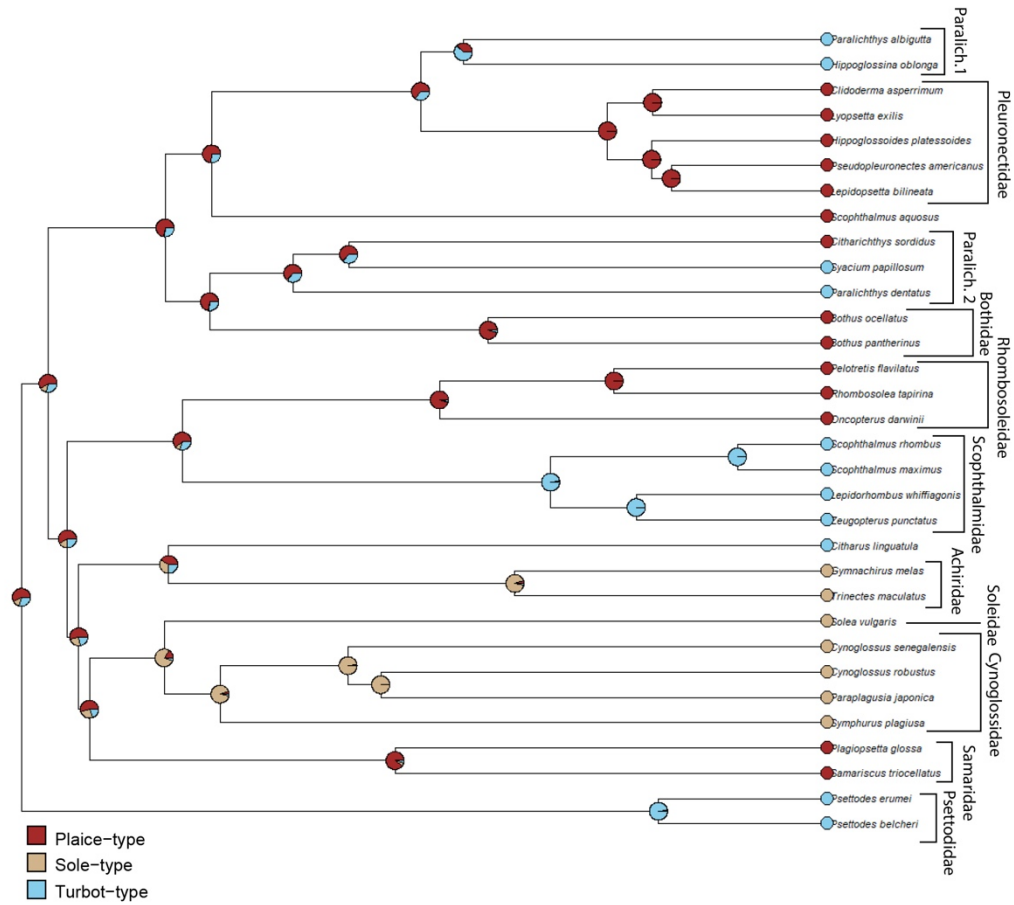
Chapter 1 Supplemental Figures



Supplemental Figure 1. Landmark-based PCA morphospace with a range of carangimorph representatives. Outgroups used: *Coryphaena hippurus*, *Mene maculata*, *Naucrates ductor*, *Leptobrama mulleri*, and *Sphyraena lucasana*. Only the transitional fossil flatfishes (†*Amphistium* and †*Heteronectes*) and Psettodidae overlap with the outgroups in the morphospace. N=71 species.



Supplemental Figure 2. Ancestral state reconstruction of feeding type using an alternate phylogeny (Byrne et al. 2018) that includes members of Rhombosoleidae. As discussed in the text, we used outgroup comparison to fossil flatfishes to establish Turbot-type feeding as the ancestral state for Pleuronectiformes. Turbot-type feeding transitioned to Plaiice-type feeding independently multiple times, including in the lineage leading to Rhombosoleidae.



