ONTOGENY OF DEFENSE:
DOES LIFE HISTORY AFFECT PREDATOR RESPONSE BEHAVIOR IN THE PYGMY OCTOPUS, *OCTOPUS BOCKI*?

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Abstract. Organisms experience physiological and ecological changes during ontogenesis, and studies have shown that such changes have an impact on behavior over the life cycle. However, little is known about how octopus behavior changes during ontogeny. The pygmy octopus, *Octopus bocki* (Adam 1941), expresses differences in chromatophore development and mantle length between developmental stages. These changes may be important in predator defense, therefore I hypothesized that predator response behavior also changes over the life cycle. Timed interactions between an octopus and a fish predator were used to compare the behaviors and color displays exhibited by three different size categories of *O. bocki*; juvenile, sub-adult, and adult. Color display diversity was analyzed using the Shannon-Weiner diversity index. The analysis revealed a negative correlation between color display diversity and size during predator interactions, supporting the hypothesis that behavior changes over the life cycle. Counter-intuitively color display diversity decreases while chromatophore development increases. To broaden the context in which behavior was examined, interactions between adult *O. bocki* were used to investigate how adults use color displays. The number of color/texture combinations was graphed to compare displays expressed during predator interactions with those expressed during intraspecific interactions. A Wilcoxon test revealed that adult *O. bocki* used significantly more color and texture displays during intraspecific interactions than during predator interactions. I concluded that adult octopuses use color displays for communication rather than predator defense and that these displays change during ontogenesis.

Key words: ontogeny; behavior; communication; Octopus bocki; *pygmy octopus; predator response; Moorea, French Polynesia*

INTRODUCTION

Predator avoidance is critical during any stage in an organism's life. Juvenile animals that cannot depend on parental protection are under strong selection pressure to exhibit fully developed predator avoidance behavior at an early age (Impekoven 1976, Miller and Blaich 1986, Göth 2001). Because some organisms change drastically as they age, altering size, appearance, and even habitat, that predator response behavior may also change during the life cycle is not surprising. The ontogenetic aspect of behavior has been studied in social organisms such as mammals, birds, and insects (Pongracz and Altbacker 2000, Mondor and Roitberg 2002), however little is known about how behavior changes over the ontogenetic stages of solitary marine invertebrates.

A cephalopod's life cycle involves changes in body size and chromatophore number and density (Packard 1985). These physiological changes may influence predator response behavior; for example, Hanlon and Messenger (1988) suggest that the use of chromatophores and iridophores differs between juvenile and adult cuttlefish, *Sepia officinalis* (Linnaeus 1758). Juvenile cuttlefish were more likely to exhibit inappropriate body patterning when placed on a uniform background than adults. Like cuttlefish, octopuses also have the ability to change color, pattern, and texture, yet little is known about the ontogenetic changes in octopus behavior.

In order to study the ontogeny of octopus predator response behavior, *Octopus bocki* (Adam, 1941) was used as the model species. This species is a pygmy octopus commonly found in shallow coral rubble on reef crests around Moorea, French Polynesia. Because *O. bocki* reproduces year-round (Cheng 1996), it was possible to collect juvenile, sub-adult, and

adult individuals simultaneously, facilitating life history studies.

During preliminary observations, adult *O. bocki* exhibited a large variety of possible color patterns while sub-adults and juveniles were more limited in the range of displays they could produce. This physiological difference reflects Packard's (1985) findings that chromatophore number and density increases over the life cycle of cephalopods. These observations led to the hypothesis that adult *O. bocki* will show a wider range of color patterns in response to a predator than immature individuals. My goals were to determine whether ontogeny affects the way in which an octopus responds to a fish predator, and also to investigate how color patterns and texture are used in *O. bocki*.

METHODS

Octopus collection

The specimens for this study were collected from three sites: the reef by the village of Maharepa and two locations on the Vaipahu reef crest (Fig. 1). These sites were chosen based on the abundance of *O. bocki* found in previous studies (Pearson 1994, Cheng 1996).

Site 1 is close to the Gump Research Station and very shallow (0.5 m) with many exposed slabs of coral rubble. Sites 2 and 3 are deeper (3 m) and had to be accessed by boat. At Site 1, four large garbage cans were placed on flat exposed coral rubble on the reef crest at wading distance from each other. A non-porous cinderblock was placed at the bottom of each can. Pieces of coral rubble were then selected from the surrounding crest and stacked in the cans. The rubble drained for at least 30 minutes and no more than 45 minutes, allowing the octopuses and other organisms to crawl into the shallow pool of water that collected at the bottom of the can. After 30 minutes the rubble was removed and replaced on the reef. Octopuses and potential food items such as crustaceans were collected in sealed containers and transported to the laboratory.

