

Anti-predator behavior of squid throughout ontogeny



Carly A. York ^{*}, Ian K. Bartol

Department of Biology, Old Dominion University, Norfolk, VA 23529, USA

ARTICLE INFO

Article history:

Received 23 June 2015

Received in revised form 19 March 2016

Accepted 21 March 2016

Available online xxxx

Keywords:

Behavior

Squid

Ontogeny

Anti-predator

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.

© 2016 Elsevier B.V. All rights reserved.

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 cryptic 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

^{*} Corresponding author.

E-mail address: csind001@odu.edu (C.A. York).

“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 ($Re \sim 1-10^2$) (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 ($Re \sim 10^3-10^6$) (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 heteroclitus* 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 dentatus*) (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

single squid was placed in the arena with two summer flounder. Multiple predators again were used to increase the odds of a predation event. Prior to the start of each trial, a 12 cm diameter cylinder composed of 5 mm plastic mesh was lowered into the experimental tank and a single squid was placed inside the cylinder for a 30 min acclimation period. The trials commenced when the partition was raised above the tank and the flounder and squid were allowed to interact. Each trial lasted 10 min, after which the squid was removed from the arena and returned to its holding tank. Video frames from the camera were stored in real time on hard disk using a CL160 capture card (IO Industries, London, ON, Canada) and Video Savant 4.0 software (IO Industries, London, ON, Canada).

2.3. Behavioral responses and kinematics

Kinematic variables were measured using National Institute of Health's public domain software ImageJ (<http://rsb.info.nih.gov/ij/>). Predator–squid distance (d), angular orientation of squid to approaching predator (θ), angular orientation of the squid escape trajectory (ϕ) and orientation (i.e., tail-first or arms-first body position) were measured at the initiation of each squid defensive response to the predator (i.e., at the beginning of inking, posturing, and/or body patterning). Predator–squid distance (d) was measured from the predator upper rostrum to the closest component of the squid. Angle θ was the angle between the longitudinal body axis of the squid and the line connecting the tip of the predator upper rostrum to the squid center of mass, whereas ϕ was the angle between the line connecting the tip of the predator upper rostrum to the squid center of mass and the path of escape over multiple frames (Fig. 1). Interactions were divided into four groups of angular orientations for both θ and ϕ : (1) $< 45^\circ$, (2) 46° – 90° , (3) 91° – 135° , (4) 136° – 180° . These groupings were useful in determining whether the predator approached the squid from an anterior, lateral or posterior direction, as well as for determining the direction of the squid escape trajectory (Fig. 2).

Body postures were grouped into two categories: (1) 'splayed arms' where the arms were spread apart with minimal contact between the arms, and (2) 'raised arms' where unilateral or bilateral raising of the arms was observed, often with 4-arm groupings occurring on one or both sides of the body (Hanlon et al., 1994) (Fig. 3). These postures were the only two observed and therefore other postures recorded in other cephalopods were not included in this study. Several types of body patterns were examined in juveniles and adults including: (1) clear body, where chromatophores are retracted, rendering the animal mostly translucent; (2) dark body, where most chromatophores are expanded producing a dark body coloration; (3) banded pattern, where chromatophores are expanded in striped sections across the mantle, fins, head and/or arms; and (4) dark arms and clear body, where chromatophores are retracted on the body but expanded along the head and arms. Unlike juveniles and adults, the range of body patterns displayed by paralarval *D. pealeii* has not been previously reported. Based on our observation of paralarvae in the presence of a predator, three body patterns were identified: (1) clear body, where the chromatophores are retracted, rendering the animal mostly translucent; (2) intermediate body, where the chromatophores are partially expanded; and (3) dark body, where the chromatophores are expanded fully, producing a dark body coloration. The mean areas of three chromatophores on the mantle were measured on each individual ($N = 60$) to determine the body pattern category during the predator encounter.

Inking patterns were grouped according to the following categories: (1) 'ropes', (2) 'pseudomorph', (3) 'puffs', and (4) 'clouds'. Ink 'ropes' consist of long continuous streams of ink (Bush and Robison, 2007), while a 'pseudomorph' is described as a dense blob of ink that is approximately the same size and shape as the cephalopod (Hanlon and Messenger, 1996). 'Puffs' are defined as short releases of ink that quickly dissipate (Bush and Robison, 2007). Cephalopods can also create diffuse 'clouds' during escape responses that generally involve large volumes of

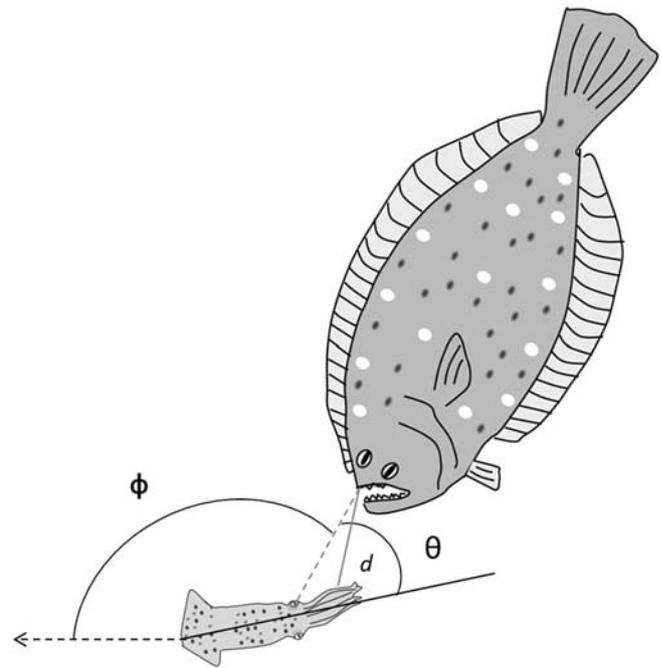


Fig. 1. Angular orientation of squid during interactions with predator. The angular orientation of squid to the approaching predator (θ) was the angle between the longitudinal axis of the squid (solid black line extending from squid) and the line connecting the tip of the predator upper rostrum to the center of mass of the squid (dashed gray line). The angular orientation of the squid escape jet (ϕ) was the angle between the line connecting the tip of the predator upper rostrum to the squid center of mass and the path of the escape over multiple frames (dashed arrow extending from squid). Predator–squid distance (d) was measured from the predator rostrum to the closest component of the squid (solid gray line).

ejected ink with irregular borders (Hanlon and Messenger, 1996) (Fig. 4). The swimming velocity of squid during escape jets, which occur in conjunction with inking, was also measured. Several kinematic components of the predator approach were considered, including the velocity of the predator during the interaction, overall distance traveled by the predator and the distance traveled before a response was initiated for paralarval, juvenile and adult squid.

2.4. Statistical analysis

Statistical analysis was performed in SPSS (v. 18 SPSS Inc., Chicago, IL, USA). Paralarval squid ($N = 60$) were treated as a single ontogenetic group as they consistently had a dorsal mantle length of 0.18 cm. Juvenile squid were those animals 3.0–3.9 cm DML ($N = 9$), while adults were animals 4.0–7.0 cm DML ($N = 11$). When individuals had multiple predator–prey interactions, mean proportions were calculated for body pattern type, posture type, and ink shape. Different predator–prey interactions were considered for postural encounters and inking encounters. Before analysis, all data were tested for normality using Shapiro–Wilk tests. The proportion data deviated from normality (all $p < 0.05$) and thus were arcsine transformed prior to parametric analysis. No differences were found between juveniles and adults (t -tests: all $p > 0.05$) for any of these variables, and therefore data were combined for further analysis of this group ($N = 20$). Kinematic variables (d , θ , ϕ , body orientation) associated with inking events and posturing events were compared via independent two-tailed t -tests. For analysis of squid behaviors and predator distance, comparisons were made between behavioral events that occurred in the beginning of the interaction ($\leq 50\%$ total distance) and the end of the interaction ($> 50\%$ total distance).