Supplemental Figure 3. Reconstruction of feeding type (without imposing a prior likelihood at the root-node) infers a Plaice-type ancestral state. There is convergent evolution of Sole-type feeding. Plaice-type transitioned to Turbot-type multiple times in the lineages leading to Psettodidae, Scophthalmidae, and Paralicthidae.

Chapter 1 Supplemental Tables

Supplemental Table 1.1. Specimens Studied and Vertebral Number.

ID	Family	Genus	Species	Vertebral count
USNM_228345	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
USNM_345415	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
USNM_361607	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
USNM_361618_2	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
USNM_423622	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
AMNH_51012	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
AMNH_15902	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
AMNH_15896	Psettodidae	<i>Psettodes</i>	<i>erumei</i>	23.00
AMNH_286357	Psettodidae	<i>Psettodes</i>	<i>belcheri</i>	25.00
USNM_137409	Citharidae	<i>Lepidoblepharon</i>	<i>ophthalmolepis</i>	29.00
USNM_137708	Citharidae	<i>Brachypleura</i>	<i>novaezeelandiae</i>	31.00
USNM_00109495	Citharidae	<i>Citharoides</i>	<i>macrolepis</i>	32.00
USNM_075670	Citharidae	<i>Citharoides</i>	<i>macrolepidotus</i>	32.00
MCZ168314_1	Citharidae	<i>Citharus</i>	<i>linguatula</i>	35.00
MCZ168318_1	Citharidae	<i>Citharus</i>	<i>linguatula</i>	33.00
MCZ168318_2	Citharidae	<i>Citharus</i>	<i>linguatula</i>	35.00
MCZ168318_3	Citharidae	<i>Citharus</i>	<i>linguatula</i>	36.00
MCZ168318_4	Citharidae	<i>Citharus</i>	<i>linguatula</i>	36.00
MCZ168320_1	Citharidae	<i>Citharus</i>	<i>linguatula</i>	37.00
MCZ168320_2	Citharidae	<i>Citharus</i>	<i>linguatula</i>	36.00
MCZ168320_3	Citharidae	<i>Citharus</i>	<i>linguatula</i>	36.00
USNM_409171	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	42.00
USNM_209733	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	40.00
AMNH_72270	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	42.00
AMNH_72270_2	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	40.00
AMNH_72532	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	41.00
AMNH_72672	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	41.00
AMNH_30919	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	40.00
AMNH_72373	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	40.00
AMNH_72480	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	40.00
AMNH_256047	Samaridae	<i>Samariscus</i>	<i>triocellatus</i>	39.00
USNM_137384	Samaridae	<i>Samariscus</i>	<i>longimanus</i>	39.00
USNM_396096	Samaridae	<i>Plagiopsetta</i>	<i>glossa</i>	38.00
USNM_154967	Cynoglossidae	<i>Symphurus</i>	<i>plagiusa</i>	46.00
USNM_154975_1	Cynoglossidae	<i>Symphurus</i>	<i>plagiusa</i>	45.00
USNM_291022	Cynoglossidae	<i>Symphurus</i>	<i>plagiusa</i>	46.00
USNM_291027	Cynoglossidae	<i>Symphurus</i>	<i>plagiusa</i>	47.00
USNM_316742	Cynoglossidae	<i>Symphurus</i>	<i>plagiusa</i>	46.00
USNM_316786	Cynoglossidae	<i>Symphurus</i>	<i>plagiusa</i>	46.00
USNM_354401	Cynoglossidae	<i>Symphurus</i>	<i>plagiusa</i>	47.00
USNM_307569	Cynoglossidae	<i>Cynoglossus</i>	<i>senegalensis</i>	61.00
USNM_072085	Cynoglossidae	<i>Paraplagusia</i>	<i>japonica</i>	52.00
USNM_323756	Cynoglossidae	<i>Symphurus</i>	<i>elongatus</i>	56.00
USNM_056386	Cynoglossidae	<i>Cynoglossus</i>	<i>robustus</i>	60.00
USNM_048971	Achiridae	<i>Trinectes</i>	<i>maculatus</i>	29.00
USNM_118881_1	Achiridae	<i>Trinectes</i>	<i>maculatus</i>	28.00

