

Visual Stimuli for the Induction of Hunting Behavior in Cuttlefish *Sepia pharaonis*

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Abstract. Cuttlefish exhibit typical hunting behavior, including elongating tentacles against specific prey such as prawn and mysid shrimp. Cuttlefish hunting behavior involves three different actions: attention, positioning, and seizure. Hunting behavior is innate and stereotypic behavior, and it is present in newly hatched juveniles. Factors associated with prey are known to induce this behavior, similar to the sign stimulus, whereby young herring chicks imitate pecking behavior against a red dot on their parent's bill. Although the hunting behavior of cuttlefish has been described and used as an indicator to test learning and memory, details of a stimulus that can elicit this behavior remain unknown. Here, we used a variety of visual stimuli presented on a computer screen to investigate the factors that induce hunting behavior of pharaoh cuttlefish, *Sepia pharaonis*. We found that the appearance of prey (western king prawn, *Melicerus laticulatus*) and their movement at a vertical angle of 45° are specific factors that can initiate hunting behavior. We also showed that the height of prey can attract cuttlefish and initiate hunting. To the best of our knowledge, this is the first report of a stimulus that elicits stereotyped hunting behavior by coleoid cephalopods.

Introduction

Coleoid cephalopods (cuttlefish, squids, and octopuses) are members of Mollusca that exhibit features such as human-like lens eyes and relatively large brains, and they are therefore

considered intelligent invertebrates (Hanlon and Messenger, 1996; Williamson and Chachri, 2004). Because coleoid cephalopods possess a sophisticated visual system, they exhibit various visual behaviors, such as complicated camouflage and dynamic coloration during reproductive contests (Hanlon and Messenger, 1996). Hunting behavior in cuttlefish, which involves the use of two tentacles, is another example of a well-known visual behavior of coleoid cephalopods. This behavior has been documented in detail and can be divided into three different actions—namely, attention, positioning, and seizure—and it is immediately elicited when a prey falls within the visual field of cuttlefish (Wells, 1958, 1962; Messenger, 1968, 1977). The three phases of hunting behavior in cuttlefish can be summarized as follows (Messenger, 1968, 1977; Hanlon and Messenger, 1996). *Attention* is the raising of the first, and sometimes second, pair of arms and movement of the eyes, head, and body, so that prey is brought onto a forward extension of the body axis with the eyes directed toward it. *Positioning* is when the cuttlefish swims toward, or occasionally away from, its prey until it achieves its attacking distance, which is equivalent to about one mantle length. *Seizure* is when the tentacles are ejected to strike at prey, which is seized and brought back to the arms and mouth. Because this behavior is exhibited by newly hatched juveniles, tentacular strike by cuttlefish is an example of an innate, stereotyped, and spontaneous behavior.

Many similar behaviors, in terms of innateness and stereotypy, are found among various animal taxa. This well-known phenomenon is termed “instinct” and was originally investigated by Niko Tinbergen and Konrad Lorenz (Manning and Dawkins, 2012). For example, chicks of the European herring gull, *Larus argentatus*, perform pecking behavior on the tip of their parent's bill, and in response, the parent feeds the chick. During this behavior, the herring gull chick is only aware of the shape of its parent's bill and the red dot, which

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Abbreviations: DML, dorsal mantle length; TL, total length.

act as simple stimuli to induce the chick to peck. Another example is provided by the three-spined stickleback, *Gasterosteus aculeatus*. Mature male fish defend their territory by reacting to the red throat coloration on other males, which they then attack aggressively. These behaviors are under neural control: the neural network responsible for detecting the simple cue (the sign stimulus or releaser) and for activating the instinct, or fixed action pattern, is termed the “innate releasing mechanism” (Manning and Dawkins, 2012).

The innate releasing mechanism does not require learning or former experience and thus must directly increase the adaptability of the animal. In cuttlefish, prey such as mysid shrimp act as a trigger for the emergence of the tentacular strike behavior (Wells, 1958, 1962; Messenger, 1968, 1977). Because this behavior continues beyond the appearance of the prey, the tentacular strike has been used as a tool to record the process of learning and memory in cuttlefish. For example, in the “prawn-in-the-tube” test, the number of strikes corresponds to the level of learning and memory (Wells, 1958, 1962; Messenger, 1968, 1977). However, the factors associated with the prey that act as stimuli to elicit tentacular strike by cuttlefish remain unknown. Like other cephalopods, cuttlefish possess well-developed vision; therefore, they must perceive particular visual information from prey and then initiate hunting behavior, in a manner similar to a typical reaction to the sign stimulus reported in other animals. In the cuttlefish *Sepia officinalis*, newly hatched animals attack living and even dead mysid shrimp moving in the water column (Wells, 1958, 1962). Young *S. officinalis* have also been shown to attack a picture resembling mysid shrimp, moving horizontally (Wells, 1958, 1962). In this case, prior experience of attacking mysid shrimp increased the frequency that the model was attacked. However, although multiple factors might affect the induction of attack behavior, these have not been determined in detail. In addition, there is no agreement on the factors that initiate hunting behavior of cephalopods among different species. For example, although octopuses are attracted to live animals, such as crabs and shrimps, for hunting, they also feed on dead (*i.e.*, motionless) specimens, indicating that the movement of prey is not necessary to initiate hunting in octopuses (Wodinsky, 1971). This was also shown in a recent study in which artificial prey (crab) were used to initiate hunting by octopuses (Amodio *et al.*, 2014). Nevertheless, it can still be stated that cephalopods usually show great interest in living organisms, probably because they comprise the main portion of cephalopod diets. Therefore, rather than being scavengers, cephalopods are key animals that actively hunt (Nixon, 1987). Regarding hunting behavior, squids and cuttlefish are popular targets for game fishing and are caught by lures, fishing gear resembling prawn or fish, which all move erratically and in an up-down motion underwater. This suggests that particular features of lures, such as shape and/or motion, elicit stereotyped tentacular strike. However, there is no clear knowledge

or consensus on the features relating to the motivation for hunting behavior by cephalopods.

