

Behavioral Responses of Mosshead and Woolly Sculpins to Increasing Environmental Hypoxia

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Behavioral responses of the rocky intertidal mosshead sculpin (*Clinocottus globiceps*) and woolly sculpin (*Clinocottus analis*) to increasing environmental hypoxia (low PO_2) were observed in a seawater aquarium with a depth gradient. The experimental design allowed observation of the response of these animals to encroaching environmental hypoxia. We observed three behaviors by which the fish dealt with hypoxia: emergence from substrate (EFS), first avoidance response (FAR), and aquatic surface respiration (ASR). With respect to EFS and FAR, mosshead sculpins were more labile in their responses than woolly sculpins. However, the mean PO_2 at which ASR was initiated was statistically indistinguishable between species. We suggest that the differences we observed represent alternate behaviors by which these related species deal with the trade-off imposed by the simultaneous needs to avoid predation and optimize respiration. We further suggest that these alternate behaviors have evolved around a conserved physiological constraint, the PO_2 at which ASR must be performed.

CALIFORNIA'S rocky coast tidepool habitats may rarely achieve homeostasis during low tide (Martin, 1995). Fishes that live in tidepools may face challenges such as increasing environmental hypoxia caused by high respiring biomass and poor wind mixing during these low tide events (Morris and Taylor, 1983; Yoshiyama et al., 1995). Many fish perform aquatic surface respiration (ASR), the inspiration of surface (1–2 mm) water that equilibrates with atmospheric air, to deal with aquatic hypoxia (Gee et al., 1978, Kramer, 1983, 1987). Additionally, several intertidal species are capable of breathing air (Martin, 1991, 1995, Yoshiyama and Cech, 1994). Both of these respiratory behaviors take advantage of the comparative O_2 richness of atmospheric air and may enhance survival and maintenance in hypoxic water when access to the surface is available (Beitinger and Pettit, 1984; reviewed by Graham, 1997; Martin and Bridges, 1999).

Tidepool dwelling sculpins of the genus *Clinocottus* represent an assemblage of fishes that may routinely experience environmental hypoxia (Yoshiyama, 1981). Field measurements indicate that the dissolved oxygen in tidepools at low tide can decrease to nearly zero in only a few hours (Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983). Such fluctuating dissolved oxygen levels can be a strong force in the selection of physiological tolerances and/or the shaping of respiratory behaviors employed to deal with respiratory hardship. Differences in the ability to cope with low dissolved oxygen, be they physiological or behavioral, can explain the differential distribution of closely related

congeners in the intertidal habitat (Gibson, 1999). For example, Yoshiyama (1981) noted that the highest proportion of mosshead sculpin (*Clinocottus globiceps*) was found in elevated wave-swept tidepools in the mid-intertidal zone, whereas a large proportion of woolly sculpin (*Clinocottus analis*) was found in a broader vertical intertidal range (high intermediate and low tidepools), with small individuals occurring in the highest of habitable tidepools. Higher tidepools should experience more extreme hypoxia than low tidepools because they are the first to be removed from wave action at low tide and the last to be re-inundated with oxygenated water when the tide returns. Because they inhabit tidepools that are more likely to become hypoxic, small woolly sculpins are therefore likely to have evolved physiological tolerance or behavioral mechanisms to counteract decreased oxygen availability. Presumably, these mechanisms would evolve to limit the exposure of these fish to aerial predators. Conversely, mosshead sculpin accustomed to relatively high-oxygen environments would be forced to deal with hypoxia less often. Thus, we hypothesized that mosshead sculpin would be more sensitive to decreased oxygen levels and act to avoid hypoxia at a higher partial pressure of oxygen (PO_2) than woolly sculpin.

Our objective was to compare the responses of the intertidal mosshead sculpin and woolly sculpin to increasing environmental hypoxia. The respiratory behavior of these species has not been quantitatively described and may shed light on their distribution differences over intertidal microhabitats. Relating the distribution,

behavior, and physiology of intertidal fishes to the unique environmental conditions they encounter offers insight into their evolutionary ecology (Gibson, 1999) because adaptive differences in behavior and/or physiology should reflect the differences in selective pressures faced by the two species.

