Anti-predator behavior of squid throughout ontogeny

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ABSTRACT

Squid utilize an array of complex anti-predator behaviors, which provide advantages to avoid high predation pressure. Although there are morphological and ecological differences among paralarval, juvenile and adult squid, no studies have examined how anti-predator behavior changes through life history stages in cephalopods. The goal of this study was to (1) document how anti-predator behavior in squid changes throughout ontogeny and (2) measure kinematic variables associated with squid-predator interactions to analyze the behavioral cues that trigger anti-predator responses. Anti-predator responses in squid were studied in a series of predator-prey trials using high-speed videography. All life histories of squid exhibited some escape jetting in response to predators, but paralarvae exhibited far fewer escape responses relative to juveniles and adults, and did not demonstrate posturing or inking behavior. For 65% of the interactions, paralarvae used stereotyped behaviors such as swimming in repetitive circles and spirals, rather than escape jetting, and clear body patterning during predator encounters. Inking responses in juveniles and adults were associated with significantly higher predator approach velocities and closer predator-squid distances when compared with postural responses. Older squid were more likely to demonstrate the banded body pattern, and the 'pseudomorph' ink shape was most commonly used. The observed differences in anti-predator behavior throughout ontogeny indicate that paralarvae rely on stereotyped swimming behaviors and translucent coloration to avoid capture, while juvenile and adults use kinematic cues of the predator approach to determine whether posturing, inking and escape jetting, or a combination of the two is the most suitable anti-predator behavior.

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1. Introduction

Throughout their lives, squids are prey targets for many marine predators, including fish, marine mammals, sea birds, and even other cephalopods, making them an integral component of marine food webs (Clarke, 1996; Mather, 2010; Piatkowski et al., 2001; Wood et al., 2008). An array of complex behaviors has evolved as components of anti-predator responses in squids (Hanlon and Messenger, 1996). Along with a jet-driven escape, a widely used strategy for predator evasion in cephalopods is camouflage (Barbosa et al., 2008; Hanlon and Messenger, 1996; Messenger, 2001). Postural displays are also commonly used to deter a predator from attacking (Bush et al., 2009; Hanlon et al., 1999; Huffard, 2006), and inking is often employed to confuse oncoming predators and allow time for escape (Bush and Robison, 2007; Hanlon and Messenger, 1996; Wood et al., 2010). Collectively, these responses provide a wide behavioral repertoire for predator avoidance.

The ability of squid to change body patterning and color quickly is central to their camouflage and postural display strategies (Hanlon and Messenger, 1996). Chromatophores, the organs largely responsible for color change and body patterning, contain a large compartment of pigment granules (Florey, 1966), including those that are yellow, orange, red, brown and black, with the pigment color combination varying with species (Fingerman, 1970; Messenger, 2001). Each organ contains an elastic sacculus with pigment granules and is surrounded by a series of 15–25 radial muscles to contract and expand the chromatophore (Messenger, 2001). These muscles are under nervous control and therefore expansion and contraction can occur rapidly and selectively to create a wide variety of patterns (Hanlon and Messenger, 1996; Messenger, 2001). The complexity of patterns that squid can produce is correlated to their habitat complexity, with species living in coral reefs, rock reefs or kelp showing the highest number of chromatic components and pattern combinations (Hanlon and Messenger, 1996). Different body patterns have also been described during mating, antagonistic displays and predator avoidance (Barbato et al., 2007; Hanlon and Messenger, 1996; Hanlon et al., 1994, 1999). Furthermore, squid perform a wide repertoire of deimatic behaviors that involve chromatic, postural and locomotor components, which are intended to signal a warning to a predator (Cornwell et al., 2009; Hanlon and Messenger, 1996; Hanlon et al., 1994, 1999; Jantzen and Havenhand, 2003; Staudinger et al., 2011).

