Adaptive coloration, behavior and predation vulnerability in three juvenile north Pacific flatfishes

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ABSTRACT

Adaptive color change in flatfish has long been of interest to scientists, yet rarely studied from an ecological perspective. Because color change can take a day or so in some species, movement between sediments with differing color or texture may render fish more conspicuous to predators. We conducted laboratory experiments to test the following hypotheses related to adaptive color change in flatfish: 1) fish which do not cryptically match sediment will be more vulnerable to predation, 2) fish will reduce activity and bury to minimize conspicuousness when on a sediment they mismatch, and 3) fish will choose a sediment they match when given a choice. Experiments were conducted using three co-occurring north Pacific juvenile flatfishes: English sole Parophrys vetulus, northern rock sole Lepidopsetta polyxystra and Pacific halibut Hippoglossus stenolepis. As per expectations, juvenile flatfish were more vulnerable to visual predators when they mismatched sediment. Mismatched fish tended to behave differently than fish which matched the sediment. Rather than burying and becoming inactive, they became more active and less likely to bury, perhaps contributing to their predation vulnerability. This increased activity may have represented search for better matching sediment, a stress response, or conspicuousness-related density dependent behavior. Fish which had acclimated to light colored sediment preferred light over dark sediment in choice trials. In contrast, fish acclimated to dark sediment demonstrated no preference. These experiments demonstrate that adaptive coloration is an integral part of the flatfish detection minimization strategy and that movement between habitats can increase risk of predation.

1. Introduction

The detection minimization strategy utilized by flatfish relies upon their flattened body shape and cryptic coloration, as well as co-evolved behavioral tactics. During daytime activity, juvenile flatfish move short distances, interspersed with lengthy pauses. When confronted by a distant predator, they cease movement, lower their body posture and/or bury (Lemke and Ryer, 2006), and are reticent to flee until the predator gets very close (Ellis et al., 1997; Ryer et al., 2004). However, both field and laboratory studies indicate that juvenile flatfish frequently make excursions into the water column during night-time hours (Hempel, 1964; Verheijen and De Groot, 1967; Hurst and Duffy, 2005), when they are presumably less vulnerable to visual predators. Such behavior may allow for migration between, or sampling of, different habitats (Hurst and Duffy, 2005) and has been suggested to facilitate tidal migrations in juvenile plaice Pleuronectes platessa and dab Limanda limanda (Burrows, 1994).

Juvenile flatfish descending from the water column after a night-time excursion will frequently find themselves on sediment that they match, as sediments are often fairly uniform over broad spatial scales. However, in some geographic regions, sediments of vastly different color and texture occur in close proximity to one another, and juvenile flatfish may descend upon sediment that they do not match well. For example, at one of our Kodiak Island Alaska study sites, ~1 km separates light colored sands from both black volcanic sands and shell hash sediments.

The adaptive significance of camouflage as an anti-predator tactic in flatfish has been assumed, but not rigorously examined. Prior experiments have addressed this topic from a fisheries enhancement perspective, posing the question: are flatfish which do not match natural sediments more vulnerable to predation than normally colored fish? Winter flounder Pseudopleuronectes americanus cultured in pale blue bottomed tanks without sediment have a blanched appearance and are more vulnerable to avian predation than cultured individuals acclimated for several weeks to a darker natural sediment (Fairchild and Howell, 2004). This would suggest an obvious anti-predator benefit associated with matching one’s environment. However, the same authors also noted
that cultured fish acclimated to natural sediment were none-the-less more vulnerable to predation than wild fish, raising the possibility that behavioral deficits associated with hatchery rearing may also compromise survival (Olla et al., 1998).

In some species, coloration change is rapid (Healey, 1999); a matter of seconds (Ramachandran et al., 1996). This change is controlled through visual stimuli projected on the upper half of the retina (De Groot et al., 1969) resulting in neurologically and hormonally mediated melanophore contraction or expansion on the fish's ocular side (Burton, 2002). Longer term 'fine tuning' of appearance takes place over days to weeks and involves movement of melanophores and production of additional melanin and/or melanophores (Burton, 2002). Although under the same control mechanisms, initial coloration change in other species can be slower and take hours or days (e.g. winter flounder Pseudopleuronectes americanus; Fairchild and Howell, 2004). English sole Parophrys vetulus, northern rock sole Lepidopsetta polyxystra and Pacific halibut Hippoglossus stenolepis juveniles exhibit initial changes to match sediment in several hours to a day or so, but require several weeks for more precise color matching (personal observation). As a consequence, juveniles of these species encountering a seafloor they do not match may be more conspicuous to predators, unless mitigating behavior is adopted.