MATERIALS AND METHODS

Animals.—Experiments were conducted during summer and fall of 1996. Mosshead sculpin ($n = 10$) and woolly sculpin ($n = 10$) were captured from rocky intertidal habitat in Horseshoe Cove at the Bodega Marine Reserve in Bodega Bay, California. Animals were captured by dipnetting bailed or unbailed tidepools during low tides. All fish used in the experiment were taken from tidepools of intermediate tidal elevation (0.5–1.2 m above mean low water). Tidepool elevations were estimated by following the tide down and recording the time that each tidepool surface was at sea level. Then, the predicted tide elevations were calculated for the relevant time for each pool. The calculation for the tide height at the time sea level and tidepool surface level matched is considered to be the tidepool tidal elevation (P. Connors pers. comm.). Immediately after capture, animals were placed in a 19-liter bucket containing fresh seawater and transferred to holding aquaria at the University of California, Bodega Marine Laboratory (BML). Aquaria were supplied with a natural photoperiod (38°19'N) and a constant seawater flow (10–12 C, 32 ppt salinity). Fish were maintained on a diet of chopped mussels, live brine shrimp, chopped squid, and algae. After experimentation, fish (no mortalities) were weighed, measured (standard length) and released at their capture site.

Experimental aquarium.—A 98-liter glass aquarium was situated with one end elevated on an indoor BML wet table with natural lighting. The bottom of the aquarium was thus inclined and covered with small cobble for substrate. Three sides of the aquarium were insulated with a jacket of bubble-wrap sheet, and temperatures were maintained via a continuous spray of ambient seawater between the aquarium walls and the insulating jacket. The front (long side) and top of the aquarium were left unwrapped for observations and a grid of 3.5 × 4 cm rectangles was drawn on the front to fix the animal's location on the slope and bottom. Before each experiment, fresh seawater was added to the aquarium to a depth of 24.5 cm at the deep end and 5 cm at the shallow end. Five water-sampling

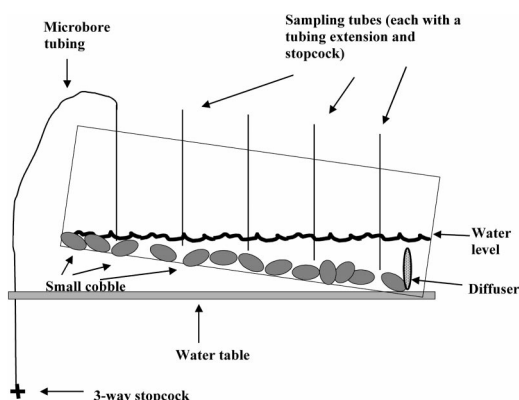


Fig. 1. The experimental aquarium. Nitrogen was introduced to the tank via the diffuser. Each sampling tube was attached to a three-way stopcock for water analysis. The sides and back of the aquarium were insulated with aluminized bubble wrap (not shown), and a grid was drawn on the front of the aquarium to record location of fish (not shown).

tubes (Tygon Microbore) were spaced along the length of the aquarium, 20 cm apart. They sampled water from just above the substrate and ran up, out of the tank. The end from which water was collected hung below the bottom of the outside of the aquarium, allowing water sampling to occur without disturbing the fish. Each water sampling tube was fitted with a blunt needle, three-way stopcock, and syringes for sampling water without atmospheric contamination. The PO_2 of sampled water was measured (nearest mm Hg) using a Radiometer PHM 71/D616/E5046 oxygen analyzer system. Figure 1 graphically depicts the experimental tank.

A dissolved oxygen gradient along the aquarium's long axis was created by slowly bubbling nitrogen into the deep end of the aquarium. Nitrogen was allowed to diffuse slowly out of a porous teflon tube (PTFE Tubing, IMPRA Inc., Arizona) at a rate that caused minimal surface disturbance and was controlled by eye or by using a gas flow meter (Manostat).

Experimental protocol.—The species to be tested in an individual experiment was chosen randomly by flipping a coin, and an individual fish was quickly netted from one of the holding aquaria and placed into the experimental aquarium. Once released into the test aquarium, most fish immediately sought cover among the rocks of the substrate. The fish were allowed to acclimate to the normoxic conditions for at least 40 min before experiments began. Forty minutes appeared to be enough time for fish placed in the aquarium to "settle down" and

show no outward signs of stress such as increased respiration rate. After the acclimation period, initial seawater PO₂ was checked in both the deep and shallow ends of the aquarium, and the experiment was started if initial PO₂s were > 87% air saturation (135 mm Hg). During the experiment, PO₂ was monitored to assess the level of aquatic hypoxia and to identify the PO₂s at which fish exhibited respiratory distress behaviors. Each measurement episode entailed sampling the water for PO₂ (mm Hg) nearest the fish's location, and recording the time, the fish's grid location, and behavioral notes.