In addition to crypic behavior, inking events are often employed to maximize the effectiveness of an escape response by confusing predators (Hanlon and Messenger, 1996; Staudinger et al., 2011; Wood et al., 2010). A typical response to a predation threat is the

"ink-blanche-jet" maneuver, during which the cephalopod ejects ink as it jets away and blanches white (Hanlon and Messenger, 1996; Hanlon et al., 1994). Inking can occur in several forms, such as a 'pseudomorph', which is a blob of ink that is held together by mucus and approximates the volume of the cephalopod, serving to distract a predator while the animal swims off. Another method is to create a cloud of ink behind which the cephalopod can disappear (Bush and Robison, 2007; Hanlon and Messenger, 1996). Several other shapes of ink release have been observed, including 'ropes' and 'puffs' (Bush and Robison, 2007). Squid ink also contains chemicals such as L-dopa and dopamine that elicit escape responses in nearby conspecifics (Gilly and Lucero, 1992; Lucero et al., 1994; Wood et al., 2008, 2010). Not only do the chemicals in this ink potentially warn conspecifics, they may block olfactory or taste receptors in predators, causing them to abandon their approach (Caldwell, 2005; Hanlon and Messenger, 1996).

Although the various anti-predator behaviors have been well studied in some species of adult squid, little is known about how squid respond to threats throughout ontogeny. Cephalopods undergo major morphological and morphometric changes throughout their life and alter their ecological niches (Boyle and Boletzky, 1996). While cephalopods do not experience a distinct metamorphosis, and therefore do not have true larva, hatchlings are ecologically distinct from older life history stages (Robin et al., 2014; Shea and Vecchione, 2010; Young and Harman, 1988). The term 'paralarva' is used instead of 'larva', and is defined as a newly hatched cephalopod that has a unique mode of life from the adults, often with an endpoint identified by changes in morphological characteristics (Shea and Vecchione, 2010). Moreover, relative to the adult, paralarvae have a more rounded mantle, relatively smaller arms, a proportionally larger funnel, and rudimentary fins (Boletzky, 1974; Okutani, 1987; Packard, 1969). Ecologically, paralarvae differ from older squid in that they cover shorter overall distances by active swimming driven primarily by the jet (Bartol et al., 2009a), move through the water column in diel vertical migrations (Boyle and Boletzky, 1996; Robin et al., 2014), and reside in an intermediate Reynolds number (Re) regime (Re ~ $1–10^2$) (Bartol et al., 2008, 2009a; Thompson and Kier, 2002; Webber and O'Dor, 1986). Conversely, many juvenile and adult squids are capable of powerful and long distance locomotion covering significant horizontal distances, generally employ less vertical migratory behavior, though there are certainly some species that undergo significant vertical migrations (Boyle and Rodhouse, 2008), and operate in a higher Re regime (Re ~ $10^3–10^6$) (Bartol et al., 2009b; O'Dor, 1988). Paralarvae squid also have largely transparent bodies with relatively fewer chromatophores than juvenile and adult stages (Messenger, 2001; Okutani, 1987), suggesting they likely use camouflage differently than juveniles and adults. Additionally, the brain volume of squids increases exponentially with age and different regions of the brain develop at distinct points through life ontogeny (Kobayashi et al., 2013).

Although it is clear that there are large physical, behavioral, and ecological differences in the life history stages of squid, few studies have examined how anti-predator behavior changes from paralarvae to adults. The goal of this study was to (1) document how chromatic patterning, posturing and inking in squid change in response to predators throughout ontogeny and (2) measure kinematic variables associated with squid–predator interactions to better understand the behavioral cues that trigger anti-predator responses.

2. Material and methods

2.1. Animal collection and maintenance

This project was conducted in accordance with Old Dominion University's Institutional Animal Care and Use Committee (Protocol #12–016). Paralarval *Doryteuthis pealeii* (dorsal mantle length

(DML) = 0.18 cm) and juvenile/adult *Lolliguncula brevis* (DML = 3.0–7.0 cm) were used for this research. Little information is currently available on the breeding habits of *L. brevis*, and they are extremely difficult to obtain as hatchlings. Therefore, *D. pealeii* was selected to study early ontogenetic stages. *D. pealeii* is a reasonable substitute for *L. brevis* because both species have similar body size, fin size and shape, and ecological niches as paralarvae (Bartol et al., 2008). Additionally, juvenile and adult *L. brevis* demonstrate similar body patterning to juvenile and adult *D. pealeii* (Hanlon et al., 1999).