Octopus care

A dissecting microscope was used to determine sex (by the presence of the hectocotylized third right arm on males) and mantle length (from the center of the eyes to tip of mantle). The octopuses were separated into three categories based on mantle length:

juvenile (five mm or less), sub-adult (six to nine mm), and adult (ten mm or larger). Juveniles and sub-adults were kept in plastic cups and adults were kept in Tupperware containers. Each cup or container was covered with a lid to prevent escape. Seawater in the cups and containers was taken directly from the ocean and changed twice daily. Adults and sub-adults were fed crustaceans collected from the rubble, and juveniles were fed plankton or minute crabs each night. Factors such as temperature, light, salinity, and pH remained uniform for all animals.
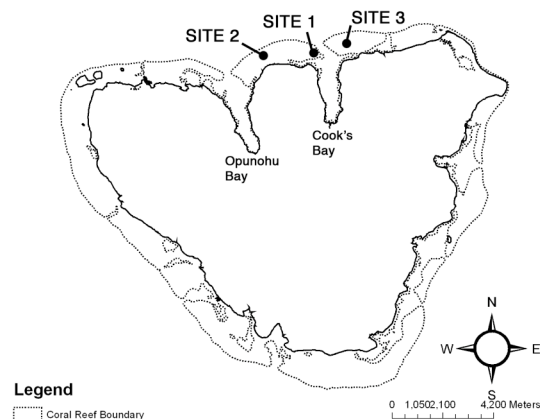

FIG. 1. Three collection sites were located on the Vaipahu reef crest (Sites 1 and 2) and the Maharepa reef (Site 3), Moorea, French Polynesia.

Response to predators

A transparent 19-liter observation tank was used for experimentation. The sample size was 21 - seven octopuses per age group. An octopus was placed in a small empty transparent jar with many holes to allow water flow, and set in the middle of the tank. Opaque barriers surrounded the observation tank so that no outside stimuli could disturb the octopus. A small window (eight x five cm) was cut to allow observation with minimal disturbance. The octopus acclimated for at least 30 mins, at which time a fish was added to the tank for a ten minute timed trial. I assumed that any species of fish is perceived as a predator to the octopus, whether it naturally eats octopuses or not. The species of fish used in these experiments was the blacktail snapper, *Lutjanus fulvus* (Scneider 1801). During the ten minute trial,

observations on the movements, color changes, texture, and other behaviors such as inking were recorded using voice recording software. These behaviors along with descriptions of each are listed in Table 1. The fish were kept up to four days before release. The order of experimentation was randomized using Excel's random number generator, and the test was repeated three times per octopus. Experiments took place during the day, and to avoid stress no single octopus was tested more than once per day.

TABLE 1. Names and descriptions of behaviors observed during interactions between *O. bocki* and a fish predator.

Behavior	Description
Colors	
Red	Red chromatophores contracted on mantle and/or arms
White	No chromatophores contracted
Red With White Spots	Chromatophores contracted on mantle except at certain locations
Flickering Red	Chromatophores rapidly contract and relax on mantle and/or arms
Flickering Blue	Iridophores rapidly contract and relax near eyes or on mantle
Flash Red	Chromatophores contract once simultaneously on mantle and arms
Textures	
Smooth	No papillae on mantle, eyes, or arms
Mantle Papillae	Small papillae on mantle
Eye Papillae	One papilla above each eye
Movements	
Still	Mantle location does not change
Crawling	Mantle location changes
Positions	
Normal	Mantle is relaxed, arms are resting
Ballooning	Mantle rises above the substrate and web forms a "balloon" or "parachute" position
Other	
Inking	Octopus releases a pseudomorph or diffuse ink

Response to intraspecifics

To observe chromatophore usage during intraspecific interactions in adult *O. bocki*, an adult was added to the home container of another adult. Hiding places such as coral pieces or shells were removed from the home container before the second adult was added. The different colors and textures displayed by the resident adult were observed and recorded for five minutes. Testing was performed during the day, and each adult was observed interacting with every other adult.

Data analysis

The average percent time each age group spent exhibiting different color patterns during predator interactions was graphed for comparison. The predator response data were then analyzed using the Shannon-Weiner diversity index. This test allowed for comparison of the color pattern diversity across a size gradient. In order to find correlations between color display diversity and mantle length, a bivariate fit analysis of the average H' values was run using JMP 5.1 (SAS Institute Inc. 2003).