Therefore, we examined the stimuli that can elicit tentacular strike behavior of pharaoh cuttlefish, *Sepia pharaonis*, by using behavioral tests. We have already confirmed the consequence of stereotyped hunting behavior, with tentacular strike also observed in *S. pharaonis*—that this is an innate behavior that can be observed in newly hatched juveniles (Sugimoto and Ikeda, 2013). In this study, we first used a computer screen to present different visual stimuli to cuttlefish, representing a potentially new experimental device for the behavioral study of cephalopods, allowing results to be compared between phylogenetically different animal groups. Newly hatched cuttlefish attack particular prey, such as mysid shrimp, with defined actions soon after hatching; therefore, hunting behavior *via* tentacular strike is considered innate (Wells, 1958, 1962). In contrast, as cuttlefish grow, they start to attack other types of organisms. Therefore, hunting behavior in later phases of life might be dependent on learning (Wells, 1958, 1962). Regardless of the presence of experience-dependent behavior, we can still hypothesize that specific factors, or sign stimuli, can also elicit hunting behavior in older cuttlefish, because the response of adult cuttlefish to mysids is similar to that of newly hatched cuttlefish (Messenger, 1968). In other words, because specific factors are imprinted in genes, they can elicit stereotyped behavior by cuttlefish through all their life stages. Based on this, we examined the sign stimuli that can elicit typical hunting behavior of *S. pharaonis* in different life stages. Because prawns can move in various angles when swimming and during the escape response (Yu *et al.*, 2009), and lures (bait logs) used in game fishing for squids and cuttlefish are jerked erratically up and down at various angles (Nair, 1985; Muniyapillai *et al.*, 2016), the angle of prey movement may be a key stimulus for the induction of hunting behavior in cuttlefish. Therefore, we set different angles of motion for images to examine the effect on tentacular strike induction.

Materials and Methods

Collection and maintenance of animals

Egg cases of *Sepia pharaonis* Ehrenberg, 1831 were collected from the coastal waters of Okinawajima Island in April 2016 and transported to the laboratory at the University of the Ryukyus. Upon arrival, they were transferred in a transparent tube (300-mm diameter, 300-mm depth, 20-l volume) soaked in a circular tank (open-field tank, Aqua, Toyko, Japan, 700-mm diameter, 330-mm depth, 120-l volume) with a closed seawater system associated with a filter tank, a combined cooler and heater, and a UV sterilizer. Hatchlings were reared in the transparent tube until they were 11 days old. Then they were transferred to the following larger tanks as they grew: a circular

tank (Multihydense, Aqua; 300-mm diameter, 355-mm depth, 20-l volume) until 24 days old; a larger circular tank (Multihydense, Aqua; 400-mm diameter, 455-mm depth, 50-l volume) until 55 days old; and, thereafter, a larger cubic plastic tank (410 mm \times 585 mm, 385-mm depth, 90-l volume), a small cubic plastic tank (380 mm \times 550 mm, 200-mm depth, 40-l volume), and a square tank (1800 mm \times 1800 mm, 600-mm depth, 1000-l volume). These tanks were associated with a filter tank, a combined cooler and heater, and a UV sterilizer. The method used to maintain cuttlefish has been described in detail previously (Sugimoto and Ikeda, 2013; Yasumuro and Ikeda, 2016). Cuttlefish 75–250 days old participated in the behavioral experiments. The dorsal mantle length (DML) of cuttlefish was measured by Image J, version 1.50i (Schneider *et al.*, 2012), using video recordings of the experiments. We were unable to measure the DML of one individual that participated in Experiment II because it remained outside the range of the recording.

Experiment I: applicability of a computer screen to examine the hunting behavior of Sepia pharaonis

Before using a virtual image to assess the hunting behavior of *S. pharaonis*, we examined the applicability of a computer screen to present visual stimuli. Eight *S. pharaonis* individuals (75–77 days old; DML 34–45 mm) participated in the experiment. A cuttlefish was acclimated in a transparent cubic glass tank (300 mm \times 300 mm \times 300 mm) with seawater. Because we previously showed that pharaoh cuttlefish prefer hunting living prawns, the following images were presented (Fig. 1A): image 1, a live western king prawn (*Melicertus latisulcatus* (Kishinouye, 1896); total length [TL] *ca.* 90 mm) caught in the coastal waters of Okinawajima Island, kept in a cubic glass tank (100 mm \times 290 mm, 320-mm depth, 9-l volume) with gravel substrate; and image 2, a video of a live western king prawn (TL *ca.* 60 mm; measured from a video recording), obtained by recording the side view of image 1 using a digital video camera (HDR-PJ800, Sony, Minato, Toyko). Image 2 was displayed on an Apple iPad Air 2 (Apple, Cupertino, CA) (Fig. 2). These stimuli were presented on a single wall of the cubic glass tank in which the cuttlefish were placed. The location of the stimulus on the screen was selected randomly, regardless of the position of the cuttlefish in the tank. Each image was presented to 4 cuttlefish for 5 min, and the behavior of the cuttlefish was recorded by a video camera (HDR-PJ800, Sony) set above the tank.