USNM_118881_2	Achiridae	<i>Trinectes</i>	<i>maculatus</i>	26.00
USNM_127436_1	Achiridae	<i>Trinectes</i>	<i>maculatus</i>	28.00
USNM_127436_2	Achiridae	<i>Trinectes</i>	<i>maculatus</i>	27.00
USNM_226359	Achiridae	<i>Trinectes</i>	<i>maculatus</i>	28.00
USNM_431104	Achiridae	<i>Trinectes</i>	<i>maculatus</i>	26.00
USNM_28950	Achiridae	<i>Gymnachirus</i>	<i>melas</i>	34.00
USNM_167720	Achiridae	<i>Hypoclinemus</i>	<i>mentalis</i>	27.00
USNM_286847	Achiridae	<i>Achirus</i>	<i>declivis</i>	28.00
USNM_167721	Achiridae	<i>Apionichthys</i>	<i>nattereri</i>	42.00
USNM_152033	Achiridae	<i>Nodogymnus</i>	<i>fasciatus</i>	34.00
USNM_101497	Soleidae	<i>Solea</i>	<i>solea</i>	47.00
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USNM_291060	Soleidae	<i>Solea</i>	<i>solea</i>	45.00
USNM_291071	Soleidae	<i>Solea</i>	<i>solea</i>	46.00
USNM_291103_1	Soleidae	<i>Solea</i>	<i>solea</i>	46.00
AMNH_13255	Soleidae	<i>Zebrias</i>	<i>japonica</i>	42.00
USNM_218770	Soleidae	<i>Pardachirus</i>	<i>hedleyi</i>	36.00
USNM_193895	Soleidae	<i>Synapturichthys</i>	<i>kleinii</i>	41.00
USNM_048290	Soleidae	<i>Michrochirus</i>	<i>ocellatus</i>	37.00
CUMV_1_1	Pleuronectidae	<i>Pseudopleuronectes</i>	<i>americanus</i>	38.00
CUMV_1_3	Pleuronectidae	<i>Pseudopleuronectes</i>	<i>americanus</i>	41.00
CUMV_1_4	Pleuronectidae	<i>Pseudopleuronectes</i>	<i>americanus</i>	38.00
CUMV_1_5	Pleuronectidae	<i>Pseudopleuronectes</i>	<i>americanus</i>	39.00
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CUMV_2_4	Pleuronectidae	<i>Pseudopleuronectes</i>	<i>americanus</i>	39.00
USNM_060168	Pleuronectidae	<i>Platichthys</i>	<i>stellatus</i>	35.00
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USNM_054302	Pleuronectidae	<i>Lyopsetta</i>	<i>exilis</i>	45.00
USNM_165431	Pleuronectidae	<i>Hippoglossoides</i>	<i>platessoides</i>	47.00
USNM_075544	Pleuronectidae	<i>Hypsopsetta</i>	<i>guttulata</i>	36.00
USNM_00071926	Pleuronectidae	<i>Clidoderma</i>	<i>asperrium</i>	45.00
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CUMV_48089_2	Paralichthyidae	<i>Paralichthys</i>	<i>dentatus</i>	38.00
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AMNH_085689_1	Paralichthyidae	<i>Syacium</i>	<i>papillosum</i>	34.00
USNM_397177	Paralichthyidae	<i>Ancylopsetta</i>	<i>dendritica</i>	39.00
USNM_214327	Paralichthyidae	<i>Hippoglossina</i>	<i>oblonga</i>	39.00
USNM_186326	Paralichthyidae	<i>Cyclopsetta</i>	<i>fimbriata</i>	36.00

