

SHORT COMMUNICATION

Lateral line analogue aids vision in successful predator evasion for the brief squid, *Lolliguncula brevis*

Carly A. York* and Ian K. Bartol

ABSTRACT

Cephalopods have visual and mechanoreception systems that may be employed to sense and respond to an approaching predator. While vision presumably plays the dominant role, the importance of the lateral line analogue for predator evasion has not been examined in cephalopods. To test the respective roles of vision and the lateral line analogue, brief squid, *Lolliguncula brevis*, were observed in the presence of summer flounder, *Paralichthys dentatus*, under light and dark conditions with their lateral line analogue intact and ablated. Hair cell ablation was achieved through a pharmacological technique used for the first time on a cephalopod. The proportion of predator–prey interactions survived was significantly higher in the light non-ablated and light ablated groups compared with the dark ablated group. The mean number of interactions survived varied across treatment groups with the light non-ablated group having significantly more success than the light ablated, dark non-ablated and dark ablated groups. These findings demonstrate that although vision is the primary sense, the lateral line analogue also contributes to predator evasion in squid.

KEY WORDS: Cephalopod, Sensory systems, Predator–prey**INTRODUCTION**

Cephalopods rely on multiple sensory systems for detection of predators (Budelman, 1996). The eyes are the most prominent sensory feature of cephalopods with the optic lobes being the dominant region of the brain (Young, 1962). The highly evolved visual system of cephalopods probably plays a large role in predator detection and initiation of an escape response. However, to date, the role of vision relative to other sensory modalities in predator evasion has not been examined in any cephalopod.

In addition to their complex visual system, cephalopods have a sensory system that resembles the lateral line system of fishes (Bleckmann et al., 1991; Budelman, 1996; Budelman and Bleckmann, 1988). The cephalopod lateral line analogue consists of polarized epidermal hair cells that have several kinocilia and an axon extending from their base (Budelman and Bleckmann, 1988). Polarization occurs in a precise pattern (e.g. anteriorly, posteriorly, left and right), allowing the animals to respond to water movements as slow as $18.8 \mu\text{m s}^{-1}$, which is equivalent to the sensitivity of fish lateral lines (Bleckmann et al., 1991). Behavioural responses have also been elicited in cuttlefish (*Sepia officinalis*) by stimulating their lateral line analogue using a wide range of frequencies (10–600 Hz) (Komak et al., 2005). While fish can react swiftly to the flow field produced by an attacking predator using their lateral line system

(Stewart et al., 2013), it is unknown whether the lateral line analogue of cephalopods plays a similar role in predator detection. The goal of this study was to test the hypothesis that the lateral line analogue of squid *Lolliguncula brevis* (Blainville 1823) aids vision in successful predator evasion.

RESULTS AND DISCUSSION

To test the respective roles of vision and the lateral line analogue, brief squid, *L. brevis*, were observed in the presence of summer flounder *Paralichthys dentatus* (Linnaeus 1766) under light and dark conditions with their lateral line analogue intact and ablated. Hair cell ablation was achieved through a pharmacological technique, which was validated for the first time in a cephalopod. All of the squid that were tested in the light non-ablated group ($N=10$) successfully evaded the predators with a total number of 60 interactions survived (4–11 interactions survived per individual). In the light ablated group ($N=10$), however, only six squid successfully evaded the predators with a total of 34 interactions survived (0–5 interactions survived per individual). Successful evasion was further reduced in the dark condition with only five squid surviving in the non-ablated group ($N=10$; 22 total interactions survived; 0–4 interactions survived per individual) and two squid surviving in the ablated group ($N=10$; 15 total interactions survived; 0–4 interactions survived per individual).

The proportion of interactions survived significantly differed across treatment groups ($F_{3,36}=6.16$, $P=0.002$; Fig. 1). Tukey *post hoc* comparisons showed the light non-ablated group (mean \pm s.d., 1.00 ± 0.00) had a higher proportion of interactions survived than the dark ablated group (0.33 ± 0.44 , $P=0.001$). Additionally, the light ablated group (0.78 ± 0.34) showed higher survivability than the dark ablated group (0.33 ± 0.44 , $P=0.046$). While not significant at $\alpha=0.05$, the light non-ablated group exhibited a trend of a higher proportion of interactions survived relative to the dark non-ablated group (0.57 ± 0.50 , $P=0.056$). The mean number of interactions survived differed significantly across treatment groups ($F_{3,36}=8.69$, $P<0.001$). Tukey *post hoc* comparisons of the four groups indicated that the light non-ablated group (6.00 ± 2.20) had a significantly higher mean number of interactions survived than the light ablated group (2.90 ± 1.52 , $P=0.031$), the dark non-ablated group (2.20 ± 1.62 , $P=0.002$) and the dark ablated group (1.50 ± 1.27 , $P<0.001$).