Three respiratory distress behaviors were recorded: emergence from the substrate (EFS), first avoidance response (FAR), and ASR. EFS was the action by which a fish first relinquished its hiding spot among the cobble substrate. Although it was difficult to quantify without disturbing the fish, it was obvious that the fish's gill ventilation frequency increased as seawater PO₂ decreased. The FAR constituted the first movement across at least one grid line toward shallower water and higher PO₂. Thus, there is a subtle distinction between EFS and FAR, in that EFS did not require a fish to move toward a less hypoxic region of the aquarium. ASR was the first active inspiration of the water surface, when the fish placed its mouth into the surface meniscus. Seawater PO₂s at the site (along the grid gradient) of the initiation of each behavior were recorded.

We compared the lengths and weights of mosshead and woolly sculpins used in the experiment with a Student's *t*-test. Water temperature at which mosshead and woolly sculpins were tested was compared using a Student's *t*-test. Because we hypothesized that mosshead sculpins would act to avoid hypoxia at higher PO₂s than small woolly sculpins, we used a one-tailed statistical analysis on the behavioral data (Zar, 1996). Data for EFS, FAR, and ASR were checked for equality of variances using Levene's test for variances and the means for each species for each behavior were tested against each other using either Welch's test (in the case of unequal variances) or a Student's *t*-test in JMP for Windows version 4 (SAS Institute, Cary, NC, 2000). Statistical tests were evaluated at a significance value of *P* = 0.05.

RESULTS

Mosshead and woolly sculpins responded differently to encroaching environmental hypoxia. EFS was observed at a slightly higher though not significantly different PO₂ for mosshead sculpins (Welch's test, *P* = 0.062) than for wool-

TABLE 1. MEAN ± SE WITH (*n*) SEAWATER PO₂S (MM HG) AT WHICH MOSSHEAD SCULPIN AND WOOLLY SCULPIN FIRST RESPONDED TO GRADUALLY INCREASING HYPOXIA IN A LABORATORY AQUARIUM.

Species	EFS PO ₂	FAR PO ₂	ASR PO ₂
Mosshead	37 ± 6 (10)	37 ± 6 (10)	26 ± 2 (10)
Woolly	27 ± 2 (10)	26 ± 2 (10)	23 ± 2 (10)

Abbreviations: EFS: Emergence from Substrate, FAR: First Avoidance Response, ASR: Aquatic Surface Respiration.

ly sculpins (Table 1). The FAR was also observed at a slightly higher PO₂ for mosshead sculpins (Welch's test, *P* = 0.0572) than for woolly sculpins. The small sample size resulted in low power (power = 0.3517 and 0.3641 for EFS and FAR, respectively), and taken alone, these results are unconvincing. However, the variance in PO₂ of response among mosshead sculpins (EFS SD = 19.12; FAR SD = 18.20) was much higher than the variance in PO₂ of response among woolly sculpins (EFS SD = 6.82; FAR SD = 7.51) for both EFS and FAR (Levene's test, *P* = 0.0145 and 0.0272, respectively). In contrast, the PO₂s at which both species first showed the most pronounced respiratory distress behavior, ASR, were statistically indistinguishable (Student's *t*-test, *P* = 0.1711). The variance in PO₂ of response for ASR was also indistinguishable between species (mosshead SD = 7.56; woolly SD = 5.55; Levene's *F* test, *P* = 0.2394).

There were no differences in the lengths and weights of fish of the two species used in the experiment (Student's *t*-test for length *P* = 0.66; for weight *P* = 0.57). The average length and weight of mosshead sculpins in the experiment was 60 mm (SD = 8.9) and 5.7 g (SD = 2.1). Woolly sculpins averaged 58 mm in length (SD = 7.4) and 4.7 g (SD = 1.7). All fish were tested at similar temperatures (Student's *t*-test *P* = 0.81). Water temperatures ranged from 9.3 C to 12.6 C in the experiment.