Experiment II: significance of prey movement

To investigate whether prey movement could initiate hunting behavior by cuttlefish, seven videos, each using a frame-grabbed image of a real western king prawn, were presented to *S. pharaonis* as follows (Fig. 1B): image 3, western king prawn (*i.e.*, motionless); image 4, western king prawn mov-

ing 100 mm forward horizontally (*i.e.*, 0°) for 5 s; image 5, western king prawn moving forward, 22.5° upward for a distance of 55 mm, then 22.5° downward for a distance of 55 mm for 7 s; image 6, western king prawn moving forward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s; image 7, western king prawn moving forward, 67.5° upward for a distance of 105 mm, then 67.5° downward for a distance of 105 mm for 11 s; image 8, western king prawn moving 90° upward for a distance of 90 mm, then 90° downward for a distance of 90 mm for 7 s; and image 9, western king prawn moving backward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s. The moving distance of the shrimp was prescribed by the trajectory of the tip of the telson. In images 4–7 and 9, the western king prawn was moved leftward on the screen and with a repeated upward and downward motion until it disappeared from the edge of the screen (Fig. 1B, upper line). Then the western king prawn reappeared from the edge of the screen, and it was moved rightward on the screen (Fig. 1B, lower line). These images were developed using PowerPoint on an Apple Macintosh computer (Mac Book Pro) and were displayed on an Apple iPad Air 2 (display size: height 149 mm, width 198 mm). Each image was presented repeatedly for 5 min to 6 cuttlefish (140–200 days old; DML 63–128 mm). All cuttlefish were tested only once; hence, a total of 42 individuals participated in this experiment. In all images, the TL of the king prawn was 50 mm. The picture moved continuously at a speed of approximately 4 cm s⁻¹ for images 4–9, which is consistent with the walking speed of brown tiger prawns, *Penaeus esculentus* (4–6 cm s⁻¹; Hill, 1985). Stimuli were presented and cuttlefish behavior was recorded as described in Experiment I.

Experiment III: significance of prey shape

To investigate whether the shape of prey initiates hunting behavior by cuttlefish, five video images involving forward movement, 45° upward and downward, were presented to *S. pharaonis* (Fig. 1C). The angle toward the moving target was set at 45° to eliminate any effects due to differences in the direction of the target. This angle was determined from the image most frequently (although not statistically significantly) attacked in Experiment II (see Results). The images used were as follows: image 10, silhouette of western king prawn (TL 50 mm); image 11, silhouette of lure (length 50 mm); image 12, dark ellipse (50-mm length, 10-mm height); image 13, black rectangle (50-mm length, 10-mm height); and image 14, black square (23-mm side). These images were moved leftward on the screen and with repeated upward and downward motion until they disappeared from the edge of the screen (Fig. 1C, upper line). Then they appeared from the edge of the screen and were moved rightward on the screen (Fig. 1C, lower line). Because cuttlefish attack a model mysid moving along its long axis (Wells, 1962), we speculated that

