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Exploratory II: Crypsis, substrate preference and prey detection in the red octopus, *Octopus rubescens* (Berry, 1952)

Octopus rubescens is a small octopus ranging from Alaska to Baja, California. They are carnivores, eating small crabs, hermit crabs, molluscs and fishes. They are in turn eaten by wolf-eels, lingcod, seabass, rockfishes, flatfishes, scorpionfishes, euphausiids and scuba divers (Taylor and Chen, 1969). When mature they are often found to weigh less than 100 grams. While usually a red-orange color, they are able to go from black to light ochre. As both their predators and prey are often dependent on vision to spot *O. rubescens*, the ability to change shape, color and texture is an important adaptation for this octopus.

The patterns on the dorsal surface of *O. rubescens* are made up of morphological and physiological elements. These elements are chromatophores, iridocytes, leucophores and melanophores. All of these elements are under neural control, and as they are manipulated "a skin-deep view of the cephalopod brain" is revealed (Packard and Hochberg, 1977). Chromatophores produce colors of yellow, orange, brown and red. Melanophores (a type of chromatophore) produce black. Chromatophores are regularly distributed and fill in the spaces between melanophores. A dark cell represents activity in the chromatophore nerve fiber while a light cell represents no activity. Iridocytes can be thought of as "mirror-cells" and are used to literally reflect the background of the octopus. These cells are used to produce colors like blue, yellow and green. Leucophores produce white (Hanlon and Messenger, 1996).

Along with these specialized cells, *O. rubescens* has a flexible soft body and small papilla on its skin enabling it to morph into multiple shapes and textures. All of these components together allow the animal to display rapid, neurally-controlled polymorphisms. The development of search images by predators has undoubtedly had an important influence on the development of polymorphism in octopuses. These patterns are mainly used for crypsis in order to reduce detection. Once detected, however, this ability to quickly change shape and color can also be used to startle a predator or confuse it and thereby reduce its ability to identify the octopus as prey. By displaying multiple morphs, octopuses can increase their "apparent rarity" and thereby reduce encounters with predators who often differentially prey upon the more common phenotypes (Endler, 1991). This ability to quickly change shape and color has also been suggested to be a method of intraspecific communication in octopuses, but most of these animals are solitary and intraspecific signaling is thought to be poorly developed (Hanlon and Messenger, 1996).

Octopus rubescens has the ability to produce 15 patterns: 3 of these are known to be chronic (long-lasting) and the rest of them are acute (Hanlon and Messenger, 1996). This number of patterns is on the low-end as far as rock reef and kelp octopuses go. This may be due to the fact that *O. rubescens* is active mainly at night and it often lives in the intertidal zone where the water is less clear due to turbulence and wave action.

A specimen of *O. rubescens* was found under a rock in the sand at the North Cove of Cape Arago Park. At first glance, the octopus appeared to be some kind of strange worm, as all that was visible was an arm with rows of suckers. When the specimen was first picked up and placed into a plastic bag it inked into the surrounding water. These observations brought to mind several questions. How does such a small organism find prey at night under rocks? Is the octopus able to lift rocks and overcome barriers in order to find and attack its prey? What are the octopus's other modes of defense besides inking? Does it have a preferred substrate to defend itself in? With regards to the first question, it was hypothesized that *O. rubescens* uses its sense of smell, as well as sight when hunting at night under rocks because visibility would be limited in that environment. The second hypothesis was that *O. rubescens* would be unable to lift any of the barriers given to it because all of the containers found in the laboratory to trap prey were thought to be too heavy for the octopus to lift. Also, it was hypothesized that the octopus would be able to use crypsis in any environment it was put into. This was expected because *O. rubescens* has so many complex cells perfectly adapted for the task. Finally, it was hypothesized that the octopus would prefer the fine sand as a substrate because this is the substrate it was found in.

Materials and Methods:

<u>Animals and Tests</u>

The same specimen of *Octopus rubescens* was used in all of the tests. The specimen weighed 15.4 grams, had a 3 cm-long head and 6 cm-long arms. During the week of testing the octopus ate 2 sand shrimp (*Crangon alaskensis*), 2 mole crabs (*Emerita analoga*), 4 purple shore crabs (*Hemigrapsus nudus*), and 2 striped shore crabs (*Pachygrapsus crassipes*). The octopus was kept in a running-seawater tank with an aerator.

Four studies were done with *O. rubescens*: a Y-maze olfactory test, a barrier manipulation test, a substrate crypsis study and a substrate preference study. The Y-maze study was designed to test whether the octopus used smell as well as sight in its search for prey. The barrier manipulation test was designed to test whether the octopus was strong enough (and smart enough) to go after prey separated from it by heavy objects. The substrate crypsis study was designed to test whether the octopus would be able to camouflage itself against all environments given to it, while being chased and while being left alone. Finally, the substrate preference study was designed to test which substrate the octopus preferred, also while being chased and while being left alone.

Y-maze olfactory test

A specimen of *Pachygrapsus crassipes* was placed in one side of the Y-maze and a gentle stream was turned on to let the scent of the crab run through the water maze. The octopus was placed at the other end of the maze and a long piece of plexi-glass was placed on top of the maze to prevent the octopus from escaping. Three trials, each lasting ten minutes, were conducted. The activity level and placement inside the maze of the octopus were noted.