Independent two-tailed t -tests were used to compare orientation, posture, inking events and body patterns between the two size class groups. Analysis of variance (ANOVA) was performed to compare

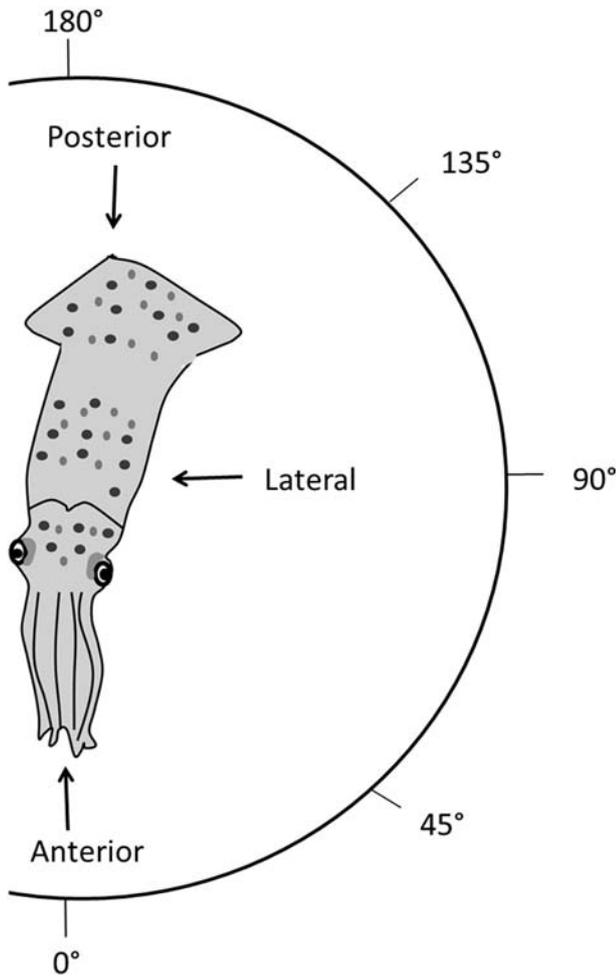


Fig. 2. Diagram of squid orientations. Interactions were divided into four groups of angular orientations for both ϕ and θ : 1) $< 45^\circ$, (2) $46^\circ\text{--}90^\circ$, (3) $91^\circ\text{--}135^\circ$, (4) $136^\circ\text{--}180^\circ$.

groups of angular positions and behavioral responses (body patterning, posture events, inking events). The average proportion of interactions with inking events and with body patterning (i.e., posturing and/or chromatic changes) were calculated for each individual squid and ANOVAs were performed on these data to determine preference when confronted with a predator. Significance was tested at $p < 0.05$ and all means are presented \pm standard deviation unless otherwise noted.

3. Results

3.1. Paralarvae kinematics

Throughout the predator–prey interactions, paralarval squid did not demonstrate posturing and only one inking event was recorded. Significantly more predator–prey interactions occurred while paralarval squid were oriented with arms toward the predator versus tail-first relative to the approaching predator (t -test: $t_{118} = 16.4$, $p < 0.001$), with only five total interactions (8.0%) occurring while the paralarvae were oriented tail-first. Paralarvae responded to an approaching predator with an escape jet in only 35% of total interactions. When paralarvae did respond, the mean average swimming velocity during escape jetting was 33.51 ± 13.79 DML s^{-1} . Distance and velocity of the predator did not determine whether an escape jet was performed (distance t -test: $t_{58} = -0.36$, $p = 0.71$; velocity t -test: $t_{58} = -1.4$, $p = 0.17$). The distance that the predator traveled, however, played a role in whether an escape jet was performed by the squid (t -test: $t_{58} = -2.55$, $p = 0.01$), with escape responses occurring at larger predator travel distances (mean travel distance = 0.67 ± 0.31 predator body lengths (BL)) than non-escape responses (mean travel distance = 0.45 ± 0.39 BL). No difference in θ or ϕ was found between escape and non-escape responses (θ t -test: $t_{58} = 1.3$, $p = 0.20$; ϕ t -test: $t_{58} = -0.12$, $p = 0.91$).

For the 65% of predator–prey interactions that did not result in an escape jet, the paralarvae performed stereotyped swimming behaviors that potentially could aid in predator avoidance. These behaviors included swimming repetitively in a circle in either the xy or xz plane with circle diameters of 1–3 DML (Fig. 5A). In some cases the paralarvae exhibited no net displacement during these circular motions (i.e., starting and ending points of the loop were the same), whereas in other cases, net displacement was present, resulting in movement in a spiral pattern (Fig. 5B). Additionally, several different forms of jetting were performed. These included short pulsed sequential jets, distinct from escape jets, in which the squid moved rectilinearly through the water column in either an arms-first or tail-first orientation (Fig. 5C). Erratic zig-zag jetting, in which the paralarvae employed short vectored jets with multiple changes in direction, were also found in both arms-first and tail-first orientations (Fig. 5D). The paralarvae also exhibited rolling and pitching, but these behaviors were unlikely to have anti-predator benefits as there was no translational motion.

3.2. Juveniles/adults kinematics

Response initiation distance was significantly greater for those juvenile/adult squid exhibiting only postural responses versus those

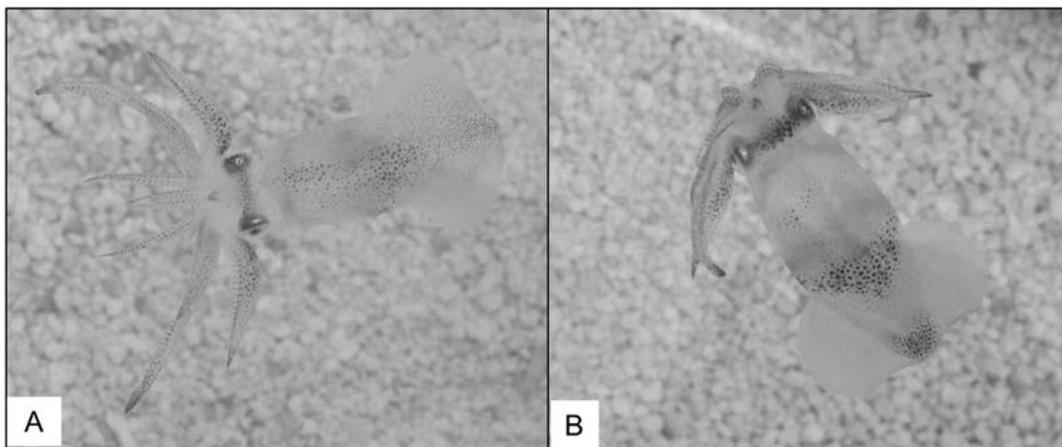


Fig. 3. Squid body postures examined in this study: (A) “splayed arms” where all eight arms are spread outward, (B) “raised arms” where there is unilateral or bilateral raising of groups of arms.

