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# The Influence of Substrate Color on the Alarm Response of Tidepool Sculpins (*Oligocottus maculosus;* Pisces, Cottidae)

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## Abstract

For animals that use crypsis to avoid predators, immobility reduces the risk of detection. The magnitude of this immobility benefit depends upon the probability that a predator is present, since a predator must be present for crypsis to be valuable. Thus, cryptic animals typically reduce their movement rates upon detection of a nearby predator or signs of its activity. Such a response occurs in tidepool sculpins (*Oligocottus maculosus*) when presented with water-borne compounds released from the skin of injured conspecifics (HUGIE et al. 1991). The benefit of immobility should also depend upon the animal's background, or substrate, since animals on a matching substrate achieve a higher level of crypticity than those on a nonmatching substrate, and have more to gain by remaining still. Therefore, we predicted that the response of tidepool sculpins to conspecific skin extract would involve a greater reduction in movement rates for fish on sand (matching) than for those on white (nonmatching) substrate. The results of a laboratory experiment supported this prediction, with fish on sand showing a large decrease in movement rates in response to skin extract, while the movement rates of those on white substrate remained unchanged.

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## Introduction

Prey that rely on crypsis to avoid detection use a variety of behavior patterns to enhance the cryptic effect. For example, they commonly choose backgrounds upon which they will appear most cryptic (DONNELLY & DILL 1984; FELTMATE & WILLIAMS 1989; MERCURIO et al. 1985; MOREY 1990; STEEN et al. 1992; review in EDMUNDS 1974). FELTMATE & WILLIAMS (1989), MERCURIO et al. (1985), and MOREY (1990) provide experimental evidence that cryptic animals are less at risk on their chosen substrates than on rejected ones.

injured conspecifics (HUGIE et al. 1991). This "alarm response" includes decreased movement and feeding rates and increased use of cover and burrowing into the substrate (HUGIE et al. 1991), and apparently indicates that the animals perceive a predator to be nearby. In this experiment, water flushed over the lacerated skin of sculpins ("skin extract") was added during trials to increase the subjects' perception of the likelihood that a predator was present.

## Methods and Materials

Sculpins were collected on Jul. 21, 1989, from tidepools at First Beach, on the east side of Trevor Channel, Barkley Sound, B.C., using dip nets and a 120 × 180-cm pole seine. Immediately after collection, the fish were inspected and any injured fish returned to the tidepools. The remaining fish were then transported to laboratory facilities at Simon Fraser University in white, 20-l buckets. In the lab, the sculpins were held in 20-, 40-, and 80-l aquaria with loose sand substrates and fed a diet of brine shrimp (*Artemia salina*) and broken mussels (*Mytilus edulis*). Water temperature was maintained between 11 and 14 °C.

The skin extract was prepared in a single batch on Aug. 9, before the start of the experiment, and 5-ml aliquots frozen. This batch preparation method was used to reduce variation in skin extract aliquot potency caused by differences between donor fish or by differences in length and depth of lacerations (see below). Aliquots required cryo-preservation to prevent the potential deterioration of skin extract compounds. A preliminary experiment performed in Jul. 1989 indicated that freezing did not affect skin extract potency: changes in movement rates in response to fresh and previously frozen preparations did not differ significantly (Mann-Whitney $U = 70.5$, $p > 0.6$, $n = 12$ fish each).

For the present experiment, 10 donor fish (5 males and 5 females, 49—67.5 mm) were used to prepare 22 aliquots of skin extract. Each fish was killed by a blow to the skull, placed in a clean petri dish, and lacerated 50 times on each flank with a clean razor blade. Each flank was flushed with approximately 6 ml of sea water from a 5-ml syringe. The liquid from all 10 donors was poured into a 125-ml Erlenmeyer flask, and stirred at low speed for 1 min using a magnetic stirrer. 22 5-ml disposable syringes were filled, capped, and frozen at −14 °C. The entire procedure took 25 min, and was done in a cold room to minimize decay and evaporation rates.

Before each trial, the entire experimental apparatus was rinsed with hot tap water followed by two rinses with cold sea water. Four 20-l glass aquaria, each divided into two 23.9 × 19.8 × 19.8 cm experimental chambers by a watertight, opaque white wall were used for the tests. Experimental substrates (see below) were placed in the chambers and the chambers filled to a depth of 10 cm with sea water passed through a hobby brewing filter (pore size < 50 $\mu$), to remove all potential food items. Filling was done the day prior to use, to allow the water temperature to equilibrate with that of the cold room (11—14 °C). The tanks were placed in well-lit surroundings with white blinds on all sides. Aquarium airstones, adjusted to provide a moderate bubbling rate, were placed against the rear walls of each chamber, to ensure that the skin extract became well mixed upon addition to the tanks. Skin extract was introduced to the chambers through Tygon tubes leading from behind the blind to the water surface immediately above the airstones.