In this study we examined the potential consequences of a mismatch between flatfish and sediment coloration, as well as behavior that would mitigate increased predation vulnerability resulting from such a mismatch. These studies were carried out on juvenile English sole, northern rock sole and Pacific halibut, the dominant species making use of shallow water nursery areas around Kodiak Island Alaska during summer months (Abookire et al., in review). In our first experiment we tested the hypothesis that mismatched fish would be more vulnerable to a visually oriented piscine predator than fish which matched the sediment. In a second experiment we tested the hypothesis that mismatched fish would exhibit lower activity and remain buried in an attempt to reduce their conspicuousness. Lastly, we tested the hypothesis that juvenile flatfish would demonstrate preference for sediment that they already match.

2. Materials and methods

2.1. Fish collection and maintenance

Age-0 Pacific halibut and northern rock sole (hereafter halibut and rock sole) were collected from Chiniak Bay, Kodiak Alaska, with a beam trawl (2 m wide, 3 mm mesh), then air transported to the Hatfield Marine Science Center in Newport, Oregon. Age-0 English sole were similarly collected from Yaquina Bay in Newport. Age-3 halibut, utilized as predators, were grown out in the laboratory from Kodiak Island Alaska during summer months (Abookire et al., in review). In our first experiment we tested the hypothesis that mismatched fish would be more vulnerable to a visually oriented piscine predator than fish which matched the sediment. In a second experiment we tested the hypothesis that mismatched fish would exhibit lower activity and remain buried in an attempt to reduce their conspicuousness. Lastly, we tested the hypothesis that juvenile flatfish would demonstrate preference for sediment that they already match.

2.2. Sediments

The dark sediment was a well sorted medium black sand with a range of grain sizes from 0.1 to 4.0 mm (median=0.4 mm). The light colored sediment was light beige in coloration and approximates what juvenile fish encounter in most Kodiak nurseries. It was a well sorted medium sand with particles ranging from 0.2 to 2.0 mm (median particle size=0.4 mm). As both sands consisted of crushed silicate rock, particles were angular, as opposed to the more rounded particles normally encountered in marine environments. Nonetheless, fish displayed typical burial behavior, feeding and growth (personal observation).

2.3. Predation experiment

Age-0 flatfish were acclimated to respective sediments (light or dark) for 4 to 6 wks in 2 m diameter (3000 l) holding tanks. Predation trials were conducted in 2.9 m diameter (6400 l) arenas with a 3 cm deep covering of light sand and provided with flow-through 9°C (±1°C) seawater. Each arena contained 2 age-3 halibut (365 – 390 mm total length) which had not been fed for 48 h prior to the trials. Tethering data indicates that larger flatfish are the dominant predator upon juvenile flatfish in Kodiak nurseries (Ryer & Laurel, unpublished data). Illumination in the arenas was approximately 5 μmol photons m−2 s−1.

Knowledge of activity patterns is desirable when using point measurements of predation vulnerability or habitat preference (Gibson and Robb, 2000). Prior research indicates that halibut and rock sole bury and remain inactive for 24 – 36 h after feeding to satiation (Stoner and Titgen, 2003), but at 48 h are generally active yet still elicit a strong anti-predator response (i.e. burial and/or motionlessness). Accordingly, fish were not fed during the 48 h preceding trial initiation in this and subsequent experiments. This insured that fish were motivated to explore their surroundings, but not so hungry as to ignore predatory risk (Lemke and Ryer, 2006).

