1 **Undulation frequency affects burial performance in living and model flatfishes**

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Abstract

Flatfishes bury themselves under a thin layer of sand to hide from predators or to ambush prey. We investigated the role of undulation frequency of the body in burial in five species of flatfishes (*Isopsetta isolepis*, *Lepidopsetta bilineata*, *Hippoglossoides elassodon*, *Parophrys vetulus*, and *Psettichthys melanostictus*). High-speed videos show that undulations begin cranially and pass caudally while burying, as in forward swimming in many other fishes. The flatfishes also flick the posterior edge of their dorsal and anal fins during burial, which may increase the total surface area covered by substrate. We built a simple physical model – a flexible, oval silicone plate with a motorized, variable-speed actuator – to isolate the effect of undulation frequency on burial. In both the model and actuated dead flatfish, increased undulation frequency resulted in an increase in the area of sand coverage. Complete coverage required an undulation frequency of no more than 10 Hz for our models, and that was also sufficient for live flatfishes. The model shows that undulation is sufficient to bury the animal, but live flatfishes showed a superior ability to bury, which we attribute to the action of the median fins.

Keywords: Pleuronectiformes; Burial performance; Crypsis; Undulation frequency

1. Introduction

Many animals bury themselves, or are buried by circumstance, including both vertebrates and invertebrates. Burial occurs when an organism is covered by substrate and remains submerged without moving beneath the surface. Many species, including some crabs and squid (Bellwood, 2002; Rodrigues et al., 2010) bury themselves to avoid predation or to ambush prey. Some lizards use different burial kinematics when burying in wet or dry sand and generally use undulation to “swim” through the sand (Sharpe et al., 2013, 2015). Burial is common in ray-finned fishes (Actinopterygii), where body form among buriers is diverse. Examples include the sand lance (*Ammodytes spp.*), midshipman (*Porichthys notatus*), sand fish (*Trichodon trichodon*), and staghorn sculpin (*Leptocottus armatus*) (Arora, 1948; Eschmeyer et al., 1983; Pinto et al., 1984; Morioka, 2005; Gidmark et al., 2010). Thin, elongate fishes such as sand lances dive headfirst into the sand to bury themselves (Gidmark et al., 2010). More deep-bodied fishes, such as midshipman, sand fish, and sculpin, combine movements of their bodies and their fins to scoop sand out from beneath them (Arora, 1948; Eschmeyer et al., 1983; Morioka, 2005). Stingrays (Droge and Leonard, 1983) and flatfishes bury themselves with rapid undulations that cause a cloud of substrate to fall onto the body (Kruuk, 1963).

The 678 species of flatfishes in the order Pleuronectiformes (Nelson, 2006) are laterally compressed, asymmetrical fishes that bury themselves in the substrate, typically with only their eyes showing. The diversity of their body shapes ranges from narrow ovals (e.g., *Microstomus pacificus*) to nearly perfectly circular fishes (e.g., *Pleuronichthyes coenosus*), with rhomboidal deviations from the circular-oval continuum (e.g., *Platichthys stellatus*). The substrates on which the fishes are found include mud, silt, fine to coarse sand, and fine to medium gravel. Some species spend substantial time foraging in the water column (e.g., *Hippoglossus stenolepis*), but most either forage in the substrate or ambush prey from hiding. This diversity in body shape, substrate and habit makes flatfishes useful models for understanding the mechanics, tradeoffs and constraints of burial. Burial in flatfishes is a complex behavior that includes coordinated movements of the body and fins, generation of powerful flow patterns, and sensory feedback about substrate and burial stage. Flatfishes perform this behavior for a variety of purposes, including to avoid predation (Kruuk, 1963; Ellis et al., 1997), to ambush prey (Stoner and Ottmar, 2003), and to conserve energy by reducing an individual’s activity levels (Gibson and Robb, 2000). A burial event resembles the undulation seen in normal locomotion, but rather than

proceeding forward the fish remains in place while fluidizing substrate from underneath it and around the edges of its body. When the fish stops undulating, the agitated substrate falls through the water column to conceal the fish.