D. pealeii paralarvae were purchased from the Marine Biological Laboratory, Woods Hole, MA, and maintained in a recirculating seawater system at a salinity of 30–32‰ and at temperatures of 19–24 °C until hatching. *L. brevis* used in this project were captured by otter trawl in Wachapreague, VA, USA. Trawls were conducted in August, September and October as the catch probabilities are highest in these months (Bartol et al., 2002). After capture, squid were transferred to a 114 L, circular holding tank (Angler Livewells, Aquatic Eco-Systems, Inc., Apopka, FL, USA) fitted with a portable battery powered aerator (Model B-3, Marine Metal Products Co., Inc., Clearwater, FL, USA) for transport to the lab. Squid were maintained in 450-gallon seawater systems with several forms of filtration (e.g., BioBalls, protein skimmers, ozone filtration, etc.). Seawater was maintained at temperatures and salinities equivalent to those of the capture sites (19–22 °C; 30–35‰). A moderate current flow was maintained to promote active swimming and squid were fed a diet of live *Palaemonetes pugio* and *Fundulus heteroclitos* as suggested by Hanlon et al. (Hanlon, 1990; Hanlon et al., 1983). Squid were allowed to acclimate for at least 2 h prior to experimental trials. Only those animals that appeared healthy and exhibited normal behaviors were used. In total, 60 paralarval squid and 20 juvenile/adult squid were selected for this study.

Two summer flounder (*Paralichthys denatus*) (13.2 cm and 15.5 cm total length) and mummichogs (*Fundulus heteroclitus*) (1.3 cm and 1.5 cm total length) were purchased from the Marine Biological Laboratory, Woods Hole, MA, and maintained in a recirculating seawater system at a salinity of 30–32‰. The flounder and mummichogs were fed live squid (*L. brevis* and *D. pealeii*, respectively) for one week prior to experimental trials so that they could become proficient in squid capture before data collection. Although we used different fish species for the paralarvae and juvenile/adult trials, the species chosen reflect predators that the squid are most likely to encounter in each ontogenetic phase in the waters of the mid-Atlantic region, with the goal of documenting behaviors that reflect natural conditions.

2.2. Predator–prey experiments

Paralarvae trials were conducted in a 10 × 10 × 10 cm clear acrylic tank. A DALSA Falcon video camera (DALSA Corp., Waterloo, ON, Canada; 1400 × 1024 pixel resolution, 100 frames per second) outfitted with a 25 mm lens (FOV = 2.7 × 3.7 cm) was positioned above the arena. A 500 watt halogen light provided illumination for the experimental trials. Video frames from the camera were stored in real time on hard disk using a CLSAS capture card (IO Industries, London, ON, Canada) and Streams 5 software (IO Industries, London, ON, Canada). At the beginning of each trial, 5-10 paralarvae were placed in the arena for a 10 min acclimation period. After the acclimation period, two small mummichogs were added and the experiments commenced. Multiple predators were used to increase the frequency of predation events. Each trial lasted 10 min, after which the fish were removed and surviving squid were returned to their holding tank.

Adult and juvenile trials took place in a 1.2 m diameter × 0.76 m deep round tank with a crushed coral substrate. The arena was lined with curtains to avoid disturbing acclimating animals. A UNIQ UP-685 CL high-speed color camera (Uniq Vision; 659 × 494 pixel resolution, 110 frames per second) outfitted with a 5 mm lens (FOV = 130 cm × 170 cm) was suspended from scaffolding over the tank. Four 500-watt halogen lights provided illumination for the experimental trials. For each experiment, a