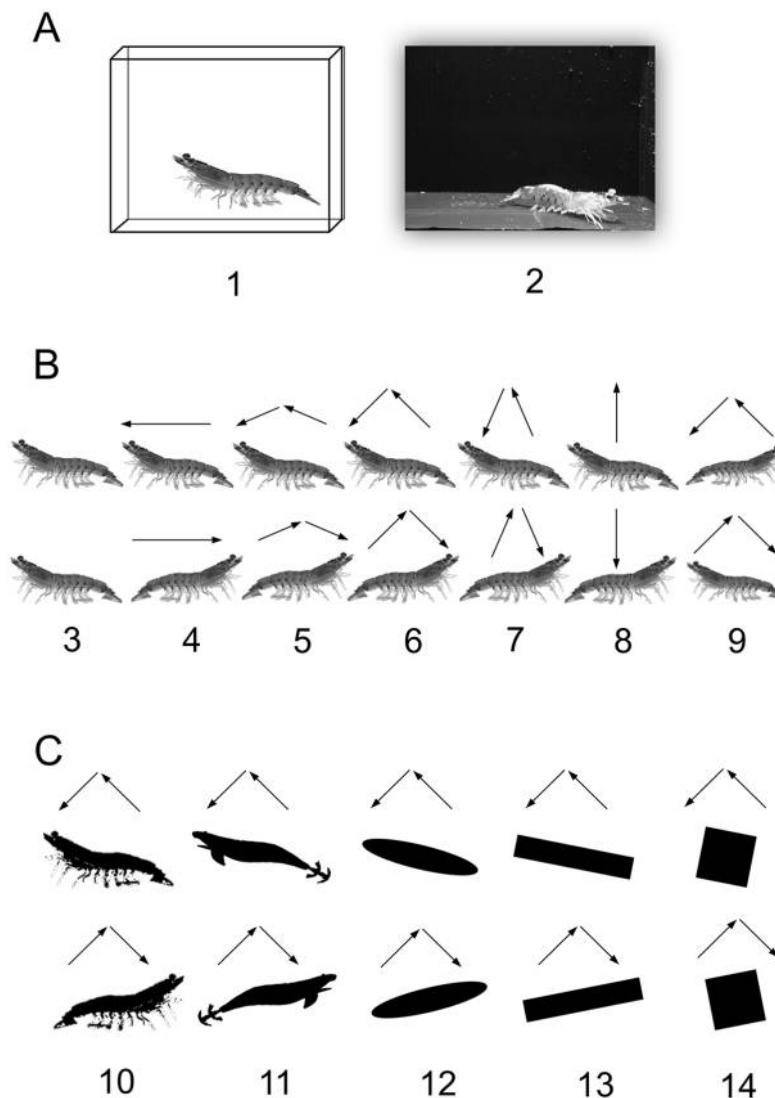


Figure 1. Images of the western king prawn, *Melicerus laticulatus*, presented to *Sepia pharaonis*. (A) Experiment I. (B) Experiment II. (C) Experiment III. The number below each image indicates the image code. 1. A live western king prawn. 2. Video of a live western king prawn. 3. Video of a motionless image of a real western king prawn. 4. Video of an image of a real western king prawn moving 100 mm forward horizontally (*i.e.*, 0°) for 5 s. 5. Video of an image of a real western king prawn moving forward, 22.5° upward for a distance of 55 mm, then 22.5° downward for a distance of 55 mm for 7 s. 6. Video of an image of a real western king prawn moving forward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s. 7. Video of an image of a real western king prawn moving forward, 67.5° upward for a distance of 105 mm, then 67.5° downward for a distance of 105 mm for 11 s. 8. Video of an image of a real western king prawn moving 90° upward for a distance of 90 mm, then 90° downward for a distance of 90 mm for 7 s. 9. Video of an image of a real western king prawn moving backward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s. 10. Silhouette of a western king prawn moving forward, 45° upward, then 45° downward. 11. Silhouette of a lure moving forward, 45° upward, then 45° downward. 12. Dark ellipse moving forward, 45° upward, then 45° downward. 13. Black rectangle moving forward, 45° upward, then 45° downward. 14. Black square moving forward, 45° upward, then 45° downward. In images 4–14 but not 8, the image was moved leftward on the screen with repeated upward and downward motions until it disappeared from the edge of the screen (upper line). Then the image appeared from the edge of the screen, and it was moved rightward across the screen (lower line).

cuttlefish would not be attracted by a non-elongated object. Therefore, we used images 13 and 14 to examine this possibility. These images were displayed on an Apple iPad Air 2 and presented repeatedly to 6 cuttlefish (200–250 days old;

DML 103–139 mm) for 5 min. All cuttlefish were tested only once; hence, a total of 30 individuals participated in this experiment. Stimuli were presented and the behavior of cuttlefish was recorded as described for Experiment I.

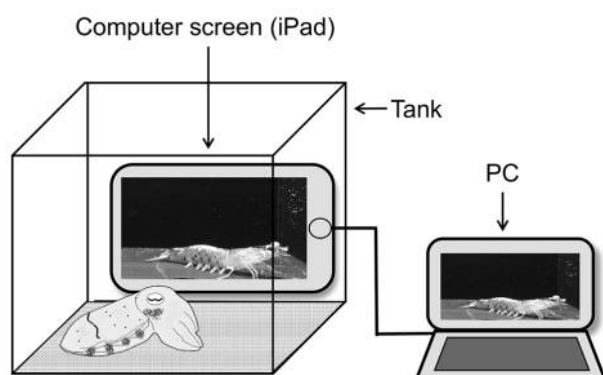


Figure 2. Schematic diagram of the apparatus used to present images of *Melicerus latisulcatus* to *Sepia pharaonis* in Experiments I–III.

Data analysis

The behavior of *Sepia pharaonis* was analyzed from the recorded videos. To evaluate whether hunting behavior occurred, we focused on two stepped actions, *Positioning* and *Seizure* (Messenger, 1968, 1977). In *Positioning*, the 10 arms of the cuttlefish are closed, and the tip is directed toward the image on the screen. This indicates that the cuttlefish are interested in the image. In *Seizure*, cuttlefish radically elongate their tentacles toward an image. *Attention* is defined by Messenger (1968, 1977) as a reaction in which the cuttlefish turns its body toward the target, coinciding with the onset of hunting. We did not include *Attention* in the present measurement because our procedure differs from that of Messenger (1968, 1977). In our study, the direction of cuttlefish was not artificially controlled, because we did not want to stress the animals at the start of the experiment. Additionally, because the attention reaction is of short duration, it would be negligible when evaluating cuttlefish hunting behavior, which mainly consists of positioning and seizure. Conversely, we observed that cuttlefish often swam with or without positioning. Messenger (1968) also noted that cuttlefish swim forward or backward in order to achieve the correct distance for attack during positioning. Therefore, we measured this swimming behavior with the following definition to analyze its association with motivation for hunting. In *Swimming*, cuttlefish move their fin, regardless of their direction toward an image. Duration of positioning and the number of seizures were measured to evaluate hunting behavior. Duration of swimming was also measured to analyze its association with hunting behavior. Then duration of attention and swimming and number of attacks were averaged for each of Experiments I–III.

Student's *t* tests or one-way ANOVAs with Bonferroni's *post hoc* tests were used to determine whether statistical differences ($P < 0.05$) existed across the images for the duration of attention and swimming and for the number of attacks. Statistical analyses were conducted using KaleidaGraph, version 4.1.4 (HULINKS, Tokyo).

Results

Applicability of a computer screen to examine the hunting behavior of *Sepia pharaonis* (Experiment I)

Sepia pharaonis positioned themselves toward live and virtual western king prawns presented on the screen (images 1 and 2, Fig. 3). Regardless of whether the prawns were live, *S. pharaonis* exhibited positioning behavior for more than 50% of the time during which the prawn was presented (Fig. 3). There was no significant difference in the duration of positioning by cuttlefish between live western king prawns and virtual prawns (Student's *t* test, $t = -0.498$, $df = 6$, $P > 0.05$). *Sepia pharaonis* exhibited seizure behavior when exposed to the virtual western king prawn, whereas no seizure behavior was observed when exposed to the live prawn, although they positioned themselves toward it (Figs. 3, 4). These results may indicate that *S. pharaonis* recognizes the image on the computer screen as prey; therefore, this device can be used to test the hunting behavior of cuttlefish.