Burial requires moving substrate from beneath and around an animal to above it, ideally leaving an even distribution of particles that completely conceals. Several factors may affect burial time and coverage, as well as the energy needed to bury. Strong currents of water must be generated by body movements, and these currents are shaped and accelerated by both the fins and the body of the fish. Therefore, we expect that both the kinematics of body movement and the shape of the body have significant effects on burial. Also, in flatfishes, the dorsal and anal fins form a perimeter around the body. The amplitude and frequency of the undulatory wave of the body is likely the most important determinant of flow, but we expect small movements of the fins to have substantial effects on flow direction. The substrate must be fluidized by the flow, so in addition to velocity, the particle size distribution and density will also determine burial parameters.

We used a combination of high-speed videography and physical models to determine the effects of body shape and kinematics on burial performance. Based on observations of the body movements of live fishes, we developed a physical model to determine if undulation frequency could have a significant role in burial. The goals of the present study were four-fold: (1) to quantify time to burial, percent coverage and frequency of undulation in five species of flatfishes; (2) to look for patterns in burial performance that can be explained by morphology or kinematic variation; (3) to undulate dead flatfishes to isolate the role of frequency on burial performance; and (4) to actuate canonical models of flatfish to determine whether the patterns seen in the live fishes (see goal 2) were reflected in generalized flapping foils.

2. Materials and methods

2.1. Animal collection and care

Flatfishes were collected at Friday Harbor Laboratories (FHL) from June 17 to July 2, 2014 via beach seines (Jackson Beach 48°31'13.0"N 123°00'35.1"W) and trawls (San Juan Channel 48°35'10.9"N 123°02'18.7"W; Orcas Eastsound 48°38'26.9"N 122°52'14.0"W; Lopez Upright Head 48°34'45.4"N 122°53'03.2"W). We used five species of flatfishes that vary in shape and ecology (*Isopsetta isolepsis* (n = 6), *Lepidopsetta bilineata* (n = 6), *Hippoglossoides elassodon* (n

= 3), *Parophrys vetulus* (n = 4), and *Psettichthys melanostictus* (n = 3); see Table 1). Fishes were kept in flow-through seawater tanks (11–13 °C) without substrate with similarly collected fishes of other species and fed mysid shrimp every 2–3 days.

The animals were maintained according to animal care practices outlined in University of Washington IACUC protocol 4208-03 and were released at the conclusion of the study. Two *L. bilineata* individuals died in captivity prior to video data collection and were frozen for later use.

2.2. High-speed videography

We recorded each individual using a high-speed camera (Troubleshooter LE500MS; Fastec GmbH, Paderborn, Germany) positioned over a tank with approximately 5–7 cm of fine-grain sand (collected at Eagle Cove, San Juan Island, WA, USA) on the bottom. Individuals were transferred to the filming tank and an acrylic divider was added to restrict the movement of the fish to within the frame of the camera. One burial event was recorded for each fish using high-speed videography (250 frames per second, shutter speed of 1/2500 s, 640 x 480 resolution, gamma of 1.5). Before and after burial, photographs were taken with a scale bar in the frame. All fishes were videotaped within five weeks of collection.

2.3. Video analysis

Duration of behavior(s) was measured between the start of the first undulation and the end of all motion. Time to burial was defined as the amount of time from the start of the first undulation to when the body was completely covered. Undulation frequency (Hz) was the ratio of the number of observable undulations (before the animal was obscured by sand) and the time taken to perform those undulations. We used ImageJ (Rasband, 1997–2014) to measure the percent of surface area that was buried during each trial by analyzing photographs using the pictures taken before and after the burial. Because flatfishes are known to have sand particle preferences that may affect their burial behavior (Moles and Norcross, 1995; Phelan et al., 2000; Stoner and Ottmar, 2003), all trials were run on the same sand, rinsed between uses. An estimate of the shape of the flatfish was found by taking the fineness ratio (ratio of a fish's length to its maximum diameter) of each fish. Two fineness ratios were determined, one which included the width of the fins, and one which included the width of the body without the fins.