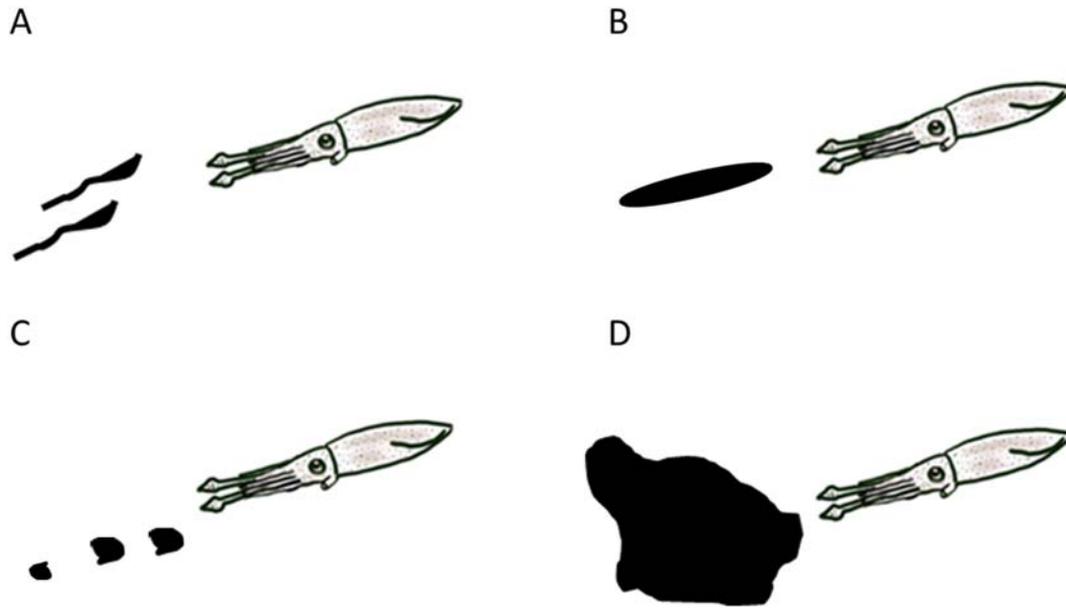


Fig. 4. Images of inking patterns examined in predator–prey experiments (modified from Bush and Robison, 2007). Inking patterns include: (A) ink ‘ropes’, which consist of long continuous streams of ink; (B) a ‘pseudomorph’, which is described as a dense blob of ink that is approximately the same size and shape as the cephalopod; (C) ‘puffs’, which are short releases of ink that quickly dissipate; and (D) ‘clouds’ that generally involve large volumes of ejected ink with irregular borders.

demonstrating ink/escape responses (t -test: $t_{17} = -7.1$, $p < 0.001$; Fig. 6A). Squid showing only postural displays (i.e., raised or splayed arms) also were associated with lower predator approach velocities than those exhibiting an ink/escape response (t -test: $t_{19} = -3.03$, $p = 0.006$) (Fig. 6B). Interestingly, squid using ink/escape responses were also associated with greater overall predator travel distances than those showing only postural responses (t -test: $t_{18} = -5.25$, $p < 0.001$; mean predator travel distance = 2.52 ± 0.34 BL (ink/escape response), 0.68 ± 0.35 BL (postural response)). No significant differences in response initiation distance were found between ‘splayed arm’ postural responses, which were only displayed during tail-first orientations, and ‘raised arm’ postural responses, which were only observed during arms-first orientations (t -test: $t_{19} = 0.25$, $p = 0.80$),

nor was there a significant difference in the velocity of the approaching predator between the two behaviors (t -test: $t_{10} = 1.34$, $p = 0.21$). The squid was more likely to actively position itself anteriorly facing the predator when the predator was within 2.5 body lengths of the squid (t -test: $t_{16} = -2.20$, $p = 0.046$). Predator–prey distance did not affect the specific body pattern or inking pattern selected (all $p > 0.05$).

3.3. Inking and escape jet differences through ontogeny

The juvenile and adult squid in this study responded to an oncoming predator with an inking event in approximately 60% of all interactions. Inking events were always exhibited in sequence with an escape jet,

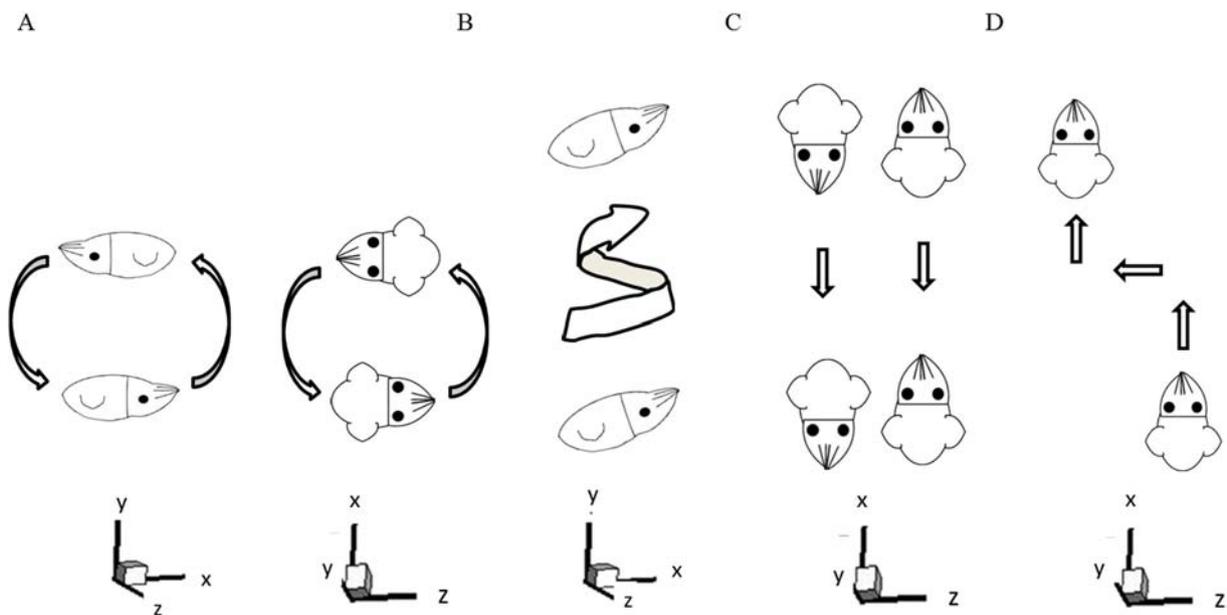


Fig. 5. Stereotyped swimming behaviors of paralarvae squid: (A) swimming repetitively in a circular pattern in both the xy and xz plane; (B) swimming in a spiral pattern; (C) pulsed sequential jets in which the squid moved rectilinearly through the water column in either an arms-first or tail-first orientation; and (D) erratic zig-zag jetting, in which the paralarvae employed short vectored jets with multiple changes in direction (squid perform this pattern in both tail-first and arms-first orientations; arms-first orientation is depicted here).

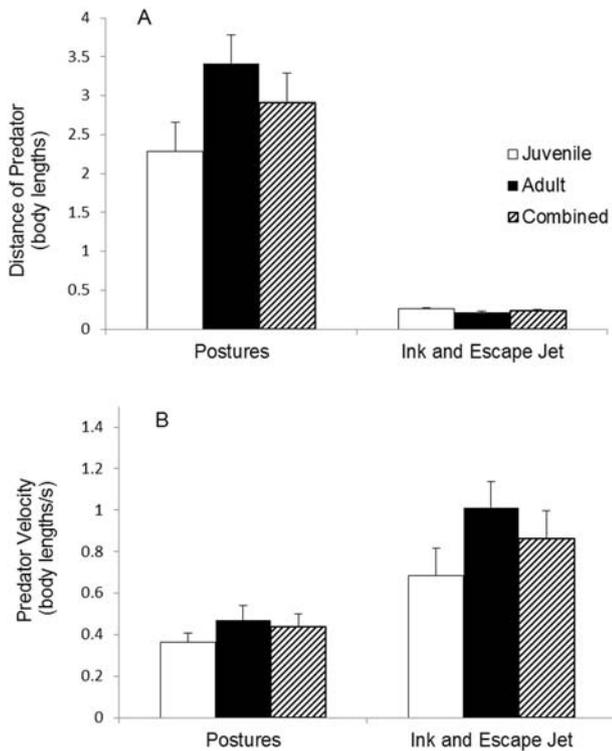


Fig. 6. The mean (A) distance of predator from the squid when the squid initiated a behavioral response, expressed in predator body lengths (BL), and (B) velocity of the predator approach when squid initiated a behavioral response, expressed in BL s^{-1} . Significant differences between juvenile and adult squid were not evident, and thus juvenile and adult data were pooled for analysis (gray bars). Paralarval squid did not demonstrate postures or frequent inking behavior and were therefore excluded from this figure. Mean \pm S.E. is presented.