DISCUSSION

Kramer (1987) postulated that a fish would engage in ASR when it could acquire more oxygen at the water surface than it could from aquatic respiration. In this manner, a fish behaves optimally with respect to respiration. If the fish in our experiment were respiring optimally, they would have been expected to switch to ASR much earlier (higher PO₂) than they did because of the higher availability of oxygen in the surface layer of the water (Burggren, 1982). Behavioral decisions, however, are made based

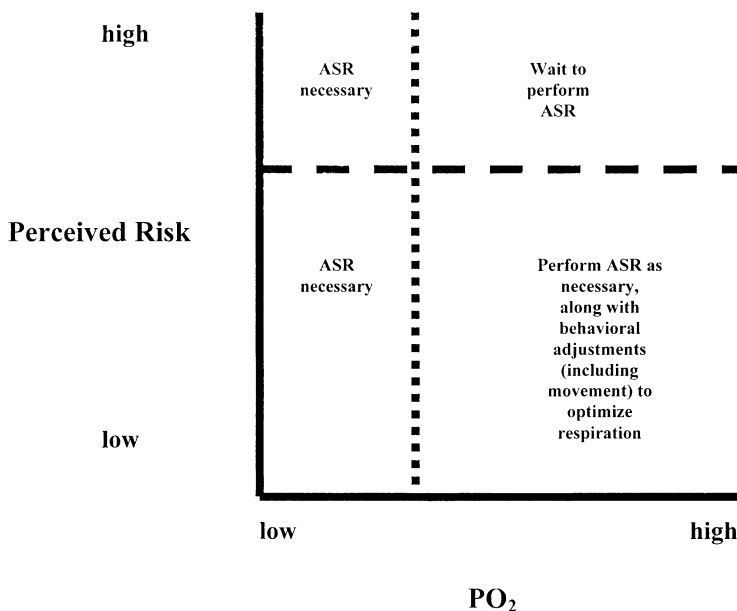


Fig. 2. A graphical model depicting the hypothesized relationship between perceived risk, water PO₂, and aquatic surface respiration (ASR) behavior in tidepool-inhabiting sculpins. At low levels of perceived risk, fish are expected to perform ASR as needed to optimize respiratory oxygen intake. At high levels of perceived risk, fish are expected to perform ASR as demanded by some physiological threshold PO₂ below which the behavior must be performed. At some threshold level of risk, fish are expected to put off ASR until physiological demands provide motivation to perform the behavior.

on complex interactions between perceived risk and physiological demands (Smith and Kramer, 1986; Lima and Dill, 1990). The perceived (high) risk in this study resulted from the exposed, illuminated conditions in the experimental aquarium and the presence of the observer who was visible to the fish. We placed severe physiological demands on the fish by lowering the aquatic PO₂ in the experimental tank. Limiting PO₂s presumably occur where oxygen demand is such that metabolic requirements take precedence over the need to seek cover, and they, therefore, represent a proximate constraint on the distribution of these fishes in the tidepool environment (Beitinger and Pettit, 1984). Additionally, Yoshiyama et al. (1995) indicated that intertidal sculpins were reluctant to emerge for aerial respiration in laboratory aquaria suggesting that the fish perceive a high-risk situation. Our experiment allowed us to examine one side of the trade-off between perceived risk of predation and metabolic requirements. By creating an environment in which the fish would otherwise choose not to perform ASR, we noninvasively determined these limiting PO₂s for two species of sculpin (Fig. 2).

Our experimental design also made it possible to observe the behaviors that may arise as a result of different selective pressures in closely

related species. Although our small sample sizes resulted in low statistical power, it appears that woolly and mosshead sculpins deal differently with encroaching environmental hypoxia. Indeed, our data suggest that these two species have evolved different behavioral responses around a shared physiological constraint (identical limiting PO₂s). Thus, even with a physiological constraint that limits the ability to respire in hypoxic water, the two species are able to respond to similar changes in dissolved oxygen while minimizing different levels of predation risk. For example, the high variance in the response of mosshead sculpins suggests that this species is inclined to deal with hypoxia through subtle behavioral changes. Variation in response in this species would be expected because of the more biologically diverse, lower elevation tidepools in which it lives. These tidepools contain cover and allow mosshead sculpins to optimize respiration through subtle behavioral changes such as moving about to locate and take advantage of areas where dissolved oxygen remains high, while still remaining concealed from predators. The higher elevation pools in which woolly sculpins are found have less cover and represent a "higher risk" environment, for which the less variable "sit-and-wait" behavior for coping with environmental hypoxia is well suited.

In this way, the woolly sculpin may be able to wait out the low tide and avoid exposure to aerial predators altogether.

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