Barrier manipulation test

Five glass containers were placed inside the home tank of the octopus, each of them filled with seawater and a specimen of *Emerita analoga*. Two of the containers were small watchglasses (4.8 cm in diameter and 2.5 cm deep). Each of them weighed 47.20 grams. These were flipped upside-down on the bottom of the tank, trapping a mole crab underneath. Another one of the containers acting as a barrier between the octopus and its prey was a small glass vial (6 cm long and 2 cm wide). A mole crab was placed inside the vial and enough seawater was allowed into the vial so that it would sink to the bottom of the tank. A 50 ml beaker was also placed upside-down into the tank with a mole crab trapped underneath. Finally, a petri dish with two mole crabs inside was placed at the bottom of the tank with a rock holding it closed. These containers covering mole crabs were left inside the tank for a week. They were checked every day to see if they had been overturned or if the mole crabs had died.

Substrate crypsis study

A plastic box measuring 38 cm long, 25 cm wide and 11 cm deep was used as the container for all substrate trials. The box was filled up to 7 cm deep with seawater. While the trial was running an aerator was placed in a corner of the box. Each trial lasted 20 minutes and every morph change was noted. Two trials were run for each substrate and halfway through each trial the octopus was agitated with the plastic net used to remove it from its tank. Enough substrate was placed in the box to cover the bottom. There were six substrates used in the study: branching coralline algae (*Calliarthron sp. Corallina sp.* And *Bossiella sp.*), black pine (*Neorhodomela larix*), feather boa kelp (*Egregia menziesii*), grainy black and white sand, dark fine sand, medium-sized rocks and light blue aquarium rocks. The species of coralline algae used are full of branches and ranges from a pink to a deep-purple color. Black pine is brownish-black and it has brush-like branches. Feather boa kelp is olive green and brown with irregular branches along its stalk. A trial was also run in the empty plastic box, using the creamy color of the water table reflecting through the sides. The morphs of the octopus while it was inside its home tank were also noted. An attempt was made to catalog all 15 morphs that had been cited in previous literature (Hanlon and Messenger, 1996).

Substrate preference study

The same box as used in the substrate crypsis study was used for these trials as well. The box was divided in half the long way with a piece of plexi glass and one substrate was poured into each side. Once the substrates were in place, the box was filled 7 cm deep with seawater. The substrates compared were: light blue aquarium rocks vs. grainy sand, grainy sand vs. rocks and grainy sand vs. fine sand. At the beginning of each trial the octopus was taken from its tank and placed in the middle, on the line between the two substrates. Each trial lasted for 10 minutes and it was observed which half of the box the octopus spent more time in. Halfway through each trial the octopus was agitated with the plastic net. Each comparison was run for six trials each.

Observations and Results:

The results of the Y-maze tests were inconclusive. The octopus was very active in the Y-maze and swam back and forth through both sides of the maze until the end of the ten-minute trial every time. At the end of each of the three trials *O. rubescens* sat at the end of the maze with the crab and stared at it, trying to touch it with its tentacle through a hole in the dividing wall. *Pachygrapsus crassipes* did not seem to be noticed by *Octopus rubescens* until this end point when the octopus crouched down in the corner next to it.

The octopus opened two of the five pieces of glassware covering mole crabs (Table 1). The two opened pieces were both small watch glasses. The first small watchglass was found tipped up on a rock and the remnants of the mole crab were found on the other side of the tank. The second empty watchglass was found in the same position it had been left in except the mole crab was missing. Its remnants were also found in the other corner of the tank. The jar with the twist-off cap and the petri dish and lid held shut with a rock were left unopened by the octopus. The 50 ml beaker was turned over by the mole crab.

Container	**State of container after one week**
Small watchglass	Open and mole crab eaten
Small watchglass	Open and mole crab eaten
Petri dish with lid	Closed, mole crabs alive
50 ml beaker	Opened by mole crab
Small jar with twist-off cap	Closed, mole crab dead

Table 1: results of the week-long barrier manipulation experiment in which *O. rubescens* was only able to access mole crabs under the small watchglasses

The results of the substrate crypsis study support the idea that the octopus is able to use its chromatophores and leucophores to blend in with its substrate, but no use of iridocytes was ever observed. The octopus was able to change color and texture instantaneously. No distinct pattern was observed in the usage of the different morphs observed, but a few of the morphs seemed closely tied to specific activities or levels of agitation (Table 2).