USNM_00158336	Paralichthyidae	<i>Cyclopsetta</i>	<i>chittendeni</i>	36.00
USNM_00155579	Paralichthyidae	<i>Etropus</i>	<i>rimosus</i>	34.00
USNM_005156	Paralichthyidae	<i>Paralichthys</i>	<i>albigutta</i>	37.00
USNM_63610_1	Paralichthyidae	<i>Citharichthys</i>	<i>sordidus</i>	38.00
USNM_156278	Bothidae	<i>Bothus</i>	<i>ocellatus</i>	34.00
USNM_282639	Bothidae	<i>Bothus</i>	<i>ocellatus</i>	36.00
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USNM_416465	Bothidae	<i>Bothus</i>	<i>ocellatus</i>	38.00
AMNH_76415_1	Bothidae	<i>Bothus</i>	<i>ocellatus</i>	35.00
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USNM_260375	Bothidae	<i>Crossorhombus</i>	<i>sp</i>	36.00
USNM_055255	Bothidae	<i>Chascanopsetta</i>	<i>crumenalis</i>	57.00
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USNM_037026	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	35.00
USNM_045211	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	35.00
USNM_396325	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	35.00
USNM_423940	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	34.00
USNM_423945	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	34.00
USNM_423952	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	33.00
USNM_433053	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	34.00
USNM_286245	Scophthalmidae	<i>Scophthalmus</i>	<i>aquosus</i>	35.00
USNM_286608	Scophthalmidae	<i>Scophthalmus</i>	<i>rhombus</i>	36.00
USNM_211791	Scophthalmidae	<i>Lepidorhombus</i>	<i>wiffiagonus</i>	42.00
USNM_022996	Scophthalmidae	<i>Scophthalmus</i>	<i>maximus</i>	30.00
USNM_17361	Scophthalmidae	<i>Zeugopterus</i>	<i>punctatus</i>	36.00
AMNH_242161	Poecilopsettidae	<i>Poecilopsetta</i>	<i>natalensis</i>	31.00
USNM_395254	Poecilopsettidae	<i>Poecilopsetta</i>	<i>beanii</i>	40.00
USNM_286556	Poecilopsettidae	<i>Poecilopsetta</i>	<i>colorata</i>	39.00
USNM_138004	Poecilopsettidae	<i>Poecilopsetta</i>	<i>praelonga</i>	40.00
USNM_406764	Rhombosoleidae	<i>Azygopus</i>	<i>flemingi</i>	47.00
USNM_318393	Rhombosoleidae	<i>Pelotretis</i>	<i>flavilatus</i>	41.00
USNM_214778	Rhombosoleidae	<i>Rhombosolea</i>	<i>tapirina</i>	33.00
USNM_177042	Rhombosoleidae	<i>Ammotretis</i>	<i>sp</i>	40.00
USNM_086732	Rhombosoleidae	<i>Oncopterus</i>	<i>darwinii</i>	29.00
USNM_362525	Achiropsettidae	<i>Mancopsetta</i>	<i>maculata</i>	54.00
USNM_362466	Achiropsettidae	<i>Achiropsetta</i>	<i>tricholepis</i>	51.00
USNM_193278	Outgroup	<i>Coryphaena</i>	<i>hippurus</i>	31.00
USNM_307872	Outgroup	<i>Mene</i>	<i>maculata</i>	23.00
USNM_185929	Outgroup	<i>Naucrates</i>	<i>ductor</i>	26.00
USNM_218542	Outgroup	<i>Leptobrama</i>	<i>mulleri</i>	25.00
USNM_6353	Outgroup	<i>Sphyræna</i>	<i>lucasana</i>	14.00
MNHNFBOL86	Fossil	<i>Amphistium</i>	<i>paradoxum</i>	22.00
NMNH_1974-1639-2	Fossil	<i>Heteronectes</i>	<i>chaneti</i>	24.00

Supplemental Table 1.2.