The results of this study indicate that both vision and the lateral line analogue provide sensory information for successful predator evasion. The light non-ablated group survived a higher number of interactions than the light ablated and dark treatment groups, indicating the importance of both sensory systems. The observed higher proportion of interactions survived for the light non-ablated group relative to the dark ablated group and the trend of a higher proportion of interactions survived for the light non-ablated versus the dark non-ablated group demonstrate that vision is the most important modality (of the two tested here) for predator avoidance.

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

*Author for correspondence (csind001@odu.edu)

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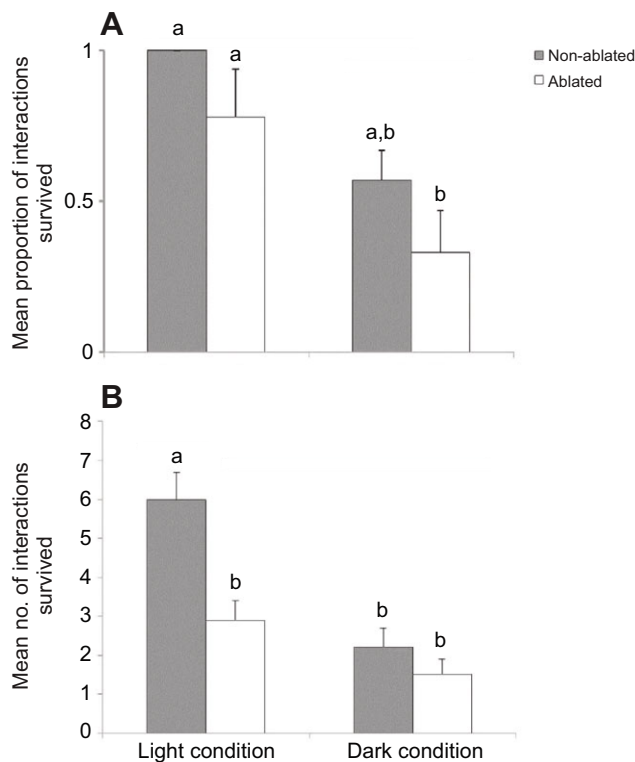


Fig. 1. Survivorship for non-ablated and ablated squid during light and dark conditions. (A) Proportion of interactions survived in each treatment group. (B) Number of interactions survived in each treatment group. Non-transformed means and s.e.m. are presented, as opposed to transformed data used in statistical analyses, to facilitate easier interpretation of data. Bars with the same lowercase letters are not significantly different (Tukey *post hoc* comparison tests).

The higher mean proportion of interactions survived in the light ablated than in the dark ablated group provides further support for this conclusion. The increased number of interactions survived in the light non-ablated than in the light ablated group, however, suggests that the lateral line analogue also plays a role in predator evasion even when vision can be used. Although the results for the dark non-ablated and dark-ablated groups were not significantly different, there was a 30% decline in the total number of animals that survived when the lateral line analogue was ablated, indicating that having the sensory hair cells intact aided survival in dark conditions as well.

The lateral line analogue appears to play a similar role to the fish lateral line, serving as a sensory component to predator evasion (Stewart et al., 2013). Although vision and the lateral line analogue clearly contribute to survival, there are other sensory modalities that could potentially add to successful predator evasion. Olfactory cues were probably present and could have alerted the squid to the predator's presence. Additionally, mechanoreceptors within the muscle or on the fins could have detected a pressure wave created by an approaching predator (Budelmann, 1996). It is possible that several sensory components not tested here contribute to successful predator avoidance; therefore, further research is needed to examine other potential sensory modalities involved in predator detection and evasion in cephalopods. This study, however, demonstrates for the first time that mechanoreceptor ablation techniques can be performed successfully on cephalopods and the lateral line analogue, together with the visual system, is important for predator detection and survival.