where inking occurred either at the initiation of the escape jet (61%) or at another point throughout the escape jet (39%). Inking in paralarvae only occurred in 2.0% of all interactions. Significant differences were found in relative swimming velocities during escape responses between paralarval squid and juvenile/adult squid (ANOVA: $F_{3,121} = 89.36$, $p < 0.001$). Tukey post-hoc analysis revealed that paralarvae had significantly slower escape responses (mean = 33.5 ± 13.7 DML s^{-1}) than juveniles/adults (mean = 47.9 ± 1.41 DML s^{-1} , $p = 0.001$; Fig. 7). No significant differences in predator approach velocities were found between paralarvae (mean = 1.75 ± 3.17 DML s^{-1}) and juveniles/adults (mean = 1.14 ± 0.43 DML s^{-1} , $p = 0.98$) trials. The squid escaped at speeds significantly greater than the predator approach for both age groups (paralarvae: $p < 0.001$; juveniles/adults: $p < 0.001$; Fig. 7). Paralarvae reached a mean velocity of 6.03 ± 2.48 cm s^{-1} while the predator only reached 1.64 ± 1.04 cm s^{-1} . Juveniles and adult squid were also faster than the predator approach (15.04 ± 5.69 cm s^{-1}) reaching a mean velocity of 191.6 ± 111.61 cm s^{-1} . Although the escape response of the paralarvae was fast enough to evade the predator, an escape was only initiated in 35% of interactions, leading to an overall survival rate of only 40%, whereas juveniles and adults survived all interactions in these trials.

The proportion of inking events during encounters varied significantly with the angular orientation of the squid relative to the approaching predator for juveniles/adults (ANOVA: $F_{2,16} = 14.1$, $p < 0.001$); inking events were more prevalent when θ were $46\text{--}90^\circ$ (mean proportion of inking events = 0.90 ± 0.25) compared with $\theta < 45^\circ$ (mean proportion of inking events = 0.45 ± 0.33 , $p < 0.001$). Juvenile/adult squid demonstrated a significantly higher proportion of inking events when displaying a clear body pattern than when displaying other body patterns (ANOVA: $F_{2,16} = 5.47$, $p < 0.015$). No significant differences were found between squid postures, i.e., splayed

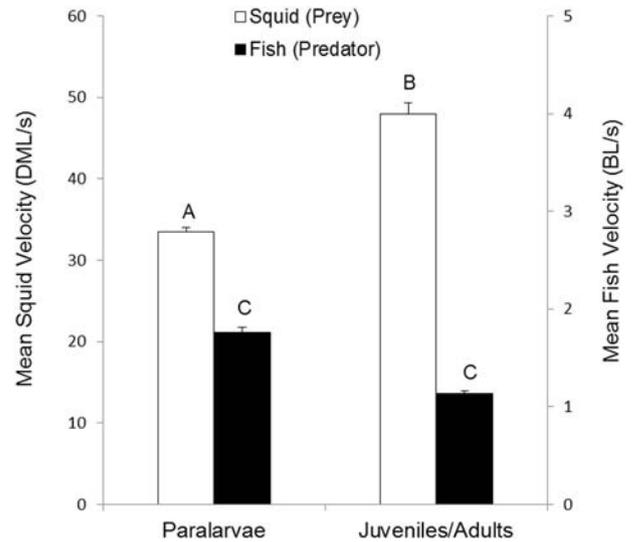


Fig. 7. The mean velocity of the fish and the squid during predator–prey interactions for paralarval and juvenile/adult trials normalized to body lengths s^{-1} . 100% of the juvenile squid survived the encounters; 40% of paralarvae survived the encounters. Bars with different letters are significantly different. Mean \pm S.E. are presented.

arms vs. raised arms, based on θ (all $p > 0.05$). The average angular orientation of the squid escape trajectory (ϕ) was $152.34 \pm 35.23^\circ$. ϕ did not influence the selection of postures (ANOVA: $F_{1,14} = 0.09$, $p = 0.77$), orientation (ANOVA: $F_{1,16} > 0.16$, all $p > 0.69$) or proportion of ink and escape events (ANOVA: $F_{2,16} = 0.49$, $p = 0.62$).

3.4. Body pattern differences through ontogeny

Different body patterns were observed for paralarvae and juveniles/adults. Paralarvae demonstrated clear body patterning ($N = 33$, mean area of chromatophores = $2.74 \times 10^{-5} \pm 1.13 \times 10^{-5}$ cm 2), intermediate body patterning ($N = 18$, mean area of chromatophores = $1.45 \times 10^{-4} \pm 9.86 \times 10^{-6}$ cm 2), and dark body patterning ($N = 9$, mean area of chromatophores = $2.50 \times 10^{-4} \pm 5.50 \times 10^{-5}$ cm 2). The mean area of the chromatophores in the three body classifications was significantly different, indicating that they are indeed three distinct body patterns (ANOVA: $F_{2,57} = 379.7$, $p < 0.001$; Fig. 8). The proportion of clear, intermediate and dark body patternings displayed during predator–prey responses were significantly different for paralarvae (ANOVA: $F_{2,177} = 12.4$, $p < 0.001$). Tukey post-hoc tests revealed that clear body patterns (mean proportion = 0.55 ± 0.50) were used significantly more often than intermediate body patterns (mean proportion = 0.31 ± 0.43 , $p < 0.001$) and dark body patterns (mean proportion = 0.15 ± 0.36 , $p = 0.007$, Fig. 9A). The velocity of the approaching predator did not affect the body pattern selection of the paralarvae (all $p > 0.05$), nor did the angle of the predator approach, distance of the predator or distance traveled by the predator at the time of the interaction (all $p > 0.05$). When responding to an approaching predator, juvenile and adult squid were significantly more likely to demonstrate the banded pattern than the dark body, dark arms with clear body, or clear body pattern (ANOVA: $F_{3,76} = 26.1$, $p < 0.001$; Fig. 9B). When the juvenile/adult squid responded with an inking event (something that is very rare for paralarvae), it was significantly more likely to be in the form of a ‘pseudomorph’ than a ‘cloud’ or ‘puff’ (ANOVA: $F_{2,57} = 91.2$, $p < 0.001$). The rope shape was not observed in these experiments.