Supplemental Table 2. Sources of information on diets, primary prey type, and categorization into one of three feeding types for 33 species of Pleuronectiformes. |

Species	Diet and Behavior Sources	Primary Prey	Feeding Type
<i>Psettodes erumei</i>	Kizhakudan et al., 2013	Fish and Small Crustacean	Turbot-type
<i>Psettodes belcheri</i>	Maigret and Ly, 1986	Fish and Small Crustacean	Turbot-type
<i>Citharus linguatula</i>	Carpentieri et al., 2010; Redon et al., 1994	Fish and Small Crustacean	Turbot-type
<i>Samariscus triocellatus</i>	Kuiter and Tonoizuka, 2001	Data deficient	Plaice-type
<i>Symphurus plagiosa</i>	Stickney, 1976	Small Crustacean and Mollusk	Sole-type
<i>Cynoglossus senegalensis</i>	Lowe-McConnell, 1987	Polychaete and Small Crustacean	Sole-type
<i>Paraplagusia japonica</i>	Nakane, 2011	Small Crustacean, Bivalve, Polychaete	Sole-type
<i>Cynoglossus robustus</i>	Baeck et al., 2011	Polychaete and Small Crustacean	Sole-type
<i>Trinectes maculatus</i>	Derrick and Kennedy, 1997	Polychaete and Small Crustacean, Bivalve	Sole-type
<i>Gymnachirus melas</i>	Robertson and Van Tassell, 2015	Polychaete and Small Crustacean	Sole-type
<i>Achirus declivis</i>	Duarte and Andreata, 2003; Couto, 2001	Fish, Polychaete and Small Crustacean	Plaice-type
<i>Solea solea</i>	Kruuk 1963; Allen 2005; Applebaum 1983	Polychaete and Small Crustacean	Sole-type
<i>Synapturichthys kleinii</i>	Dallavalle and Chanet, 2009	Fish	Turbot-type
<i>Pseudopleuronectes americanus</i>	Stehlik 2000; Olla 1969	Polychaete, Bivalve, and Small Crustacean	Plaice-type
<i>Lepidopsetta bilineata</i>	Onate, F.C., 1990. NOAA Fisheries	Echinoderm and Small Crustacean	Plaice-type
<i>Lyopsetta exilis</i>	Pearcy and Hancock 1978	Polychaete and Small Crustacean	Plaice-type
<i>Hippoglossoides platessoides</i>	Holmes and Gibson, 1983	Echinoderm and Small Crustacean, Fish	Plaice-type
<i>Clidoderma asperrimum</i>	Mikawa, 1953; Tokranov and Orlov, 2001	Echinoderm and Small Crustacean	Plaice-type
<i>Paralichthys dentatus</i>	Staudinger & Juanes 2010, Olla et al. 1972	Fish and Small Crustacean	Turbot-type
<i>Hippoglossina oblonga</i>	Sedberry, 1983	Fish and Mollusk	Turbot-type
<i>Paralichthys albigutta</i>	Ashton 1982, Gloeckner and Luczkovich 2009	Fish and Small Crustacean	Turbot-type
<i>Citharichthys sordidus</i>	Pearcy and Hancock, 1978; Kravitz et al., 1977	Fish and Small Crustacean	Turbot-type
<i>Bothus ocellatus</i>	Robertson and Van Tassell, 2015; Randall, 1996	Small Crustacean and Fish	Plaice-type
<i>Bothus pantherinus</i>	Fischer et al., 1990	Small Crustacean and Fish	Plaice-type
<i>Scophthalmus aquosus</i>	Chang et al., 1999	Fish and Small Crustacean	Turbot-type
<i>Scophthalmus rhombus</i>	Vinagre et al., 2011	Fish	Turbot-type
<i>Lepidorhombus whiffiagonis</i>	Santic et al., 2009	Fish	Turbot-type
<i>Scophthalmus maximus</i>	Holmes and Gibson, 1983; Vinagre 2011	Fish	Turbot-type
<i>Zeugopterus punctatus</i>	Holmes and Gibson, 1983	Fish and Small Crustacean	Turbot-type
<i>Poecilopsetta beanii</i>	Evseenko, 2004	Data deficient	Plaice-type