MATERIALS AND METHODS

Animal collection and maintenance

This study was conducted in accordance with Old Dominion University's IACUC (protocol no. 12-016). *Lolliguncula brevis* ranging from 2.5 to 6.0 cm dorsal mantle length (DML) were used in this project. Squid were captured by otter trawl in Wachapreague, VA, USA, and kept in 1.2 m diameter circular tanks using protocols described previously (Hanlon, 1990). Animals were allowed to acclimate for at least 2 h before experiments. Only animals that appeared healthy and exhibited normal behaviours were considered for the experiments. For the lateral line analogue ablation validation, *Doryteuthis pealeii* (Lesueur 1821) paralarvae were purchased from the Marine Biological Laboratory, Woods Hole, MA, USA, and maintained in a recirculating seawater system at a salinity of 30–32‰ and at a temperature of 19–24°C until hatching.

Lateral line analogue ablation validation

Antibiotic solutions have successfully been used for lateral line ablation in fish studies (Harris et al., 2003; Stewart et al., 2013); however, the technique had never been performed on cephalopods and therefore validation of this approach was required. To determine the appropriate concentration of the neomycin sulphate solution for lateral line analogue ablation, a series of antibiotic trials was conducted. *Doryteuthis pealeii* paralarvae were used in the antibiotic trials because of the large number available and small body size, which is conducive for scanning electron microscopy (SEM) preparation. Paralarvae were divided into four treatment groups ($N=15$ per group): 0, 150, 250 and 500 $\mu\text{mol l}^{-1}$ neomycin sulphate. Paralarvae were placed into the antibiotic solution for 1 h. Prior to fixation, squid were over-anesthetized using water at 5°C for ~15 min.

SEM was employed to survey the success of ablation within each of the experimental groups. For SEM, the squid were placed overnight in a fixative (3% glutaraldehyde, 6% sucrose, 0.5% tannic acid, 0.065 mol l^{-1} Sørensen's buffer), rinsed and stored in a buffer (1% glutaraldehyde, 6% sucrose, 0.065 mol l^{-1} Sørensen's buffer) and then dehydrated in a graded ethanol series. Specimens were dried using the chemical drying agent hexamethyldisilazane and mounted on aluminium stubs with double-sided adhesive tape. Specimens were sputter-coated with 15–30 nm gold and examined with a Hitachi S-3400N JEOL 6300-F field emission scanning electron microscope at an accelerating voltage of 15 kV. SEM images revealed consistently successful lateral line ablation after treatment with 500 $\mu\text{mol l}^{-1}$ neomycin sulphate solution (Fig. 2). Similar protocols were used with *L. brevis* juveniles to confirm lateral line ablation at this treatment concentration. Based on successful ablation in *D. pealeii* hatchlings and *L. brevis* juveniles, a 500 $\mu\text{mol l}^{-1}$ concentration was selected for predator–prey experiments. Although this treatment was effective at decreasing the number and integrity of lateral line hair cells, the lateral line regeneration capabilities of squid were not tested and are not currently known. Observation of both *D. pealeii* and *L. brevis* after treatment showed that squid maintained normal behaviours. Ablated squid maintained typical pitch, roll and yaw behaviours during swimming in the holding tanks, were able to hover effectively, and were able to successfully approach and attack prey, indicating that ablation does not impact the function of the statocysts or routine swimming and feeding behaviours.

Predator–prey experiments

Predator–prey interaction experiments were used to evaluate the use of vision and mechanoreception in predator evasion. Trials took place in a 1.2 m diameter, 0.76 m deep round tank with gravel substrate. The arena was lined with curtains to avoid disturbing acclimating animals. Black plastic sheeting was used to block light during the dark trials. For each experiment, a single squid was placed in an arena with two flounder (*P. dentatus*; 13.2 and 15.5 cm total length). Flounder have shown successful captures for the relative prey size presented in this study (Staudinger and Juanes, 2010), and were chosen as predators because of their exceptional vision in both bright and dark conditions (Horodysky et al., 2010). Multiple predators were used to increase the odds of a predation event. The flounder were fed live squid prior to the trials so that they could become proficient in squid capture before data collection. Food was withheld 24 h prior to the start of all trials to standardize predator hunger.