Twenty-four hours prior to trials fish were measured for total length. English sole, halibut and rock sole ranged in size (total length) from 51 – 80 mm, 45 – 69 mm and 47 – 65 mm, respectively, with English sole approximately 10 mm larger than either halibut or rock sole (Tukey paired comparisons, P<0.05, F1,22=119.89, P<0.001). During measurement, each fish received a minute clip made with scissors on either the upper or lower lobe of the caudal fin to allow identification of acclimation treatments without relying upon fish color. Treatment clip location was alternated with each trial, precluding any potential performance bias being associated with a particular treatment. Before each trial, the lights in the room were turned off and the juvenile flatfish were released into the arena. As age-3 halibut are visual predators, this allowed the juveniles to settle to the bottom in safety. After 15 min the lights were turned on, and the trial began. The first 3 English sole trials lasted 30 min. While observing these trials it became apparent predators were consuming nearly all the dark fish before beginning to pursue light fish. Therefore, the duration of the 3 subsequent trials was decreased to 20 min to maximize the difference in consumption of the dark versus light flatfish. Five 20 min trials each were conducted for halibut and rock sole. At the end of trials, fish were removed, enumerated, identified by fin-clip and re-measured. Fish length before and after trials did not differ (F1,49=0.66, P=0.419). Survival data for each species was analyzed using paired t-tests (Sokal and Rohlf, 1969) to test for a difference in survival between treatments. Overall survival (the sum of both acclimation treatments) was compared between species using ANOVA (Sokal and Rohlf, 1969). In this later analysis, only trials of 20 min duration were included. Parametric planned comparisons (Day and Quinn, 1989) were utilized to test for differences in mean survival between species.

2.4. Activity experiment

Fish were acclimated to respective sediments (light or dark) for 4 to 6 wks in 1 m diameter holding tanks. Similar 1 m diameter tanks were used for the experimental trials and contained light sand. These tanks could be viewed from overhead via video cameras. Fish were unfed for approximately 48 h prior to initiation of trials. For English sole trials, a group of 5 light fish (matched treatment) or 5 dark fish (mismatched) was transferred to an experimental tank in darkness at 2200 h on the afternoon prior to trial initiation. The lights remained off until 0615 h the following morning. This presumably allowed fish...
to recover from any minimal stress incurred during transfer, but as it was dark, the mismatched fish could not change their coloration to match the light sediment (De Groot et al., 1969). Observations on burial and videotaping for activity scores started at 0645 h. For the next 1 h, at 15 min intervals, an observer viewed the tank through a slot in the blind surrounding it, and scored each fish according to a 5 point burial scale; 0=0% buried, 1=25%, 2=50%, 3=75% and 5=100% (Ryer et al., 2004). From videotape recordings, activity was scored using two 5 min intervals per trial: from the beginning of each tape (30 min after lights on) and 30 min later (60 min after lights on). Scoring consisted of recording the number of times fish crossed 4 straight lines on an acetate sheet taped to the video monitor. Each line passed through the center of the tank, with 45° angles separating adjacent lines. For rock sole and halibut, fish were placed into the experimental tank at 1600 h in darkness, and the lights came on at 0630 h. Video taping began as soon as the lights came on, but no tank side observations were made to score burial. Activity was scored during 5 min intervals, as previously described but using three 5 min intervals per trial: at 0, 30 and 60 min after lights on. Eight replicate trials were conducted for each acclimation history for English sole, while six trials were conducted for halibut and rock sole. English sole ranged in total length from 64 – 81 mm (mean=74 mm, SD=5). Halibut total lengths ranged from 52 – 61 mm (mean=56, SD=3). Rock sole ranged from 61 – 68 mm (mean=65 mm, SD=3). Length did not differ between acclimation histories for any species (F [1,30]=0.04, P=0.836), although English sole were larger than halibut, which were in turn larger than rock sole (Tukey multiple comparisons, Pb0.05, F[2,30]=71.39, P<0.001).

Because of the differing protocols, English sole were analyzed separately from halibut and rock sole. English sole activity data were natural log transformed to achieve homogeneity of variance. English sole data were analyzed using ANOVA, with acclimation history as a between group (subject) factor and time a repeated measures factor (Hicks, 1982). Halibut and rock sole were analyzed together using similar repeated measures ANOVA, but with species as an added between group factor. Parametric planned comparisons (Day and Quinn, 1989) were utilized to test for differences in mean activity between acclimation treatments at each time period.