4. Discussion

The results of this study reveal the unique differences in anti-predator strategies of squid as they undergo morphological and

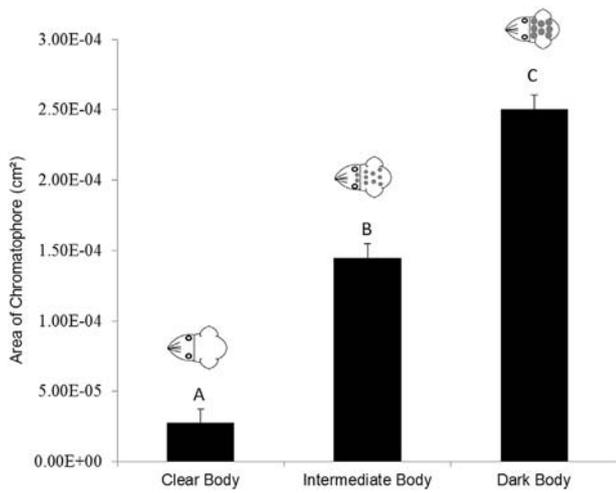


Fig. 8. Distinct body patterns of paralarvae during predator–prey interactions and the associated mean area of chromatophores in the clear body, intermediate body and dark body patterns. Bars with different letters are significantly different. Mean \pm S.E. presented.

ecological transformations throughout ontogeny. Paralarval squid did not react to an oncoming predator with posturing or consistent inking responses, as only one inking event occurred throughout all of the trials. Instead, they either produced an escape jet (35% of encounters) or demonstrated routine stereotypical behaviors (65% of encounters) in the presence of a predator. In contrast, juvenile and adult squid exhibited frequent inking/escape jetting and postural responses to an oncoming predator, with the selection of anti-predator behavior being impacted by characteristics of the predator approach. During the slower predator approaches, the juvenile/adult squid were more likely to posture, while faster approaches triggered inking and escape jet responses. In

juveniles/adults, postural responses were favored when the predator was positioned at large distances from the squid while inking responses were selected when the predator was close to the squid. Additionally, postural displays were selected when the predator traveled only a short distance toward the squid, while ink responses were used when the predator traveled a significantly greater distance toward the squid. Postural displays are likely an attempt to dissuade the predator at the beginning of the attack while minimizing energy expenditure associated with an inking response (Wood et al., 2008). Inking is then utilized only when crypsis or posturing has failed and the predator continues with its approach, as was seen in this study.

In many cephalopods, inking provides a distraction to oncoming predators, allowing them to escape from reach (Hanlon and Messenger, 1996). Additionally, the chemical properties of ink can act as a predatory deterrent as it disrupts the sensory systems of the predator (Derby, 2007; Gilly and Lucero, 1992; Wood et al., 2010). The juvenile and adult squid in this study responded to an oncoming predator with an inking event in approximately 60% of all interactions, whereas only one inking event was recorded in the paralarvae (2.0% of all interactions). It is possible that paralarvae are more selective in their use of inking events given the high energetic requirements of producing ink (Wood et al., 2008) coupled with the need to allocate high levels of energy toward development (Russo et al., 2003). In addition, juveniles and adults showed proportionally more inking events with clear body patterns relative to other body patterns and inking and escape jetting were always coupled, a sequence of behaviors that is consistent with the “ink-blanche-jet” maneuver described earlier. In juveniles/adults, the ‘pseudomorph’ shape was produced more often than ‘rope’, ‘puff’ or ‘cloud’ shapes. This is consistent with previous observations, where shallow-dwelling cephalopods commonly use ‘pseudomorphs’ (Hanlon and Messenger, 1996), but a wider variety of shapes are evident in deep sea species (Bush and Robison, 2007). In several instances, the flounder were distracted by the ‘pseudomorph’ and directed their approach toward the ink instead of the squid. Ink has also been shown to act as a conspecific alarm cue

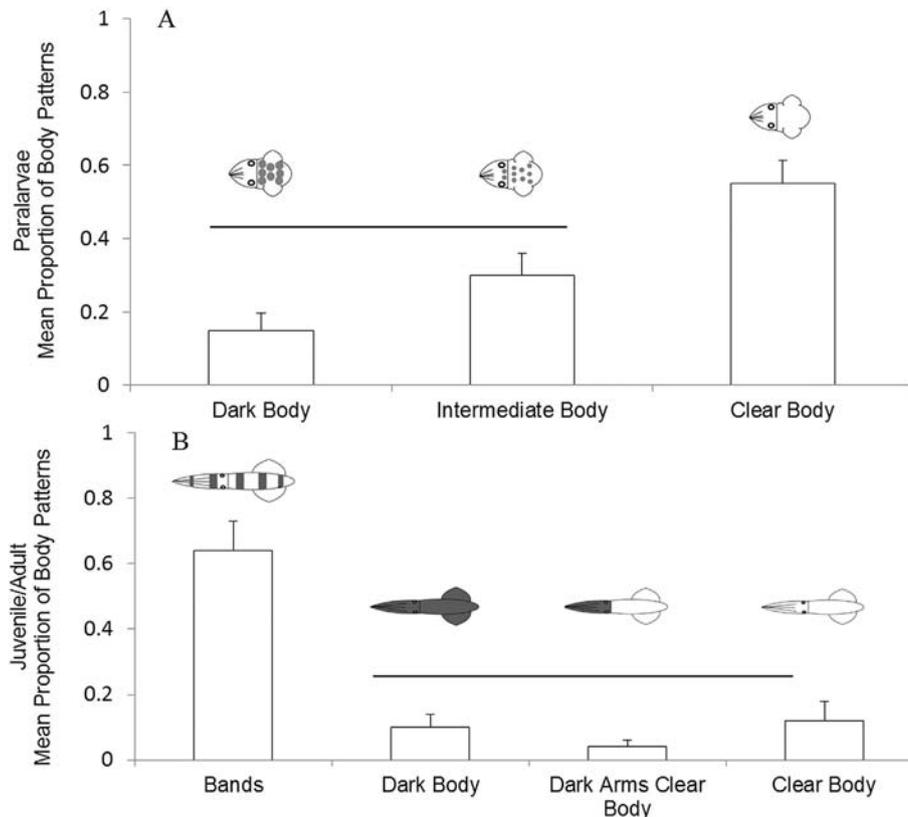


Fig. 9. Mean proportion of body patterns demonstrated in (A) paralarvae, and (B) juvenile/adult squid during predator approaches. Mean \pm S.E. is presented.

that could be used when swimming in schools (Wood et al., 2008, 2010). Brief squid do school in their natural habitat (I. Bartol, pers. obs.), and it is possible that the 'pseudomorph' not only acts as a decoy but also a social warning cue. Although 'pseudomorphs' were found to be the most common ink response observed in the present study, it is conceivable that the 'rope' and 'puff' shapes, which involve smaller sequential ink release patterns, are used as conspecific alarms. Since the experimental trials only considered one juvenile/adult squid per trial, this social alarm possibility was not tested directly.

Both paralarvae and juveniles/adults showed impressive average escape velocities of 33.5 ± 13.79 and 47.9 ± 1.47 DML s^{-1} (6.03 ± 2.48 $cm s^{-1}$ and 191.60 ± 111.61 $cm s^{-1}$), respectively. In fish, average escape velocities range only from approximately 14–23 BL s^{-1} in larvae (Williams et al., 1996) to roughly 20–28 BL s^{-1} in adults, depending on the species (Gibb et al., 2006). The juvenile/adult squid reached significantly higher relative swimming velocities than the paralarvae in these experiments, which is likely reflective of the unique hydrodynamic and morphological conditions that squid encounter throughout ontogeny. Paralarvae operate at low to intermediate Reynolds numbers and must overcome high viscous forces, while juveniles and adults operate at higher Reynolds numbers where inertial forces are greater and gliding through the water column is more prevalent (Bartol et al., 2008). Additionally, relative to paralarvae, juvenile and adult squid have more streamlined bodies and larger, more developed fins to provide additional propulsive forces to complement the jet, at least at the start of the escape jet (Bartol et al., 2009a,b; Hoar et al., 1994; Stewart et al., 2010). Both paralarvae and juveniles/adults escaped at speeds greater than the approaching fish, leading to a 100% survival rate for juvenile and adults, but only a 40% survival rate for paralarvae. The low survival rate of paralarvae is a reflection of the absence of an escape response in many encounters, not their inability to escape a predator, as their swimming velocity during escape jetting was significantly higher than the velocity of the predator approach when they did initiate an escape response. Paralarvae only responded with an escape jet in 35% of all interactions, whereas the juveniles and adults always responded with an escape jet when a posture failed to deter the approaching predator. The absence of any escape response in such a high percentage of paralarvae is intriguing and may relate to a reduction in the effectiveness of epidermal hair cells in early ontogeny, which have been shown to play an important role in predator wake sensing (York and Bartol, 2014) (see below).