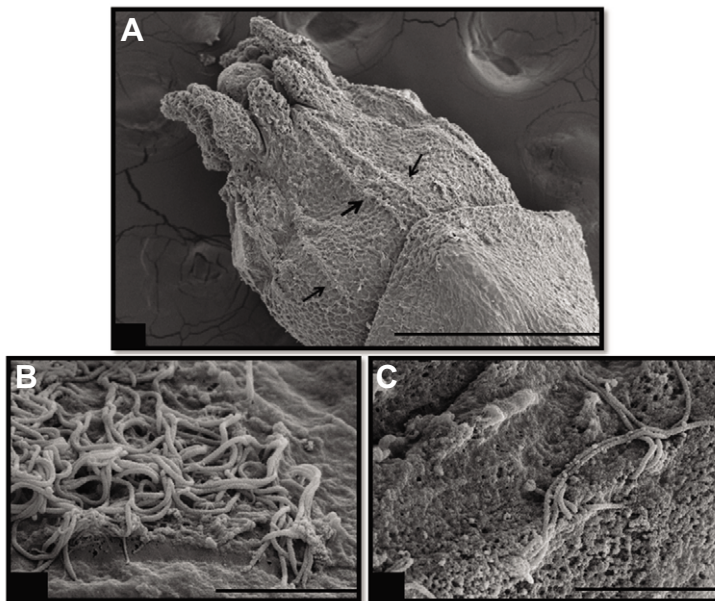


Fig. 2. SEM images of the lateral line analogue shown on a *Doryteuthis pealeii* paralarvae. (A) Lines of hair cells on the head indicated with arrows. (B) Close-up views of sensory hair cells of the lateral line analogue. (C) Sensory hair cells after treatment with a $500 \mu\text{mol l}^{-1}$ solution of neomycin sulphate. The majority of hair cells were destroyed completely after treatment, with the remaining hair cells being porous and heavily damaged. Scale bars: A, 400 μm ; B, C, 5 μm .

One hour prior to trial acclimation (see below), squid were placed in a container that contained either the neomycin sulphate solution for ablation groups or untreated water for the non-ablation groups. Prior to the start of each trial, a cylinder made of 5 mm plastic mesh was lowered into the experimental tank and a single squid was placed inside 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 ran for 10 min before surviving squid were removed. Four different conditions were tested: (1) light non-ablated, (2) light ablated, (3) dark non-ablated and (4) dark ablated. Ten separate squid were tested in each treatment condition. Each group contained squid of similar sizes (mean \pm s.d.: light non-ablated 4.2 ± 0.3 cm DML, light ablated 3.9 ± 0.3 cm DML, dark non-ablated 3.9 ± 0.4 cm DML, dark ablated 3.9 ± 0.3 cm DML). All interactions were recorded by a single observer. In darkened conditions, the observer was easily able to identify interactions by tracking the flounder silhouette against a light-coloured tank bottom and observing surface water disturbances, which were frequently present during interactions. After each interaction, a flashlight was turned on briefly to confirm squid capture or escape. Interactions used for subsequent statistical analysis were defined as: (1) successful predator strikes, (2) unsuccessful predator strikes and (3) approaches toward the squid but where a strike was not initiated because of an escape response by the squid.

Statistical analysis

Statistical analysis was performed in SPSS (v. 18 SPSS Inc., Chicago, IL, USA). The proportion of interactions survived for each squid was calculated to show success relative to the number of capture attempts. As this measure does not reveal the total number of interactions survived, the sum of interactions survived for each squid in each treatment group was also calculated. All data were tested for normality using Shapiro–Wilk tests. Data from several groups varied from normality (all $P \leq 0.02$) and therefore all data were transformed prior to parametric analysis. The proportion of interactions survived was adjusted via arcsine transformation, while the sum of interactions survived was adjusted with a square-root transformation. A regression was performed on the total number of interactions survived and the mantle length of the squid in each condition to determine the relationship between size and survivability. No significance was found (all $P \geq 0.10$), and thus all sizes were pooled for further analysis. ANOVA was performed on the transformed values of the total number of interactions survived and the proportion of interactions survived in each treatment group. Although ANOVA tests were performed on transformed data, the means reported in

the Results and figures are not transformed to facilitate easier interpretation of the data.

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Competing interests

The authors declare no competing financial interests.

Author contributions

C.A.Y. and I.K.B. collaborated on the experimental approach, data analysis and preparation of this manuscript. C.A.Y. performed the experiments and collected the data.

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