Significance of prey movement (Experiment II)

Sepia pharaonis exhibited positioning behavior toward the virtual western king prawn, regardless of whether it remained motionless or moved vertically at various angles, for an average of 30%–50% of the time that each image was presented (images 3–9, Fig. 5). There was no significant difference be-

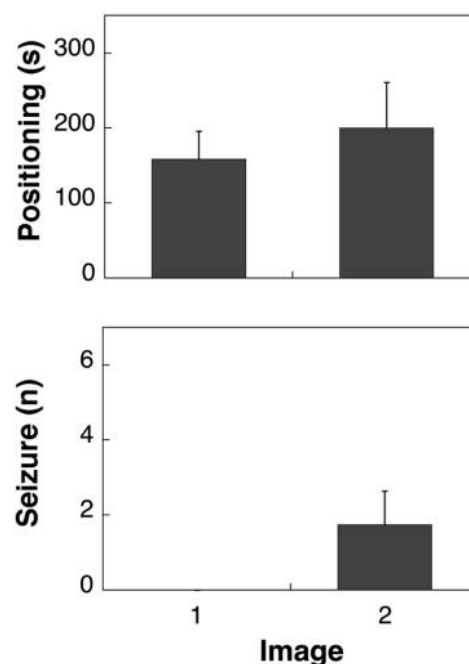


Figure 3. Behavioral assessment of *Sepia pharaonis* against two images (images 1 and 2) of the western king prawn, *Melicerus latisulcatus*, in Experiment I. The number below the x-axis indicates the image code. 1. A live western king prawn. 2. Video of a live western king prawn. The bar and line represent the mean and standard error, respectively.



Figure 4. *Sepia pharaonis* attacking virtual image of a western king prawn, *Melicertus latisulcatus*, on the iPad screen. Note that tentacles are elongated toward the image.

tween images (one-way ANOVA, $F = 0.643$, $df = 6, 35$, $P > 0.05$). *Sepia pharaonis* exhibited seizure behavior to all western king prawns except those that remained motionless (Fig. 5). One-way ANOVA revealed a significant group effect ($F = 3.238$, $df = 6, 35$, $P = 0.012$). Image 6 (western king prawn moving forward and vertically at 45°) was attacked most by *S. pharaonis*, and this was significantly different from the attack of image 3 (motionless western king prawn) (Bonferroni's *post hoc* test, $P < 0.01$; Fig. 5).

Significance of shape of prey (Experiment III)

Sepia pharaonis positioned themselves toward all types of stimuli displayed on the computer (images 10–14); the duration of positioning was shortest toward image 10 (silhouette of western king prawn moving forward vertically at 45°) (Fig. 6). *Sepia pharaonis* spent more than 50% of the time positioning themselves toward the images, with the exception of image 10, and cuttlefish were positioned for significantly longer periods toward images 11–13 compared to image 10 (one-way ANOVA, $F = 5.236$, $df = 4, 25$, $P = 0.003$; Bonferroni's *post hoc* test, $P < 0.05$; Fig. 6). *Sepia pharaonis* attacked all images except for image 14. Cuttlefish attacked image 10 most frequently, and the frequency gradually decreased from image 11 to image 13. One-way ANOVA revealed a significant group effect ($F = 5.903$, $df = 4, 25$, $P = 0.002$). Significant differences in the number of attacks were observed between images 10 and 13 and between images 10 and 14 by using Bonferroni's *post hoc* test ($P < 0.01$ for both; Fig. 6).

Swimming and motivation for hunting behavior

Cuttlefish often exhibited swimming behavior when images were presented. In Experiment I, *S. pharaonis* swam in front of the western king prawn regardless of whether it was live or virtual (Fig. 7). In Experiment II, *S. pharaonis* swam for about 50% of the time when presented with the images, with the exception of the image of the motionless western king

prawn (Fig. 7). There were significant differences in swimming duration between images 3 and 7 and between images 3 and 9 (one-way ANOVA, $F = 3.160$, $df = 6, 35$, $P = 0.014$; Bonferroni's *post hoc* test, image 3 vs. image 7, $P < 0.01$, and image 3 vs. image 9, $P < 0.05$; Fig. 7). In Experiment III, *S. pharaonis* spent the longest time swimming when image 10 was presented and the shortest time swimming toward image 14 (black square moving vertically at 45°); and this was statistically significant compared with images 11, 13, and 14 (one-way ANOVA, $F = 7.710$, $df = 4, 25$, $P < 0.001$; Bonferroni's *post hoc* test, image 10 vs. image 11, $P < 0.05$, image 10 vs. images 13 and 14, $P < 0.01$; Fig. 7). Swimming duration was positively correlated with the number of seizures

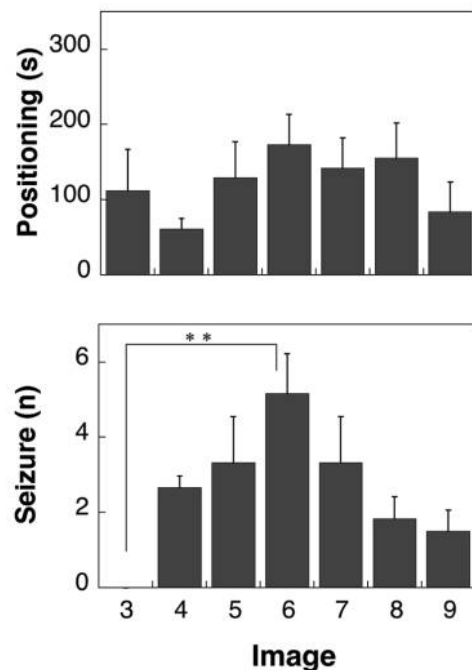


Figure 5. Behavioral measurements of *Sepia pharaonis* against seven images (images 3–9) of the western king prawn, *Melicertus latisulcatus*, in Experiment II. The number below the x-axis indicates the image code. 3. Video of a motionless image of a real western king prawn. 4. Video of an image of a real western king prawn moving 100 mm forward horizontally (*i.e.*, 0°) for 5 s. 5. Video of an image of a real western king prawn moving forward, 22.5° upward for a distance of 55 mm, then 22.5° downward for a distance of 55 mm for 7 s. 6. Video of an image of a real western king prawn moving forward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s. 7. Video of an image of a real western king prawn moving forward, 67.5° upward for a distance of 105 mm, then 67.5° downward for a distance of 105 mm for 11 s. 8. Video of an image of a real western king prawn moving 90° upward for a distance of 90 mm, then 90° downward for a distance of 90 mm for 7 s. 9. Video of an image of a real western king prawn moving backward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s. In images 4–7 and 9, the image was moved leftward across the screen with repeated upward and downward motions until it disappeared from the edge of the screen. Then the image reappeared from the edge of the screen and was moved rightward across the screen. The bar and line represent the mean and standard error, respectively. Bonferroni's test, significant, two asterisks, $P < 0.01$.