Morphs observed	**Substrate(s) where observed: Activity**
Light tan, non-papillate	All substrates: swimming, *O. rubescens* switched to this morph immediately after being touched by the net in all substrates
Brown with black and white spots, papillate	Black Pine *Neorhodomela larix*: Disturbed by net; Home tank on rocks
Cream-colored with eye-rings, non-papillate, "clouds" of dark brown float over head region	Coralline algae *Calliarthron sp. Corallina sp.* and *Bossiella sp.*; empty box: crouching in box
Dark brown and papillate	Black Pine *Neorhodomela larix*: hiding under seaweed, when initially disturbed
Beige with dark brown "zebra" stripes	Empty box, Black Pine *Neorhodomela larix*: resettling after being disturbed by net
Half brown and half cream down the middle (brown half papillate)	Black Pine *Neorhodomela larix*; empty box; grainy black and white sand: toward end of timed trials after being disturbed
Beige with dark spots, eye-rings blinking	Grainy black and white sand; light blue aquarium rocks: always with arms curled up and head flattened against arms, right after inking
Dark maroon with beige spots on head	Home tank: hiding in upper corner near lid
Dark maroon	Home tank: flattened posture on rocks
Dark brown with large rings of white, papillate	Large rocks: upright in corner
Olive Green	Caught by net

Table 2: All of the morphs observed during the substrate crypsis trials: a subtle pattern in morph and activity/agitation level was observed

In the substrate preference tests *O. rubescens* showed a preference for the grainy black and white sand most often (Table 3). However, tests comparing the grainy and the fine sand were inconclusive.

Substrates compared	Results
Grainy black and white sand vs. light blue aquarium rocks	Chose grainy sand 83% of the time
Grainy black and white sand vs. large dark rocks	Chose grainy sand 100% of the time
Grainy black and white sand vs. fine dark sand	Chose grainy sand 50% of the time

Table 3: Percentage of trials (6 total) in which the octopus chose the grainy sand substrate which was chosen most often

Discussion:

It was hypothesized that *O. rubescens* is able to detect its prey through olfaction as well as sight, using the sensory organs on its suckers to "taste" the water. This hypothesis was not supported. From the Y-maze test *Octopus rubescens* showed no indication of using olfaction in detection of the crab, *Pachygrapsus crassipes*, but this is most likely because the octopus was so frightened and agitated by its new environment which did not supply any crevices for hiding. Also, it is known that octopuses rely mainly on their sight for prey detection and it is probable that the octopus was able to see the crab even from the beginning of the maze, but hunting was not on its mind. The maze seemed to be too small for such an intelligent and quickly moving organism. In order to adequately test this hypothesis the octopus would have to be put into an environment where it felt less threatened and where it would be unable to find the prey item through sight.

It was also hypothesized that *O. rubescens* would not be able to open any of the containers because they were too large for such a small specimen to handle. This hypothesis was not supported as two of the watchglasses were overturned. They each weighed three times more than the octopus itself, so this feat is rather remarkable. Also, the diet of the octopus was supplemented with *Hemigrapsus nudus* and *Pachygrapsus crassipes* so the octopus was not too desperate for food and may not have tried too hard, if at all, to open the other containers. The details of the overturnings of the containers and the possible attempts made to overturn the others were not observed because *O. rubescens* is a shy, nocturnal animal and spent most of the day crouched in a corner, avoiding the light.

The hypothesis concerning the use of crypsis by *O. rubescens* was partially supported. The octopus specimen demonstrated the ability to camouflage itself with all of the substrates it was presented with except it seemed unable to match the light blue aquarium rocks it was given as a substrate. This may be due to the fact that the octopus was scared, or its apparent inability could be a result of the substrate being unnatural and thus something the octopus had never seen before. It is more likely, however, that the octopus did match the substrate, but not with color. It has been noted before that octopuses often match their substrate in brightness more often than in color. This is a logical adaptation because octopuses and many other marine organisms are in fact color-blind. As their eyes do not see that same way ours do, the octopus may have been using cryptic patterns much more often than I thought it was. Also, light levels and distances from prey and predators play major roles in the visibility of an octopus. No use of iridocytes was ever observed, except for a small patch of shimmery-green under each eye.

In addition, many of the pattern morphs observed in *O. rubescens* are disruptive in coloration (Table 2). This breaking-up of the outline of the body makes its appearance even less distinct to its predators and prey. The startling pattern observed where *O. rubescens* had a head which was exactly half cream and half papillate brown had been observed before by Boycott (1953). He termed this pattern dymantic and described it as a fright response posture, when the animal was undecided as whether to attack or escape. It has also bee previously found that individual octopuses have stereotyped patterns that are often exhibited under the same set of conditions. It was observed that the *O. rubescens* specimen turned dark red-orange every time it was released back into its tank after a trial was run (Table 2). Also, the octopus turned tan when it began to swim. Once, while it was being harassed with the plastic net the octopus inked in the plastic box. Right after inking it turned beige and its eye-rings blinked on and off for a while. Warren also found that color changes in *O. rubescens* are often tied to locomotion and postural adjustments (1974).

The ability to produce rapid, neurally-controlled polymorphisms is important to *O. rubescens* because they spend their early months as plankton, drifting into all sorts of different habitats. An octopus has to be able to find different prey and avoid different predators, depending on where it settles out as a larva. Without this ability to become different, survival would be a significant challenge.

The last hypothesis, that the octopus would prefer the fine sand substrate, was not supported. Instead, it appears that the octopus prefers the grainy black and white sand. This may be because the octopus is better able to camouflage itself in this substrate than in the uniformly dark sand. This would be especially important to the octopus in the environment it was presented with: full daylight with no crevices to crawl into.

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