A count of the number of color and texture combinations was totaled to compare the displays exhibited by adults when encountering a predator to the displays exhibited by adults when encountering another adult. A Wilcoxon test was then run using JMP 5.1 to find significant differences between the number of colors and textures displayed during intraspecific interactions and predator interactions.

RESULTS

Response to predators

During interactions between the octopus and fish a total of 14 behaviors were observed, and six of these behaviors were color patterns. The average percent time each age group spent displaying each color pattern is graphed in Fig 1.

The Shannon-Weiner diversity index analysis compared color pattern diversity across a range of octopus mantle lengths. A bivariate fit of the average H' values showed a significant correlation between color display diversity and mantle length ($R^2 = 0.65$, $p = 0.003$). These results are shown along with a linear fit line in Fig. 2.
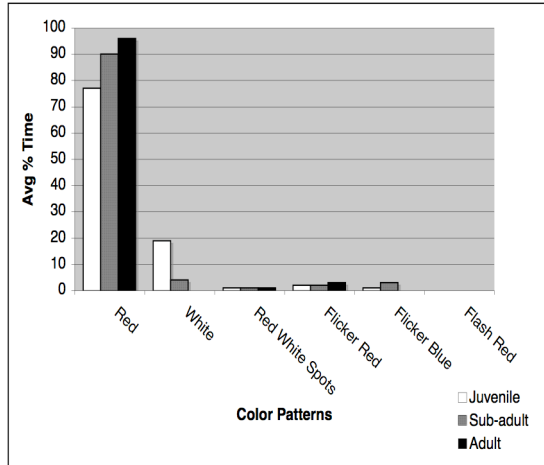
FIG. 1. The average % time each age group spent displaying each color pattern during predator response trials.
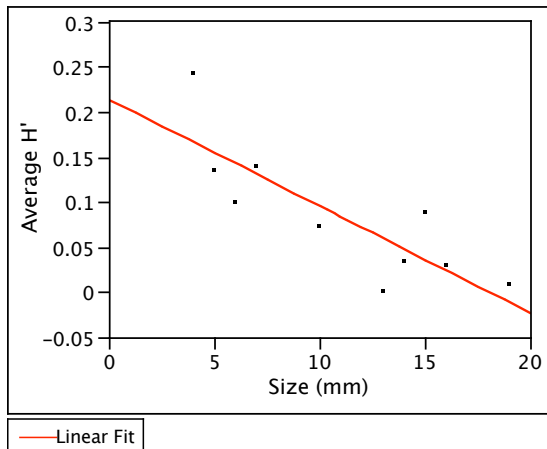

Linear Fit

FIG. 2. Bivariate fit of average H' values is graphed by size (mm) ($R^2=0.65$, p=0.003), illustrating a negative correlation between octopus size and color display diversity in response to predators.

Response to intraspecifics

During adult intraspecific interactions, nine color patterns, four textures, and three body positions were observed. I present a comparison of behaviors that were seen in predator interactions with those seen in intraspecific interactions in Table 2. Three colors, one texture, and two body positions were observed which were not seen in predator interactions. Iridophores, producing blue iridescent spots, were displayed on the mantle and at the base of the arms. A single papilla was also sometimes observed on the tip of the mantle. The two new postures

observed in predator interactions were "erect mantle" and "clubbed mantle." The mantle was described as erect when it was elongated and held upright. A "clubbed mantle" described an erect mantle with a large, rounded end. In Fig. 3, I compare the average number of color/texture combinations of each individual adult recorded during predator interactions to those recorded during intraspecific interactions. A color/texture combination was defined as each unique pair of behaviors, one color and one texture, displayed simultaneously. For example, a common color/texture combination was red/smooth. The results of the Wilcoxon test found a significant difference in the number of different color/texture combinations displayed during predator interactions and intraspecific interactions (p < 0.0001).

TABLE 2. A list of the colors, textures, and positions expressed by adult *O. bocki* during predator interactions and intraspecific interactions. An "X" indicates observed behaviors and "-" indicates that the behavior was not observed.