2.5. Sediment choice experiment

Fish were acclimated to respective sediments (light or dark) for 4 to 6 wks (1 m diameter tanks). Trials were conducted in 1 m diameter tanks, equipped with partitions that could be raised or lowered to bisect the tank. One side of the tank had light sediment, the other had dark sediment. Fish were unfed for approximately 48 h prior to trials. A group of 10 fish (either light or dark) was transferred to the experimental tank on the afternoon prior to trials; English sole at 2200 h, halibut and rock sole at 1600 h (all in darkness). Lights were turned on the following morning, at 0615 h for English sole and at 0630 h for halibut and rock sole. After 2 h the partition was lowered, isolating the light and dark sediments. Fish were removed from each side, enumerated and measured for total length. For English sole, 8 replicate trials were conducted for each acclimation history, while for halibut and rock sole, 6 trials were conducted for each. English sole ranged in total length from 39 – 100 mm (mean=72 mm, SD=10). Halibut ranged from 43 – 88 mm (mean=65, SD=9) and rock sole from 41 – 61 mm (mean=51 mm, SD=5). Fish length did not differ between acclimation histories for any species (F[1,70]=0.52, P=0.474), although English sole were larger than halibut, which were in turn larger than rock sole (Tukey multiple comparisons, Pb0.05, F[2,70]=237.66, P<0.001). Initial analysis utilized ANOVA to test for the influence of acclimation history and species upon the number of fish

![Fig. 1. Predation experiment. Mean % survival (±SE) of English sole, halibut and rock sole acclimated to either dark sand (dark fish) or light sand (light fish) when subjected to predation on a light sand bottom.](image)

![Fig. 2. Activity experiment. Mean activity (±SE) of fish acclimated to dark sand (dark fish) or light sand (light fish) on a light sand bottom, measured at time intervals after lights were turned on in the morning. a - English sole, c - halibut, d - rock sole. b - mean burial score (±SE) for English sole averaged over the time duration of trials (30 – 60 min).](image)
recovered from the light sediment side. Subsequent analysis utilized paired t-tests to compare the numbers of fish recovered from the light vs dark sediment for each acclimation treatment (Sokal and Rohlff, 1969).

3. Results

3.1. Survival experiment

Survival in predation trials was strongly influenced by whether or not juvenile flatfish matched the light sand of the arena (Fig. 1). For all 3 species, fewer dark acclimated fish survived exposure to predators (English sole: t=11.95, df=4, P=0.002; halibut: t=6.78, df =4, P=0.003; rock sole: t=11.95, df=4, P<0.001). Overall survival (dark and light fish combined) differed between species (F[2,10]=16.4, P<0.001). English sole survived exposure to predators with a significantly lower survival rate than either halibut or rock sole. Halibut survival was lower than that of rock sole (Tukey paired comparisons, P<0.05).

3.2. Activity and burial experiment

Contrary to our predictions, dark acclimated fish were more active than the light acclimated fish, although the magnitude and time course of this effect differed between species. English sole were analyzed separately from the other species because no data were available at time 0. Dark acclimated English sole were more active than light acclimated fish (Fig. 2a, F[1,14]=6.85, P=0.020). There was also a decrease in activity from 30 min to 60 min (F[1,14]=4.96, P=0.043). A marginally significant acclimation×time interaction (F[1,14]=3.85, P=0.070), as well as examination of Fig. 2a, leads us to conclude that the acclimation effect was greater at 30 than at 60 min. Regardless of time, dark English sole were less likely to be buried than light English sole (Fig. 2b), as indicated by burial scores (F[1,14]=10.09, P=0.007). Halibut and rock sole differed from one another in how treatment effects were manifested over time (species×acclimation×time interaction: F[2,40]=5.06, P=0.011). For halibut (Fig. 2c), there was a tendency for activity to be greater among dark fish at 0 min, but this was not statistically significant (planned comparison, P>0.05). For rock sole (Fig. 2d), dark fish were more active than light fish at 30 and 60 min (planned comparisons, P<0.05). No burial data were recorded for either halibut or rock sole.

3.3. Sediment selection experiment

Sediment preference depended upon acclimation history (Fig. 3a–c). More fish were recovered from the light sand side of the tank in trials where fish had been acclimated to light sand and hence were light in color, regardless of species (F[1,36]=4.54, P=0.040). Subsequent analysis revealed that among light acclimated fish, more were recovered from the light than from the dark sand side of the tank (t=4.82, df=19, P<0.001). In contrast, the number of dark acclimated fish recovered from light versus dark sand did not differ (t=−0.90, df=21, P=0.378). Fish recovered on dark versus light sand did not differ in total length (F[1,70]=0.22, P=0.643).