We built artificial substrates measuring 19.6 × 19.5 cm, designed to cover the entire chamber bottom and allow easy removal for cleaning. The matching substrate consisted of a 3-mm thick layer of plexiglas, to which a solid layer of gray sand was glued using clear silicon sealant. These substrates were used with the plexiglas side on top. The sand appeared grainy and wet through the plexiglas, and provided a good simulation of the colors of loose sand and granite, tidepool substrates upon which *O. maculosus* are commonly found (NAKAMURA 1976; pers. obs.). The sculpins were able to achieve a high degree of color match with this substrate. The nonmatching substrate was also topped with a 3-mm piece of clear plexiglas, but had an opaque white sheet of the same thickness below it. White was used because it is a common substrate color in natural settings (accumulations of barnacle shells, etc.), but the sculpins could not become light enough to match it well.

The experiment consisted of 11 paired trials. Sculpins, starved between 19 and 24 h, were randomly assigned to a substrate treatment and placed singly in the appropriate chamber 2 h (± 5 min) prior to the beginning of trials, to allow acclimation. A minimum of 0.5 h prior to trial

increased slightly from 22.8 ± 3.6 before to 23.4 ± 5.3 after skin extract addition. The movement rate response to skin extract was significantly greater for fish on the sand substrate than for those on the white substrate (Wilcoxon signed-ranks test, n = 11, one-tailed p = 0.007; Fig. 1).

Thus, tidepool sculpins adjusted their movement rates in an adaptive manner in response to cues indicative of the presence of predators. Sculpins on the matching substrate became relatively inactive after detecting skin extract, presumably due to the large benefit of immobility in maintaining crypsis. In contrast, sculpins on nonmatching substrates did not change their movement rate. Against the white substrate, immobility would have provided relatively little improvement in crypticity. Therefore, movement rates did not decrease in response to skin extract, probably due to the lost opportunity costs of immobility. There may even be a benefit to movement for sculpins on nonmatching substrate upon detecting predator cues — it would allow them to search for physical cover, or matching substrate. This may have been a factor causing the sculpins on the white substrate to maintain high rates of movement after detection of skin extract, since there were no prey in the tanks, and thus no real opportunity cost of immobility.

Several other studies, all non-experimental, have examined the influence of degree of crypsis on the response to predators. KETTLEWELL (1973) searched for moths resting on trees in an area of burnt forest and an adjacent area of unburnt forest and found that moths were much easier to find in the burnt area, apparently due to different levels of crypsis on the two types of trees. The moths in the unburnt area "could be approached and captured without eliciting an escape response", while those in the burnt area "without exception . . . took flight on approach and this when I was several yards distant" (ibid. page 73). HEATWOLE (1968) determined the distance to which individuals of two species of anoles (*Anolis stratulus* and *A. cristatellus*) would allow a predator to approach before fleeing. This distance was significantly less for the more cryptic species, *A. stratulus*, than for *A. cristatellus*. Finally, RADABAUGH (1989) examined the response to predator detection by males of three darter species, differing in the degree and nature of color change between the non-breeding and breeding seasons: *Etheostoma flabellare* change very little, *E. blennioides* develop bright green colors, while *E. spectabile* develop intense and contrasting orange, blue, yellow and red areas. Non-breeding and breeding *E. flabellare* and *E. blennioides*, and non-breeding *E. spectabile* all reduced their movement rates after predator detection. However, breeding *E. spectabile* did not show a significant reduction in movement rates. Furthermore, they made more long distance moves after predator detection than any of the other darters. All of these results indicate that the extent to which an animal relies on crypsis during a predator encounter depends on the likelihood of remaining undetected during that encounter.

An unexpected result of this experiment was that prior to skin extract addition, sculpins on the white substrate had lower movement rates than those on the sand substrate. This difference was almost significant (p = 0.061, two-tailed Wilcoxon signed-ranks test). The opposite influence of substrate was expected, since when no predator cues have been detected, searching for matching substrates should be relatively cost-free for sculpins on the white substrate. In fact,

extract had been detected. Thus, referring to one or both of these components as predation risk would lead to confusion regarding the roles of the two manipulations. It is likely that experimental designs like this one, in which two or more components of predation risk are manipulated, have been overlooked because workers have not treated predation risk as a product of several components.

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