One of the most striking ontogenetic differences found in this study was the unique use of stereotyped behaviors by paralarvae, but not other life history stages. These behaviors included swimming movements such as repetitive circling, spiraling and erratic jetting that were not seen in juveniles or adults. Since the paralarvae often did not change their behavior as a predator was approaching, it is possible that they rely heavily on their repertoire of stereotyped behaviors and clear body patterning to elude potential predators in the water column until they develop better neural and motor control, which can produce more complex body patterning and enhance hydrodynamic sensing capabilities. Indeed, the ability of paralarval squid to coordinate sensory inputs and motor outputs improves within the first month of hatching (Chen et al., 1996; Preuss and Gilly, 2000), suggesting that chromatophore control and hair cell functionality also increase throughout this period. Therefore, coupling stereotyped swimming strategies with transparency in the earliest days of hatching, when posturing is less achievable and sensing capabilities are limited, is a reasonable predator avoidance strategy for paralarvae. Although brief squid vary in size from juvenile to adult, they maintain similar ecological niches and thrive in the same environment during these ontogenetic stages (Bartol et al., 2002). Therefore it is likely that similar behaviors are effective at deterring predators, which is consistent with the findings reported here. The number of chromatophores increases on the mantle and fins of squid as their size increases (Dubas et al., 1986); however, the proportion of the

body that is shaded to produce patterns stays approximately the same in juveniles and adults (banded pattern in juvenile = 34% shaded; banded pattern in adult = 41% shaded).

In this study, paralarval squid demonstrated the clear body pattern significantly more often than intermediate or dark patterns. It is likely that maintaining a clear body pattern is advantageous given the planktonic nature of paralarvae. By sustaining a transparent state, the paralarvae allow for maximum transmission of background light, making them difficult to detect in the water column (Okutani, 1987; Zylinski and Johnsen, 2011). A similar type of camouflage is also utilized by some species of adult midwater squid that have downward-directed photophores in their mantle. These squid responds to overhead illumination by turning on the photophores, which matches the intensity of the overhead illumination, reducing the overall detectability of the animals to predators (Young and Roper, 1976).

For adults and juveniles, however, the banded body pattern was demonstrated far more than a dark body, clear body or dark arms with a clear body patterns. This banded body patterning was used during both 'splayed arm' and 'raised arm' postures. Banded patterns were also seen throughout the predator attack and did not vary based on the approach distance. The banded body pattern potentially acts as disruptive coloration, making it more difficult for a predator to identify the squid as prey (Hanlon and Messenger, 1996). It is likely that the coloration, in addition to body postures, allows the squid to look larger and more threatening to predators (Hanlon and Messenger, 1996; Staudinger et al., 2011), an option that planktonic paralarvae do not have given their inherently small size and more limited coloration palette. This banded body pattern is also used by adult *D. pealeii* in combination with descending in the water column and laying on the substrate to hide from cruising predators (Staudinger et al., 2011). The *L. brevis* examined in this study did not demonstrate the behavior of dropping to the substrate, and instead remained high in the water column throughout their behavioral response to the predator. Conceivably, this was because flounder typically have an ambush style of attack, where they remain camouflaged until striking, as suggested by Staudinger et al. (2011). The majority of attacks made by the flounder in this study, however, were not ambush style. The flounder were usually active and visible prior to striking and began their attack from as far as 1.1 m away from the squid. It is likely, nonetheless, that if a different type of predator was used, the brief squid would have shown this behavior as well (i.e., dropping to substrate), as *L. brevis* and *D. pealeii* share similar ecological niches and therefore have likely adapted similar anti-predator tactics.

Although the direction of the predator approach did play a role in the behavior of the juvenile and adult squid, it did not affect the behavioral response of paralarvae. These differences are likely the result of an underdeveloped sensory system in paralarvae (Chen et al., 1996), particularly the polarized lateral line analog, which plays an integral role in successful predator detection (York and Bartol, 2014). The lateral line analog runs in an anterior–posterior direction along the head and each of the arms of the squid (Budelmann and Bleckmann, 1988). The wake of predators approaching the squid from different angles clearly will trigger different hair cells along the head and arms, potentially eliciting graded behavioral responses depending on the number and location of hair cells stimulated. Given that the paralarvae considered in the present study were only one day old and their sensory systems are not fully developed (Chen et al., 1996), it is conceivable that the lateral line analog was not yet sufficiently sensitive for predator detection and the reason why no behavioral changes were seen based on the direction of the predator approach. Clearly, further research is needed to fully understand how the hydrodynamic cues of the lateral line analog affect the escape response throughout ontogeny.

Throughout all of the predator–prey interactions, 80% of the juvenile/adult squid were oriented in an angular position between 0° and 90°, with the average angular direction of ~57°. By anteriorly facing the predator, the squid can perform a fast escape jet in the tail-first

orientation, an orientation that contributes to higher swimming speeds with maximal funnel aperture throughput for jet ejection (Bartol et al., 2001, 2009a,b, 2016), increasing their distance from the predator. Indeed the average angular orientation of the squid escape trajectory (ϕ) was $152.34 \pm 35.23^\circ$, reflecting this tail-first escape preference. Additionally, this position is advantageous given the anterior position of the squid sensory organs, including both the eyes and the lateral line analog. Previous studies have indicated that fish which move much faster or slower than a predator will orient laterally to the predator and execute a fast start at a right angle from the predator heading to maximally increase their distance from the predator (Weihs and Webb, 1984), which was supported by Stewart et al. (2014). The squid in this study, however, mostly oriented themselves anteriorly to the oncoming predator. This strategy makes sense in light of the morphological differences between the fish and squid mechanoreceptors. If squid were to position themselves laterally to the predator, a more limited portion of the lateral line analog would be receiving direct hydrodynamic cues from the predator approach. By positioning themselves anteriorly facing the predator, they are exposing the maximum area of epidermal hair cell lines in the direction of the oncoming predator. Additionally, the proportion of total inking events varied based on the angular direction of the squid relative to the approaching predator, with anterior and lateral positioning ($46\text{--}90^\circ$) triggering more inking and escape events than approaches from other angles, potentially indicating that hydrodynamic cues received by the lateral line analog play a role in inking behavior.

The anti-predator behavioral responses recorded for juveniles and adults in this study are consistent with previous research (Hanlon et al., 1994, 1999; Mather, 2010; Staudinger et al., 2011). Staudinger et al. (2011) found that the likelihood of survival when adult *D. pealeii* exhibit deimatic postures and inking events increases when confronted by a predator. In *D. pealeii* deimatic posturing involves having the arms and tentacles extended, which is similar to the 'splayed arm' posture observed in the present study for *L. brevis*, making the squid appear larger and more threatening. When this posture is exhibited in *D. pealeii*, 88% of the attacks are abandoned by the predator (Staudinger et al., 2011), indicating that it is a successful anti-predator strategy to employ in the earliest stages of the predator approach. We also found a high level of predator abandonment (51%) when a splaying arm or raised arm posture was exhibited. Posturing was employed when the predator was far away, while inking was used at shorter distances. When the predator was close and approaching at high velocities, the squid exhibited an inking response and escape jet, behaviors that have been shown to provide a high probability of escape (Staudinger et al., 2011). All of the juvenile and adult squid used in this study avoided capture, indicating that their suite of anti-predator behaviors is extremely effective.