4. Discussion

The effects of cryptic coloration on predation vulnerability have been known for many years (e.g. industrial melanism in moths; Kettlewell, 1956). Not surprisingly, juvenile flatfish that do not match their sediment are more vulnerable to visual predators. In our experiment, juvenile flatfish experienced lower survival when they were mismatched to the sediment upon which they encountered predation risk. In the only similar experiment we are aware of, winter flounder that were pale in coloration due to rearing in light blue tanks suffered lower survival in avian predation trials than did darker fish which were better matched with the dark sediment (Fairchild and Howell, 2004). As a consequence, juvenile fish engaging in nocturnal forays into the water column where currents may transport them considerable distance, may find themselves mismatching the sediment and at increased risk of predation come morning.

Predation vulnerability associated with mismatching sediment may also be attributable to behavior. We had expected that mismatched fish would bury and remain inactive so as to mitigate their conspicuousness. All three species we examined can achieve a rough match to novel sediment in a matter of a day or so, and prior works suggests this can be accomplished even if the fish is buried (Sumner, 1911). Forgoing foraging for this time would probably have minor consequences compared to increased risk of predation. However, all three species tended to be more active when they mismatched their sediment. Since both the light and dark sands we utilized had similar...
grain sizes and geometries, we concluded that change in sediment color was the most likely cue for this increased activity.

Activity makes fish more conspicuous and attractive to predators (Krause and Godin, 1995). Why would mismatched fish increase their activity? This may be an attempt to locate sediment that better matches their present coloration. However, as daytime activity typically involves short movements interspersed with pauses, the likelihood of moving far enough in the correct direction to locate sediment of appropriate color seems remote. Alternatively, this may represent a stress response; changes in activity can be indicators of stress in fish (Schreck et al., 1997). Another possible explanation involves density dependent effects. Just as mismatched fish were more conspicuous to human observers and a predator, they were also probably more conspicuous to one another. This may have been interpreted as higher con-specific density. Juvenile flatfish exhibit density dependent activity. Laurel et al. (2007) demonstrated that age-0 rock sole activity increased over a range of densities from 0.4 – 12 fish m⁻². Our activity experiment was conducted at a density of approximately 6 fish m⁻². Due to increased conspicuousness, dark fish on light sediment may have behaved as if they were at a higher density.

If increased activity in mismatched fish represents ‘search’ for sediment which offers a better match, we would expect fish to choose matching sediment in preference trials. Winter flounder prefer sediment to which they have acclimated and hence match (Fairchild and Howell, 2004). In our study acclimation history did influence sediment preference in all three species, but not in the exact manner predicted. When given a choice between light and dark sediment, light acclimated fish preferred the light sediment, as predicted. In contrast, dark acclimated fish demonstrated no preference. Lack of a strong preference for sediment providing the greatest refuge from predation is perplexing. Tank side observations confirm that fish were moving back and forth between sediments. It may be that sediment preference in these species represents a balance between acclimation history (i.e. preference for what you already match) and an innate preference for relatively light colored sediments, as these are more commonly encountered throughout their range. In the case of dark sand acclimated fish, this innate preference may have been canceled out by an acclimation effect, leading to no discernable selection between sediments. Our study would have benefited from conducting reciprocal match-mismatch trials in our survival and activity experiments (i.e. examine light and dark acclimated fish on a dark substrate). This was not included, in part, due to the difficulty in quantifying activity of dark fish on dark sand. Detection of light fish on light sand was made possible by the slight shadow cast as they moved as little as a mm or so off the sediment. This shadow was not detectable on dark sand making it impractical to quantify movement by dark fish from video. Future studies would benefit by utilization of techniques allowing for quantification of behavior on a wider range of sediment types.

5. Conclusions

The ability of flatfish to cryptically match their substrate offers a selective advantage in the form of reduced vulnerability to predation. Juvenile flatfish making nocturnal forays into the water column may settle upon sediment that renders them more conspicuous and vulnerable to predation. Our data indicates juvenile flatfish perceive when they do not match the substrate. However, rather than remain inactive and buried, mismatched fish appear to increase activity. When given a choice between light and dark sand, light colored fish were more likely to choose the ‘appropriate’ sediment than dark colored fish. Even when present, sediment preferences were rather weak, which is surprising considering the potential predation related consequences. At the initiation of this study we viewed cryptic coloration in flatfish as a behavioral trait or tactic. However, unlike other behavior, such as shelter seeking and burial, adaptive color change in flatfish is both neurologically and physiologically controlled. As such, it may not be entirely volitional, or as well integrated into the behavioral decision making process as other ontological behavior.

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