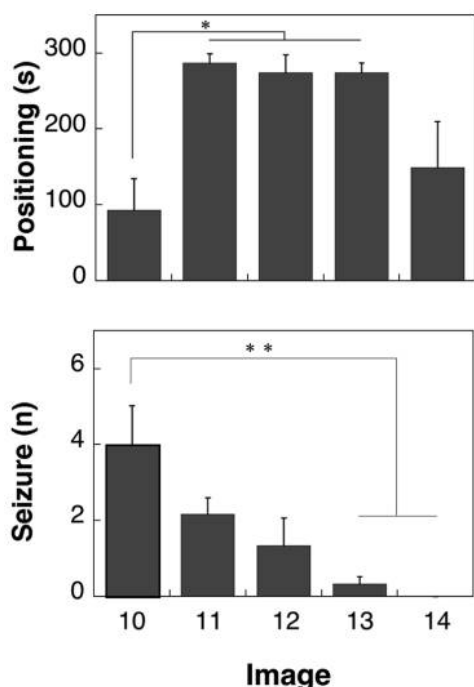


Figure 6. Behavioral measurements of *Sepia pharaonis* against five images (images 10–14) of the western king prawn, *Melicertus latisulcatus*, in Experiment III. The number below the x-axis indicates the image code. 10. Silhouette of western king prawn moving forward, 45° upward, then 45° downward. 11. Silhouette of a lure moving forward, 45° upward, then 45° downward. 12. Dark ellipse moving forward, 45° upward, then 45° downward. 13. Black rectangle moving forward, 45° upward, then 45° downward. 14. Black square moving forward, 45° upward, then 45° downward. The image was moved leftward across the screen with repeated upward and downward motions until it disappeared from the edge of the screen. Then the image reappeared from the edge of the screen and was moved rightward across the screen. The bar and line represent the mean and standard error, respectively. Bonferroni's test, significant, one asterisk, $P < 0.05$; two asterisks, $P < 0.01$.

in three experiments combined (Pearson coefficient of correlation, $r = 0.49$, $P < 0.01$; Fig. 7).

Discussion

Applicability of a computer screen to investigate cuttlefish behavior

In the present study, with the help of virtual images displayed on a computer, we confirmed that *Sepia pharaonis* will attack prey displayed on a computer, which suggests that computer images are useful to investigate cuttlefish behavior. Coleoid cephalopods possess polarized vision and can detect prey with polarization-active tissues (Shashar *et al.*, 1998, 2000). Although this may suggest that computer screens are not suitable for use in cephalopod experiments aiming to investigate behavior, there are some examples of video playback experiments in cephalopods that provide evidence for episodic personality, discrimination training, freezing behavior, and polarization sensitivity (*Octopus tetricus* [Pronk *et al.*,

2010], *Sepia officinalis* [Hough, 2014; Bedore *et al.*, 2015], *Sepia plangon*, *Sepioloidea lineolata*, and *Sepioteuthis lessoniana* [Pignatelli *et al.*, 2011]). The successful use of computer screens to induce a response in cephalopods reflects features of their visual systems. The flicker fusion threshold of cephalopods is lower than that of computer screens; therefore, they can perceive images on a computer screen without perceiving a flicker (Hough, 2014). This would be the case for *S. pharaonis*, as used in the present study.

Although *S. pharaonis* did not attack real western king prawns in Experiment I, this was because the prawn could move to the opposite side of the tank; therefore, cuttlefish could not maintain a close distance to the prawn, which is necessary for the onset of tentacle elongation (Sugimoto and Ikeda, 2013). This was shown by the lack of any statistical difference in the duration of cuttlefish positioning when genuine and virtual prawns were used, indicating the continuous attention of cuttlefish to the moving object ahead (Fig. 3).

Significance of motion in the induction of cuttlefish hunting behavior

Interestingly, *S. pharaonis* positioned themselves toward the motionless image of a prawn, with no significant difference observed with other images of prawns moving at various angles (Fig. 5). This indicates that cuttlefish display interest in the prawns regardless of their movement. Mammals exhibit a maintained sequence of involuntary eye movements to detect stationary contours (Rucci and Poletti, 2015), whereas amphibians react only to moving stimuli because they have no such eye movement mechanisms (Ewert and Borchers, 1974). On this basis, it can be suggested that cuttlefish have involuntary control of their eye movements.

Sepia pharaonis did not attack the image of a motionless prawn, whereas they attacked images of prawns moving at various angles (Fig. 5). This clearly indicates that motion is necessary for the induction of hunting behavior in cuttlefish. The way that the prey moves is important for hunting behavior in cephalopods. A model mimicking mysids was frequently attacked by young *S. officinalis* when the model was moved steadily along the direction of its long axis (Wells, 1962). However, freshly killed prawns did not induce hunting behavior in *S. officinalis*, and if there was an attention response, it quickly faded, and the later stages of the attack did not occur (Messenger, 1977). Erratic behavior of copepods tempts juvenile *Loligo opalescens* to hunt, thus affecting the learning of hunting skills during the early life phase of this squid, a time of limited duration for the critical period of imprinting (Chen *et al.*, 1996).