2.4. Modeling

To test the effect of shape and undulation frequency on burial performance, a 100 mm x 170 mm x 8 mm oval flatfish model was molded using silicone rubber (EcoFlex 0050; shore 5A hardness: 00-50, tear strength: 8756 N m⁻¹, density: 1069 kg m⁻³, elongation at break: 980%; Smooth-On, East Texas, PA, USA). The model was actuated by a variable speed orbital jigsaw (DW 318; DeWalt Industrial Tool Co., Baltimore, MD, USA) set to vertical motion only and modified with a 5 mm diameter metal rod in place of a blade (Fig. 1). The saw was clamped on either side of a wooden plank that rested over a tank of water and was held in place by two cinderblocks. The model was undulated by the jigsaw at various frequencies (3.7–31.9 Hz) over sand in the water to simulate flatfish burial. The model was undulated with an amplitude of 2.54 cm. Experiments were recorded using the same high-speed camera and settings as above, except with a shutter speed of 1/1250 s instead of 1/2500 s. We analyzed the video to determine the frequency of the undulations. Before and after burial, photographs were taken of the model to determine the surface area of the model that was buried during each trial. For comparison with the model, two frozen *L. bilineata* that died and were frozen shortly after collection were thawed and attached to the jigsaw in place of the silicone model. The dead fish were undulated in the same way as the models.

2.5. Statistics

Using the R stats package (R Development Core Team, 2012), we used the Shapiro–Wilk test to test for normality and Bartlett’s test to check for homoscedasticity in both undulation frequency and burial time data. We also used this package to conduct ANOVAs to analyze the differences in burial time and undulation frequency among species, and Tukey’s post hoc test was applied to determine which species differed significantly. We used the ‘car’ package in R (Fox and Weisberg, 2011) to conduct Pearson’s correlations to analyze the relationships between undulation frequency and other parameters (burial time, length, and burial performance). Only species means were used in Pearson’s correlations to control for the effect of species and variations in sample size. We also used the ‘nlme’ package to fit a quadratic model to the silicone model data (Pinheiro et. al., 2015) and the segmented package to fit the piecewise model to the same (Muggeo, 2008).

3. Results

3.1. Data for live fishes

The fishes began each burial event by lifting their head and then bringing it down against the substrate, beginning an undulation that traveled down their bodies cranially to caudally, creating a traveling wave. This undulation behavior appeared to kick up sand into the water column above the fish. This sand would subsequently settle on the fish, burying it. Some flatfishes appeared to move caudally right before beginning to bury, and most moved cranially very slightly during the behavior. The elongate dorsal and anal fins would undulate regionally in the anterior and posterior regions of the fins. This produced a “flicking” behavior, which appeared to allow the fish to scoop and flick sand up into the water column (Fig. 2).

Fineness ratio with fins varied significantly with species (ANOVA, $df = 4$ and 17 , $F = 7.025$, $p = 0.0015$; Shapiro–Wilk test for normality, $p = 0.95$; Bartlett’s test for homoscedasticity, $p = 0.13$).

A Tukey’s post hoc test determined that *L. bilineata* was significantly different from *H. elassodon*, *I. isolepsis*, *P. melanostictus*, and *P. vetulus* (Tukey’s post hoc test, $L. bilineata \times H. elassodon$, $p = 0.0083$; $L. bilineata \times I. isolepsis$, $p = 0.022$; $L. bilineata \times P. melanostictus$, $p = 0.015$; $L. bilineata \times P. vetulus$, $p = 0.0037$). Fineness ratio without fins varied significantly with species (ANOVA, $df = 4$ and 17 , $F = 9.689$, $p = 0.00028$; Shapiro–Wilk test for normality, $p = 0.63$; Bartlett’s test for homoscedasticity, $p = 0.80$). A Tukey’s post hoc test determined that *L. bilineata* was significantly different from *H. elassodon*, *I. isolepsis*, and *P. vetulus* (Tukey’s post hoc test, $L. bilineata \times H. elassodon$, $p = 0.0020$; $L. bilineata \times I. isolepsis$, $p = 0.00085$; $L. bilineata \times P. vetulus$, $p = 0.0013$). A summary of the shape, undulation frequency, and environment of the fish can be found in Table 1.