Cephalopods undergo enormous morphological, physiological and ecological transitions as they develop from micronektonic paralarvae to larger, more neurologically advanced adults. Each life stage has unique challenges that demand effective strategies for survival. This study is the first to examine anti-predator behavior of squid throughout ontogeny. Our findings indicate that anti-predator behavior of squids changes throughout development, with divergent strategies used in the paralarval and juvenile/adult stages. Paralarvae had fewer behavioral responses directly linked to an oncoming predator compared with juvenile/adult stages, and tended to maintain a clear body pattern while either escape jetting or demonstrating stereotyped swimming behaviors, making them elusive to predators. Juveniles and adults, on the other hand, were adept at varying their response according to the predator approach and balancing the energetic requirements of escape with the urgency of the situation. The observed variances in anti-predator strategy indicate that squid utilize suitable adaptations for their changing morphology, advancing neural development and ecological niche to maximize survival throughout ontogeny.

Author contributions

C.A. York and I.K. Bartol collaborated on the experimental approach, data analysis and preparation of this manuscript. C.A. York performed the experiments and collected the data. Both authors have approved the final version of the article.

Acknowledgments

We thank the crew of VIMS ESL and Rachel Jastrebsky for trawling assistance, and Tripp York for the photographs. This work was supported by the National Science Foundation under grant IOS 1115110 [SS].

References

- Barbato, M., Bernard, M., Borrelli, L., Fiorito, G., 2007. Body patterns in cephalopods. *Pattern Recogn. Lett.* 28, 1854–1864. <http://dx.doi.org/10.1016/j.patrec.2006.12.023>.
- Barbosa, A., Litman, L., Litman, L., Hanlon, R.T., 2008. Changeable cuttlefish camouflage is influenced by horizontal and vertical aspects of the visual background. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 194, 405–413. <http://dx.doi.org/10.1007/s00359-007-0311-1>.
- Bartol, I.K., Mann, R., Patterson, M.R., 2001. Aerobic respiratory costs of swimming in the negatively buoyant brief squid *Lolliguncula brevis*. *J. Exp. Biol.* 204, 3639–3653.
- Bartol, I., Mann, R., Vecchione, M., 2002. Distribution of the euryhaline squid *Lolliguncula brevis* in Chesapeake Bay: effects of selected abiotic factors. *Mar. Ecol. Prog. Ser.* 226, 235–247.
- Bartol, I.K., Krueger, P.S., Thompson, J.T., Stewart, W.J., 2008. Swimming dynamics and propulsive efficiency of squids throughout ontogeny. *Integr. Comp. Biol.* 48, 720–733. <http://dx.doi.org/10.1093/icb/icn043>.
- Bartol, I.K., Krueger, P.S., Stewart, W.J., Thompson, J.T., 2009a. Pulsed jet dynamics of squid hatchlings at intermediate Reynolds numbers. *J. Exp. Biol.* 212, 1506–1518. <http://dx.doi.org/10.1242/jeb.026948>.
- Bartol, I.K., Krueger, P.S., Stewart, W.J., Thompson, J.T., 2009b. Hydrodynamics of pulsed jetting in juvenile and adult brief squid *Lolliguncula brevis*: evidence of multiple jet "modes" and their implications for propulsive efficiency. *J. Exp. Biol.* 212, 1889–1903. <http://dx.doi.org/10.1242/jeb.027771>.
- Bartol, I.K., Krueger, P.S., Jastrebsky, R.A., Williams, S., Thompson, J.T., 2016. Volumetric flow imaging reveals the importance of vortex ring formation in squid swimming at different orientations. *J. Exp. Biol.* 219, 392–403. <http://dx.doi.org/10.1242/jeb.129254>.
- Boletzky, S., 1974. The "larvae" of cephalopod: a review. *Thalass. Jugosl.*
- Boyle, P., Rodhouse, P., 2008. *Cephalopods: Ecology and Fisheries*. John Wiley & Sons.
- Budelmann, B.U., Bleckmann, H., 1988. A lateral line analogue in cephalopods: water waves generate microphonic potentials in the epidermal head lines of Sepia and *Lolliguncula*. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 164, 1–5.
- Bush, S.L., Robison, B.H., 2007. Ink utilization by mesopelagic squid. *Mar. Biol.* 152, 485–494. <http://dx.doi.org/10.1007/s00227-007-0684-2>.
- Bush, S.L., Robison, B.H., Caldwell, R.L., 2009. Behaving in the dark: locomotor, chromatic, postural, and bioluminescent behaviors of the deep-sea squid *Octopoteuthis deletron* young 1972. *Biol. Bull.* 216, 7–22 (doi:216/1/7 [pii]).
- Caldwell, R.L., 2005. An observation of inking behavior Protecting adult *Octopus bocki* from predation by Green Turtle (*Chelonia mydas*) hatchlings. *Pac. Sci.* 59, 69–72.
- Chen, D.S., Dykhuizen, G.V., Hodge, J., Gilly, W.F., 1996. Ontogeny of Copepod predation in juvenile squid (*Loligo opalescens*). *Biol. Bull.* 190, 69–81.
- Clarke, M.R., 1996. The role of cephalopods in the world's oceans: general conclusions and the future. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351, 1105–1112.
- Cornwell, C.J., Messenger, J.B., Hanlon, R.T., 2009. Chromatophores and body patterning in the squid *Alloteuthis subulata*. *J. Mar. Biol. Assoc. U. K.* 77, 1243. <http://dx.doi.org/10.1017/S0025315400038789>.
- Derby, C.D., 2007. Escape by inking and secreting: marine molluscs avoid predators through a rich array of chemicals and mechanisms. 213, 274–289.
- Dubas, F., Leonard, R.B., Hanlon, R.T., 1986. Chromatophore motoneurons in the brain of the squid, *Lolliguncula brevis*: an HRP study. *Brain Res.* 374, 21–29. [http://dx.doi.org/10.1016/0006-8993\(86\)90390-2](http://dx.doi.org/10.1016/0006-8993(86)90390-2).
- Fingerman, M., 1970. *Comparative Physiology: Chromatophores* 1 1048.
- Florey, E., 1966. Nervous control and spontaneous activity of the chromatophores of a cephalopod, *Loligo opalescens*. *Comp. Biochem. Physiol.* 18, 305–324.
- Gibb, A.C., Swanson, B.O., Wesp, H., Landels, C., Liu, C., 2006. Development of the escape response in teleost fishes: do ontogenetic changes enable improved performance? *Physiol. Biochem. Zool.* 79, 7–19. <http://dx.doi.org/10.1086/498192>.
- Gilly, W.F., Lucero, M.T., 1992. Behavioral responses to chemical stimulation of the olfactory organ in the squid *Loligo opalescens*. *J. Exp. Biol.* 162, 209–229.
- Hanlon, R.T., 1990. Maintenance, rearing and culture of teuthoid and sepioid squids. In: Gilbert, D.L., Adelman, W.J., Arnold, J.M. (Eds.), *Squid as Experimental Animals*. Plenum Press, New York, pp. 35–62.
- Hanlon, R.T., Messenger, J.B., 1996. *Cephalopod Behaviour*. Cambridge University Press, New York.
- Hanlon, R.T., Hixon, R.F., Hulet, W.H., 1983. Survival, growth and behavior of the loliginid squids *Loligo plei*, *Loligo pealei*, and *Lolliguncula brevis* (Mollusca: cephalopoda) in closed sea water systems. *Biol. Bull.* 165, 637–685.