The control of hunting behavior in cuttlefish falls into two phases. The first is attention and positioning, which comprise a visually controlled closed-loop system. The second is seizure, in which there is no time for visual feedback regarding the position of the tentacles, making this an open-loop system

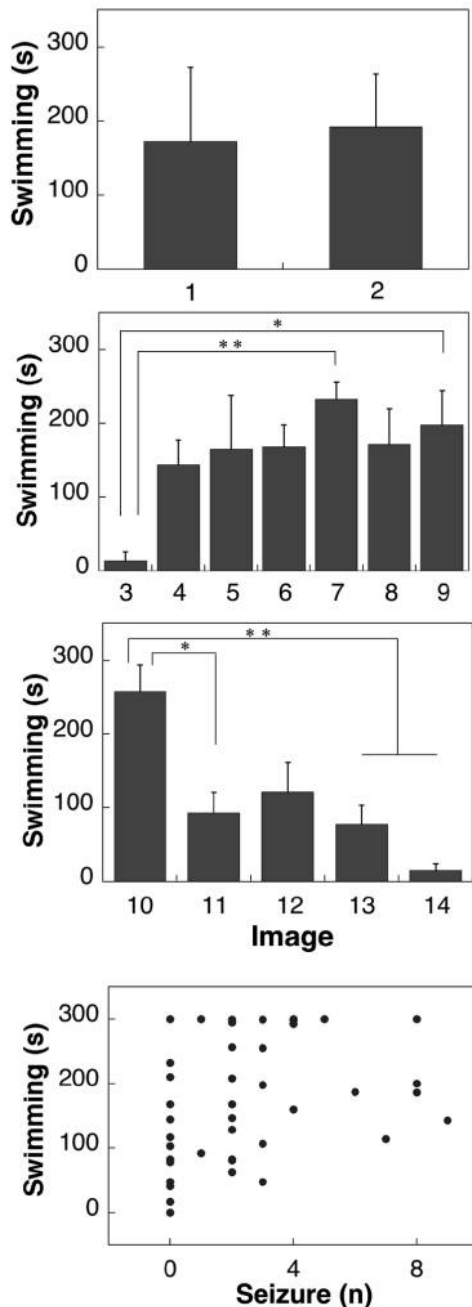


Figure 7. Duration of swimming by *Sepia pharaonis* against images of the western king prawn, *Melicertus latisulcatus*, in Experiment I (images 1 and 2), Experiment II (images 3–9), and Experiment III (images 10–14) and the relationship between swimming duration and the number of seizures for all Experiments I–III (bottom). The number below the x-axis indicates the image code. 1. A live western king prawn. 2. Video of a live western king prawn. 3. Video of a motionless image of a real western king prawn. 4. Video of an image of a real western king prawn moving 100 mm forward horizontally (*i.e.*, 0°) for 5 s. 5. Video of an image of a real western king prawn moving forward, 22.5° upward for a distance of 55 mm, then 22.5° downward for a distance of 55 mm for 7 s. 6. Video of an image of a real western king prawn moving forward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s. 7. Video of an image of a real western king prawn moving forward, 67.5° upward for a distance of 105 mm,

(Messenger, 1977). In this study, *S. pharaonis* spent less time swimming toward a motionless image compared with a moving image, as was also observed for the number of seizure behaviors (Fig. 7). This suggests that cuttlefish move or remain still when proceeding to the next phase, seizure, while they swim after positioning the image. Because the swimming duration correlates with the number of seizure behaviors (Fig. 7), short swimming duration may suggest that the cuttlefish have little interest in the image being presented.

Of note, cuttlefish tended to attack most frequently the image of the prawn proceeding forward vertically at 45°, whereas they seldom attacked the image in which the prawn was moving backward. This may suggest that the angle and direction of movement of the target induce the cuttlefish to initiate a tentacular strike. Because *S. pharaonis* is benthic and mainly uses an ambush strategy by staying on the bottom (Sugimoto and Ikeda, 2013), it appears reasonable that it is able to detect and prefers to respond to a target that is moving horizontally and in an upward direction. This is consistent with the swimming behavior of prawns. King prawn, *Litopenaeus vannamei*, frequently swim up and down in the water column at lower current velocities, and they swim backward very quickly to escape danger (Zhang *et al.*, 2006). When a prawn experiences swimming fatigue, it is swept by the water current and then falls to the bottom (Zhang *et al.*, 2006). Cuttlefish might easily be able to capture prawns that exhibit specific patterns of movement, such as free-fall motion. Prey motion-dependent hunting behavior is observed in birds. Barn owls, *Tyto alba*, can successfully capture a mouse moving directly away, whereas they frequently fail to capture prey moving sideways (Shifferman and Eilam, 2004). Similarly, cuttlefish possess an innate preference for prey motion.

Additionally, in this study, *S. pharaonis* often attacked the image when moving vertically, whereas young *S. officinalis* in Wells's (1962) study did not attack the vertically moving model. Visual sensitivity to angle is anatomically determined by the density of photoreceptor cells on the retina, and this characteristic varies among cephalopod species. For exam-

then 67.5° downward for a distance of 105 mm for 11 s. 8. Video of an image of a real western king prawn moving 90° upward for a distance of 90 mm, then 90° downward for a distance of 90 mm for 7 s. 9. Video of an image of a real western king prawn moving backward, 45° upward for a distance of 75 mm, then 45° downward for a distance of 70 mm for 7 s. 10. Silhouette of a western king prawn moving forward, 45° upward, then 45° downward. 11. Silhouette of a lure moving forward, 45° upward, then 45° downward. 12. Dark ellipse moving forward, 45° upward, then 45° downward. 13. Black rectangle moving forward, 45° upward, then 45° downward. 14. Black square moving forward, 45° upward, then 45° downward. In images 4–14 but not 8, the image was moved leftward across the screen with repeated upward and downward motions until it disappeared from the edge of the screen. Then the image reappeared from the edge of the screen and was moved rightward across the screen. The bar and line represent the mean and standard error, respectively. Bonferroni's test, significant, one asterisk, $P < 0.05$; two asterisks, $P < 0.01$.