The duration of the behavior was short (1–2.8 s) (Fig. 3B) and there was no significant difference among species in the duration of behavior (ANOVA, $df = 4$ and 17 , $F = 2.943$, $p = 0.051$; Shapiro–Wilk test for normality, $p = 0.23$; Bartlett’s test for homoscedasticity, $p = 0.51$). Time to burial was also brief, taking from 0.86 to 2.18 seconds. There was a significant difference in the time to burial between *P. melanostictus* and three other species and between *P. vetulus* and *H. elassodon* (ANOVA, $df = 4$ and 13 , $F = 10.26$, $p = 0.00056$; Tukey’s post hoc test, $P. melanostictus \times H. elassodon$, $p = 0.00047$; $P. melanostictus \times I. isolepsis$, $p = 0.0068$; $P. melanostictus \times L. bilineata$, $p = 0.0015$; $P. vetulus \times H. elassodon$, $p = 0.021$; Shapiro–Wilk test for normality, $p = 0.21$; Bartlett’s test for homoscedasticity, $p = 0.60$). Time to burial data were

not collected from four individuals (two *I. isolepsis*, one *P. vetulus*, and one *P. melanostictus*) because they did not bury their bodies completely. There was a significant correlation between time to burial and undulation frequency (Pearson's correlation, $r = -0.7817$, $p = 0.00012$; Fig. 4B).

The frequency of the undulations produced by the body of the animal during burial varied between 5.2 and 10 Hz and differed significantly among the species (ANOVA, $df = 4$ and 17 , $F = 5.968$, $p = 0.0034$; Tukey's post hoc test, *P. vetulus* X *L. bilineata*, $p = 0.040$, *P. melanostictus* × *L. bilineata*, $p = 0.0036$, *P. melanostictus* × *H. elassodon*, $p = 0.029$; Shapiro–Wilk test for normality, $p = 0.66$; Bartlett's test for homoscedasticity, $p = 0.88$) (Fig. 3C). There was no relationship between the mean species length and its mean undulation frequency (Pearson's correlation, $r = 0.0254$, $p = 0.82$). There was no relationship between the species mean of the undulation frequency and the mean duration of behavior (Pearson's correlation, $r = -0.3006$, $p = 0.39$) (Fig. 4). When surface area buried was divided by the duration of the behavior there was a significant difference found between the species (ANOVA, $df = 4$ and 17 , $F = 4.751$, $p = 0.00932$; Shapiro–Wilk test for normality, $p = 0.06$; Bartlett's test for homoscedasticity, $p = 0.40$.) These differences were found between *P. melanostictus* and *H. elassodon* (Tukey's post hoc test, $p = 0.013$), *P. vetulus* and *H. elassodon* (Tukey's post hoc test, $p = 0.021$) and between *H. elassodon* and *L. bilineata* (Tukey's post hoc test, $p = 0.019$) (Fig. 3D).

The number of undulations during burial was estimated by multiplying undulation frequency by the duration of behavior for each trial. This was barely significantly different between the species (nested ANOVA, with 'individual' nested within 'species', $df = 4$ and 16 , $F = 3.014$, $p = 0.0497$). The species means of the number of undulations did not correlate significantly with the species means of the surface area buried (Pearson's correlation, $r = 0.3156$, $p = 0.1525$).

3.2. Data for silicon model and dead fish

The actuator undulated the silicone rubber model and the dead flatfish in a way similar to live flatfishes, but with a standing wave instead of a traveling wave. By lifting the "head" end of the model and then undulating it against the substrate, the jigsaw created undulations that moved down the length of the model's "body" and kicked up sand above it. This sand subsequently settled, burying the model.

The model showed a positive, nonlinear relationship between the percent surface area buried and undulation frequency (Pearson's correlation, $r = 0.8188$, $p = 5.66 \text{ e-}06$). The data from the dead fish follow the same trend as the silicone model, but only the small flatfish showed a significant correlation (Pearson's correlation, $r = 0.7683$, $p = 0.043$) while the large flatfish did not (Pearson's correlation, $r = 0.6225$, $p = 0.13$). In all cases, percent surface area buried increased quickly with undulation frequency for frequencies between ca. 3.7 and 10 Hz. At higher undulation frequencies, the increase in percent surface area buried attenuated (Fig. 5). A quadratic model provided the best fit to the silicone model data (quadratic model: $r^2 = 0.7416$, $p = 5.144 \text{ e-}06$; linear model: $r^2 = 0.6705$, $p = 5.667 \text{ e-}06$; piecewise model: $r^2 = 0.6930$, $p = 0.00017$; exponential model: $r^2 = 0.5049$, $p = 0.00018$).