- Hanlon, R.T., Smale, M.J., Sauer, W.H.H.W., 1994. An ethogram of body patterning behavior in the squid *Loligo vulgaris reynaudii* on Spawning Grounds in South Africa. *Biol. Bull.* 187, 363–372.
- Hanlon, R.T., Maxwell, M.R., Shashar, N., Loew, E.R., Boyle, K.L., 1999. An ethogram of body patterning behavior in the biomedically and commercially valuable squid *Loligo pealeii* off Cape Cod, Massachusetts. *Biol. Bull.* 197, 49–62.
- Hoar, J.A., S. E., M., W.D., O'd.R. K., 1994. The role of fins in the competition between squid and fish. *Mechanics and Physiology of Animal Swimming*, pp. 27–43.
- Huffard, C.L., 2006. Locomotion by *Abdopus aculeatus* (Cephalopoda: Octopodidae): walking the line between primary and secondary defenses. *J. Exp. Biol.* 209, 3697–3707. <http://dx.doi.org/10.1242/jeb.02435>.
- Jantzen, T.M., Havenhand, J.N., 2003. Reproductive behavior in the squid *Sepioteuthis australis* from South Australia: ethogram of Reproductive body patterns. *Biol. Bull.* 204, 290–304.
- Kobayashi, S., Takayama, C., Ikeda, Y., 2013. Ontogeny of the brain in oval squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae) during the post-hatching phase. 93, 1663–1671. <http://dx.doi.org/10.1017/S0025315413000088>.
- Lucero, M.T., Farrington, H., Gilly, W.F., 1994. Quantification of L-dopa and dopamine in squid ink: implications for chemoreception. *Biol. Bull.* 187, 55–63.
- Mather, J.a., 2010. Vigilance and antipredator responses of Caribbean reef squid. *Mar. Freshw. Behav. Physiol.* 43, 357–370. <http://dx.doi.org/10.1080/10236244.2010.526760>.
- Messenger, J.B., 2001. *Cephalopod Chromatophores : Neurobiology and Natural History*. pp. 473–528.
- O'Dor, R.K., 1988. The forces acting on swimming squid. *J. Exp. Biol.* 442, 421–442.
- Okutani, T., 1987. Juvenile morphology. In: Boyle, P.R. (Ed.), *Cephalopod Life Cycles*. Associated Press, Miami FL.
- Packard, A., 1969. Jet propulsion and the giant fibre response of *Loligo*. *Nature* 221, 875–877. <http://dx.doi.org/10.1038/221875a0>.
- Piatkowski, U., Pierce, G.J., Morais, M., 2001. Impact of cephalopods in the food chain and their interaction with the environment and fisheries : an overview. 52, 3–8.
- Preuss, T., Gilly, W., 2000. Role of prey-capture experience in the development of the escape response in the squid *Loligo opalescens*: a physiological correlate in an identified neuron. *J. Exp. Biol.* 203, 559–565.
- Robin, J.-P., Roberts, M., Zeidberg, L., Bloor, I., Rodriguez, A., Briceño, F., Downey, N., Mascaró, M., Navarro, M., Guerra, A., Hofmeister, J., Barcellos, D.D., Lourenço, S.A.P., Roper, C.F.E., Moltshaniwskyj, N.A., Green, C.P., Mather, J., 2014. Transitions during cephalopod life history: the role of habitat, environment, functional morphology and behaviour. *Adv. Mar. Biol.* 67, 361–437. <http://dx.doi.org/10.1016/B978-0-12-800287-2.00004-4>.
- Russo, G.L., De Nisco, E., Fiore, G., Di Donato, P., D'Ischia, M., Palumbo, A., 2003. Toxicity of melanin-free ink of *Sepia officinalis* to transformed cell lines: identification of the active factor as tyrosinase. *Biochem. Biophys. Res. Commun.* 308, 293–299. [http://dx.doi.org/10.1016/S0006-291X\(03\)01379-2](http://dx.doi.org/10.1016/S0006-291X(03)01379-2).
- Shea, E.K., Vecchione, M., 2010. Ontogenic changes in diel vertical migration patterns compared with known allometric changes in three mesopelagic squid species suggest an expanded definition of a paralarva. *ICES J. Mar. Sci.* 67, 1436–1443. <http://dx.doi.org/10.1093/icesjms/fsq104>.
- Staudinger, M.D., Hanlon, R.T., Juanes, F., 2011. Primary and secondary defences of squid to cruising and ambush fish predators: variable tactics and their survival value. *Anim. Behav.* 81, 585–594. <http://dx.doi.org/10.1016/j.anbehav.2010.12.002>.
- Stewart, W.J., Bartol, I.K., Krueger, P.S., 2010. Hydrodynamic fin function of brief squid, *Lolliguncula brevis*. *J. Exp. Biol.* 213, 2009–2024. <http://dx.doi.org/10.1242/jeb.039057>.
- Stewart, W.J., Nair, A., Jiang, H., McHenry, M.J., 2014. Prey fish escape by sensing the bow wave of a predator. *J. Exp. Biol.* 217, 4328–4336. <http://dx.doi.org/10.1242/jeb.111773>.
- Thompson, J.T., Kier, W.M., 2002. Ontogeny of Squid Mantle Function : Changes in the Mechanics of Escape-Jet Locomotion in the Oval Squid, *Sepioteuthis lessoniana* Lesson, 1830. pp. 14–26.
- Webber, D.M., O'Dor, R.K., 1986. Monitoring the metabolic rate and activity of free-swimming squid with telemetered jet pressure. *J. Exp. Biol.* 126, 205–224.
- Weih, D., Webb, P.W., 1984. Optimal avoidance and evasion tactics in predator-prey interactions. *J. Theor. Biol.* 106, 189–206. [http://dx.doi.org/10.1016/0022-5193\(84\)90019-5](http://dx.doi.org/10.1016/0022-5193(84)90019-5).
- Williams, P.J., Brown, J. a., Gotceitas, V., Pepin, P., 1996. Developmental changes in escape response performance of five species of marine larval fish. *Can. J. Fish. Aquat. Sci.* 53, 1246–1253. <http://dx.doi.org/10.1139/f96-052>.
- Wood, J.B., Pennoyer, K.E., Derby, C.D., 2008. Ink is a conspecific alarm cue in the Caribbean reef squid, *Sepioteuthis sepioidea*. *J. Exp. Mar. Biol. Ecol.* 367, 11–16. <http://dx.doi.org/10.1016/j.jembe.2008.08.004>.
- Wood, J.B., Maynard, A.E., Lawlor, A.G., Sawyer, E.K., Simmons, D.M., Pennoyer, K.E., Derby, C.D., 2010. Caribbean reef squid, *Sepioteuthis sepioidea*, use ink as a defense against predatory French grunts, *Haemulon flavolineatum*. *J. Exp. Mar. Biol. Ecol.* 388, 20–27. <http://dx.doi.org/10.1016/j.jembe.2010.03.010>.
- York, C. a., Bartol, I.K., 2014. Lateral line analogue aids vision in successful predator evasion for brief squid *Lolliguncula brevis*. *J. Exp. Biol.* 2437–2439 <http://dx.doi.org/10.1242/jeb.102871>.
- Young, R.E., Harman, R.F., 1988. "Larva," "paralarva" and "subadult" in cephalopod terminology. *Malacologia* 29, 201–207.
- Young, R., Roper, C., 1976. Bioluminescent countershading in midwater animals: evidence from living squid. *Science* 191, 1046–1048. <http://dx.doi.org/10.1126/science.1251214> (80-).
- Zylinski, S., Johnsen, S., 2011. Mesopelagic cephalopods switch between transparency and pigmentation to optimize camouflage in the deep. *Curr. Biol.* 21, 1937–1941. <http://dx.doi.org/10.1016/j.cub.2011.10.014>.