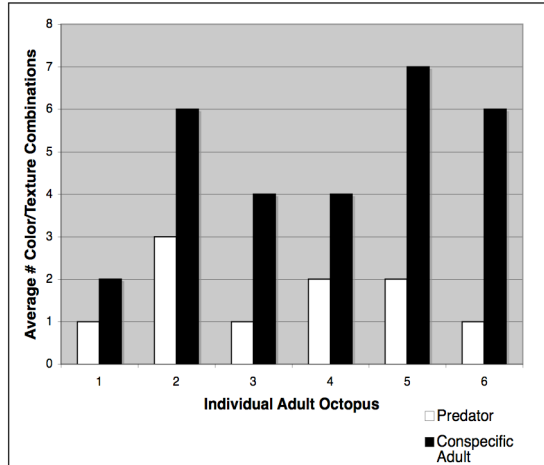|	Predator	Intraspecific
Color		
Red	X	X
Flickering Red	X	X
Flash Red	X	X
Red With White Spots	X	X
Red With Blue Mantle Spots	-	X
Red With Blue Arm Base Spots	-	X
White	X	X
White With Blue Mantle Spots	-	X
Flickering Blue	X	X
Texture		
Smooth	X	X
Mantle Papillae	X	X
Eye Papillae	X	X
Mantle Tip Papilla	-	X
Position		
Normal	X	X
Ballooning	X	-
Erect Mantle	-	X
Clubbed Mantle	-	X
Total	11	16

FIG. 3. Average # of color/texture combinations expressed during predator interactions (light bars) and intraspecific interactions (dark bars). The number of displays given during intraspecific interactions is significantly different than during predator interactions (p < 0.0001).

DISCUSSION

The resulting H' values of the Shannon-Weiner diversity index are strongly correlated with size. There was a negative correlation between mantle length and color pattern diversity (Fig. 3). Thus, as the octopus grows, the diversity of color patterns that it displays behaviorally when encountering a predator decreases linearly. These results are opposite of the proposed hypothesis that more mature *O. bocki* will display more color variations, yet support the general hypothesis that ontogeny affects predator response behavior.

The trend found in the Shannon-Weiner index prompted further exploration into how color patterns are used in adult *O. bocki*. From preliminary observations, this species has the ability to display more patterns as an adult than as a sub-adult or juvenile because chromatophores are less developed in immature octopuses (Packard 1985). However, the increase in color pattern ability as this species grows does not result in an increase in the color diversity displayed when the octopuses interacted with a predator.

The results of intraspecific interaction experiments between adult *O. bocki* suggest that color patterns are used for communication rather than predator deterrence (see Table 2 and Fig. 4). The numbers of colors, textures, and positions observed in trials where two adults were allowed to interact are greater than those observed in predator interactions (Table 2). I graphed the number of color/texture combinations for each individual (Fig. 5). In every adult, the number of color/texture combinations displayed is greater by at least double in the intraspecific interaction than in the predator interaction. I included texture in this graph because, although textures were rarely observed during predator interactions, textures such as mantle papillae were common during intraspecific interactions. The results of a Wilcoxon test showed a highly significant difference between displays observed during intraspecific interactions and predator interactions.

Moynihan (1985) defines systems of communication as the transmission and recognition of signals, which encode specific information. Octopuses and other coleoid cephalopods have an amazing ability to communicate by sending visual signals through the use of chromatophores (Moynihan 1985). Communication, rather than noncommunication (or crypsis) or aposematic display, provides an explanation supported by the results of this study. The adults use their developed chromatophores for intraspecific communication, such as sexual or hostile displays, rather than for predator deterrence.

This conclusion helps to explain why adults display less color diversity than juveniles and sub-adults when faced with a predator. One possible explanation for the trend found in Fig. 2 is that juveniles and sub-adults do not need to use their chromatophores for communication. They are not sexually mature, therefore intraspecific interactions are not an important part of their lives. The color patterns used for communication may still be developing during these early life stages, but have not been differentiated toward the correct behavioral context.

Hanlon and Messenger (1988) proposed that the cuttlefish, *Sepia officinali,s* demonstrated a shift from crypsis to communication during the life cycle. All age groups of *O. bocki* in my study exhibited red coloration for the majority of predator tests (Fig. 1), however juveniles and sub-adults exhibited red for a smaller percentage of time than adults. *O. bocki* is a nocturnal species, and red is a difficult color to detect at night. Therefore, red may represent the cryptic or normal coloration of this species. Thus, Hanlon and Messenger's (1988) study is not

supported by my data, as juveniles and sub-adults showed less cryptic coloration than adults (Fig. 1). While there is not a shift from crypsis to communication in *O. bocki*, there does seem to be a shift toward communication over the life history of this species. Furthermore, there seems to be a shift from less specific to more specific differentiated behavioral responses to certain situations as an octopus matures.

The results of these predator and intraspecific interaction experiments demonstrate the effects of ontogeny on the behavioral displays of pygmy octopuses. Display behaviors change as the octopus grows, and these changes may be attributed to sexual maturity and the development of chromatophore usage in intraspecific communication.

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