4. Discussion

The frequency at which flatfishes undulate their bodies affects the percentage of their body that becomes covered by substrate. This simple undulation, as shown by both models and dead flatfishes, is sufficient to fluidize the substrate at the perimeter and allow it to be redeposited over the exposed surface. So, a generalized flatfish shape, with extreme lateral compression and low fineness ratio, can be quickly buried using only undulations against the substrate, without requiring fin-flicking or other specialized behaviors. A similar oscillatory movement allows razor clams to burrow, and these clams also exhibit a relationship between frequency and burrowing velocity in sand (Jung et al., 2011). Also, studies of unrestrained locomotion of sandfishes through a granular medium using lateral undulations have demonstrated a linear relationship between undulatory frequency and forward speed (Sharpe et al., 2013). Both the model and the dead flatfish suggest an undulation frequency above which there is no gain in the fluidization and redistribution of particles. In the case of the model, burial performance increased with frequency up to ~20 Hz, above which there was no further improvement (Fig. 5). Dead flatfish showed a similar relationship between undulation frequency and burial performance, confirming that there are few functionally relevant differences in mechanical behavior between the model and the actuated flatfish body, despite obvious and substantial differences in morphology and material properties. However, undulation frequency is not the only factor in flatfish burial performance. Each burial event recorded from living flatfishes resulted in at least 68% coverage by substrate, and the

majority of burial events resulted in 90–100% coverage. The silicone flatfish model only reliably buried with 90–100% coverage when undulated at frequencies of 20 Hz or greater, while the living flatfishes achieved the same percent coverage at undulation frequencies of 5.2–10 Hz. When the silicone model and dead flatfishes were undulated at frequencies seen in living flatfishes (5.2–10 Hz; Fig. 5, grey region), there was considerable variation in the resulting percent coverage as compared with the consistently high burial performance seen in living flatfishes.

Behavior, morphology and biomechanical factors must enhance burial performance in live fishes, allowing lower frequency undulation to effectively bury the animal. For example, small, rapid motions of the dorsal and anal fins have been implicated in flatfish burial (Kruuk, 1963), and we noted these movements in live fishes (Fig. 6). By flicking the fins, there is both an added velocity component to the fluidizing flow and a redirection of some flow towards the central region of the fish. Neither dead fish nor models could change their mean altitude in the water column, whereas sinking into the substrate is a consistent feature of flatfish burial. As a flatfish displaces sand and sinks below the sand–water interface, undulation will produce flow that has more sand to fluidize than it did in the initial condition. Finally, our modeling used either a perfectly uniform stiffness material (silicone) or a material with non-uniform stiffness that was constant over the undulation cycle (dead flatfish). But live fishes change their flexural stiffness to control swimming kinematics and performance (McHenry et al., 1995; Long, 1998), and this will certainly affect the fluidization effects at the periphery of the fish.

The five species in our study span a wide range of habitats, morphology and ecology (Jones and Harry, 1961; Eschmeyer et al., 1983; De Ben et al., 1990; Levings and Ong, 2003; Choromanski et al., 2004), yet their burial speed and the kinematics used to achieve burial were grossly similar. It also may be useful to apply some of the tools and concepts for analyzing undulatory swimming to this unusual case of a fish undulating in place, as the positive relationship between burial performance and undulation frequency below ~20 Hz is consistent with previous observations that flatfishes swimming close to the substrate show a positive relationship between body undulation frequency and swimming speed (Webb, 2002). Furthermore, it may be that fine grain sand is such a generalized substrate that all morphologies and kinematics work well. The effects of morphology and the finer details of kinematics may make a difference when the

substrate is silt, coarser sand or fine gravel. These substrates may represent a functional extreme that challenges the flatfish to perform.

5. Conclusion

We show that the flatfish species studied all undulate their bodies at a similar range of frequencies combined with fin flicks to bury themselves. The physical model shows undulation is sufficient to bury a flatfish and undulation frequency plays an important role in determining how much of a flatfish is buried. The shape and behavior of the flatfish may have an effect on burial that may be more important in a broader range of substrates.

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Figure Captions

Fig. 1. Model setup. A DW318 variable speed orbital jigsaw was attached to a metal pole, where the blade would normally have gone, which acted as an actuator, moving a silicone fish model with fixed frequencies.