ple, *Euprymna morsei* and *Sepioteuthis lessoniana* possess a downward- and forward-directed visual axis, whereas the visual axis of *Todarodes pacificus* and *Thysanoteuthis rhombus* is completely forward-directed; and in *Eucleoteuthis luminosa* it is upward- and forward-directed, which might contribute to prey search (Makino and Miyazaki, 2010). It remains unclear which factors, including species differences, ontogeny (young [Wells, 1962] vs. sub-adult [this study]), and/or model used, lead to these differences. Further investigation is necessary to examine these possibilities.

Significance of shape for the induction of cuttlefish hunting behavior

Because newly hatched *S. officinalis* prefers to attack mysid shrimp, this preference is thought to be innate (Wells, 1958, 1962; Messenger, 1968, 1977). However, this preference is changeable, because early exposure of cuttlefish to a non-preferred prey, such as crab, can result in newly hatched juveniles showing a preference for attacking crab over shrimp, which is known as food imprinting (Darmaillacq *et al.*, 2006a, b). Because the individuals of *S. pharaonis* used in the present study were more than 70 days old and had experienced a variety of foods, such as live mysids, dead fish fry, and shrimp (Sugimoto and Ikeda, 2013; Yasumuro and Ikeda, 2016), their food preferences would be wide. Even in this situation, a “default preference” still exists for cuttlefish. The only image that *S. pharaonis* did not attack was the black square, because it was the only form that was not elongated (Fig. 6). This suggests that an innate preference for specific organism-like objects moving along the direction of their long axis is retained as cuttlefish grow, and it functions as a sign stimulus to elicit hunting behavior.

The realism of the images used in the present study may also explain the tentacular strike behavior by *S. pharaonis*. Interestingly, *S. pharaonis* frequently attacked images 10–13, in order (*i.e.*, western king prawn > lure > ellipse > rectangle, Fig. 6). This clearly indicates that cuttlefish preferred the image that was most similar to the live western king prawn. Additionally, *S. pharaonis* showed great interest in images 11–13, because they were directed at these for significantly longer durations when these images were presented. This also suggests that cuttlefish underwent conflict when deciding whether to attack images resembling prawn. The longest swimming time by *S. pharaonis* was observed toward the western king prawn silhouette (image 10). This is because cuttlefish repeatedly attacked this image; therefore, the duration of swimming in the interval between attacks increased. This also indicated that specifically moving shapes (prawn) can frequently induce hunting behavior in cuttlefish.

Our finding on stereotypic hunting behavior of cuttlefish is consistent with observations on the common toad, *Bufo bufo*. This toad responds to a moving insect or worm prey with a series of stereotyped responses, namely, orienting toward prey,

stalking, binocular fixation, and snapping with its tongue (Ewert, 1974). Toads snap at long rectangles moving directly parallel to their long axis, whereas the same object oriented perpendicularly to the direction of movement elicits no interest. This behavior is associated with ecological characteristics shared between toads and cuttlefish. They are both ambush predators, and they forage long and thin organisms, such as shrimp or worms, respectively. Additionally, toads seem to ignore prey coloration, because they are active at twilight and nighttime during their hunting season (Kondrashev, 1987). Because cuttlefish are thought to be color-blind (Mäthger *et al.*, 2006), the color of prey does not affect hunting. In our experiments, cuttlefish frequently attacked the western king prawn silhouette moving forward 45° vertically (image 10) as much as the picture of a real prawn moving in the same direction (image 6). Under these circumstances, prey orientation and movement provide the visual stimuli that elicit responses in hunting behavior.

Because mature cephalopods die soon after spawning, hatchlings do not receive parental care and must fend for themselves in terms of feeding and defense (Boyle and Rodhouse, 2005). A sign stimulus that allows juveniles to automatically elicit hunting behavior must therefore be advantageous. In our study, *S. pharaonis* eggs were raised without prey such as prawn; therefore, there was no opportunity for visual imprinting, which determines the food preference of hatched juveniles. Thus, we demonstrated that a sign stimulus elicits hunting behavior in subadult *S. pharaonis* and that it is genetically programmed, not based on prior experience.

Cephalopods live in complex and multimodal environments with many types of stimuli, where it seems advantageous if they can receive and distinguish those different stimuli. In addition to visual cues, cephalopods can detect chemical cues from prey, conspecifics, and reproductive mates (*S. officinalis* [Messenger, 1977; Boal, 1997; Boal and Golden, 1999] and *Octopus bimaculoides* [Walderon *et al.*, 2011]), and they also detect auditory cues that originate from various underwater sources (*Sepioteuthis lessoniana*, *Octopus vulgaris* [Hu *et al.*, 2009], *Doryteuthis pealeii* [Mooney *et al.*, 2016], and *S. officinalis* [Solé *et al.*, 2016]). Furthermore, cephalopods use a lateral line analogue to detect mechanical cues such as vibrations from potential predators (*Loliguncula brevis*, York and Bartol, 2014). These findings indicate that cephalopods perceive their world through many sensory modalities, suggesting possible cross-modal perception. How other sensory cues alone or in combination with vision affect the hunting behavior of cuttlefish should be examined in the future.

Ethics Statement

Research on coleoid cephalopods in Japan is not regulated by governmental rules. Nonetheless, we treated cuttlefish under the normal rearing conditions generally applied in European Union countries (Smith *et al.*, 2013).

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