Fig. 2. *Lepidopsetta bilineata* burying itself. The head of the fish beats against the sand several times creating undulations that travel down the body of the fish, and kicking up sand. At 192 ms you can see the head hitting the sand and displacing the sand around it. The posterior portion of the dorsal and anal fins flick sand as well. It is unknown what these fin flicks are doing, but they appear to be creating vortices in the sand they kick up over the body of the individual. A plume of sand is kicked up into the water column by the undulations and the flicking, which then settles down onto the body of the fish, burying it.

Fig. 3. (A) Surface area buried. These boxes show the mean and spread of the percentage of top-viewed surface area of each individual flatfish that was buried by the end of the behavior. All individuals buried more than 60% of their bodies' surface area. There was no significant difference in surface area buried among species. (B) Duration of behavior. These boxes show the mean and spread of times taken by individuals in each species to complete the burial behavior. The time taken to bury varied between 1 and 2.8 seconds overall, but was not significantly different between species. (C) Frequency of undulations during burial behavior. These boxes show the mean and spread of the undulation frequency used in each individual's burial behavior. Frequency differed significantly between species. (D) Significant differences were found among three pairs of species in percent surface area buried divided by duration of behavior (ANOVA, $p = 0.00932$; Shapiro–Wilk test for normality, $p = 0.06$; Bartlett's test for homoscedasticity, $p = 0.40$). *P. melanostictus* and *H. elassodon* were significantly different (Tukey's post hoc test, $p = 0.013$). *P. vetulus* and *H. elassodon* also differed significantly from each other (Tukey's post hoc test, $p = 0.021$). *H. elassodon* also differed significantly from *L. bilineata* (Tukey's post hoc test, $p = 0.019$).

Fig. 4. (A) No relationship was found overall between undulation frequency and duration of behavior in live subjects. The duration of a burial behavior varied between 1 and 2.8 s and did not correlate with the frequency of the undulations during the behavior. (B) A significant correlation was found between time to burial and undulation frequency. Each data point represents the mean for each species. The error bars indicate the standard error of the means.

Fig. 5. Faster undulations led to a higher percent surface area buried in the silicone model as well as the dead fish, but this increase lessened at higher undulation frequencies. A quadratic model fitted to the silicone model data shows $r^2 = 0.7416$. Values for the small and large dead fish were added to the plot to show where they lie in relation to the model fit. The shaded area indicates the range of frequencies exhibited by live flatfishes.

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413 **Table 1.** A quick comparison of the species used in this study. The fineness ratio (an estimate of
 414 shape) and the undulation frequencies were found in this study.

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Species [standard length in cm]	Undulation frequency (Hz) mean [range]	Fineness ratio including fins mean [range]	Fineness ratio excluding fins mean [range]	Environment* (range and habitat)
<i>Isopsetta isolepsis</i> [8.3–11.2]	7.98 [6.80–9.49]	1.787 [1.673–1.887]	2.840 [2.483– 3.100]	Range: Bering Sea to Ventura, CA Habitat: shallow water up to 1200 ft
<i>Lepidopsetta bilineata</i> [12.7–17.2]	9.04 [7.60–10.00]	1.512 [1.371–1.599]	2.225 [1.998– 2.387]	Range: Sea of Japan, Bering Sea to Tanner Bank, CA Habitat: shallow water to 1200 ft
<i>Hippoglossoides elassodon</i> [11.7–15.9]	8.70 [8.10–9.17]	1.896 [1.726–1.990]	2.913 [2.702– 3.126]	Range: Sea of Japan, Bering Sea to Pt. Reyes, CA Habitat: soft bottom 20–1800 ft
<i>Parophyrus vetulus</i> [11.2–15.1]	7.13 [5.58–8.06]	1.897 [1.788–2.004]	2.880 [2.626– 3.209]	Range: Bering Sea to central Baja Habitat: up to 1800 ft
<i>Psettichthys melanostictus</i> [12.0–14.7]	6.16 [5.24–6.66]	1.866 [1.602–2.184]	2.572 [2.318– 2.835]	Range: Bering Sea to Redondo Beach, CA Habitat: near shore to 600 ft

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417 *All environments were found in Eschmeyer et al. (1983) with support from the trawls described in Jones
 418 and Harry (1961), De Ben et al. (1990), Levings and Ong (2003), and Choromanski et al. (2004).