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Observational learning does not explain improvement in predation tactics by cuttlefish (Mollusca: Cephalopoda)

J.G. Boal *, K.M. Wittenberg, R.T. Hanlon

Marine Biological Laboratory, Woods Hole, MA 02543-1015, USA

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Abstract

When first presented with live crab prey, naive cuttlefish typically approached from the front and were often pinched. In subsequent trials, this initial group rapidly improved their prey capture techniques and attacked from above or behind the crab. Naive cuttlefish that first watched experienced conspecifics prey on crabs captured crabs without getting pinched. However, naive cuttlefish that first watched non-attacking cuttlefish in the same tank with crabs also avoided pinches, as did naive cuttlefish that were exposed only to crab odor. All three experimental groups were as successful on their first predation as the initial group was on its second predation, but the attack techniques they used were not as well developed as those of the initial group on their fifth trial. Results suggest that odor may serve as a primer for cuttlefish predatory attack behavior, perhaps by enhancing food arousal and improving attention. Practice was required for further improvements in predation techniques. We found no evidence that cuttlefish improved their predation techniques by observing conspecifics.

Keywords: Appetitive behavior; Trial-and-error learning; Social learning; Feeding

1. Introduction

Learning from conspecifics provides animals with the opportunity to learn about their environment without incurring the costs of mistakes; not surprisingly, social effects on learning have been demonstrated in a wide range of vertebrate species (Zentall and Galef, 1988; Robert, 1990). It is not simple to determine exactly what observers are learning, however, and problems in terminology have led to some confusion (Davis, 1973; Galef, 1976, 1988). In the experiments reported here, we were interested in whether cuttlefish improved their predation tactics by watching experienced conspecifics prey on crabs. We use the term 'observational learning' in its broadest sense, learning by observation of, or interaction with, a conspecific (see discussion of terminology in Heyes, 1994).

Cuttlefish are generalist predators (Boletzky, 1983; Guerra, 1985; Nixon, 1985, 1987; Hanlon

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* Corresponding author. Present address: Millersville University of Pennsylvania, P.O. Box 1002, Millersville, PA 17551-0302, USA. Tel.: + 1-717-8714773; fax: + 1-717-8723985.

E-mail address: jean.boal@millersville.edu (J.G. Boal).

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and Messenger, 1996); a common and preferred prey item is crab (Boletzky and Hanlon, 1983). Resting cuttlefish normally lay camouflaged and partly buried in the substrate. When they detect a prey item, in this case a crab, they perform a three-stage visual attack sequence of attention, positioning and seizure (see reviews in Messenger, 1968, 1977; Chichery and Chichery, 1991; Hanlon and Messenger, 1996). During attention, cuttlefish orient themselves to fixate binocularly on the prey item, raise their first and sometimes second pair of arms, and modify their body patterning. Body patterning changes may serve to direct attention away from the tentacles whereas arm movements may function to distract or lure prey. While positioning, cuttlefish move toward or away from the prey until reaching an appropriate attacking distance. Both attention and positioning appear to be initiated and maintained with visual feedback. The final attack phase, seizure, involves one of two tactics, striking with tentacles, the tactic used with small prey; or jumping onto larger prey and enveloping it with their arms. Cuttlefish use the latter, jumping tactic to capture large crabs such as those provided in this experiment. Once the cuttlefish captures a prey item, it manipulates it to give access to the least protected region; in crabs, these are the proximal joints of the most posterior pereiopods. The cuttlefish then uses its beak to bite the prey and inject cephalotoxin from its posterior salivary glands into the wound (Ghiretti, 1959; Chichery and Chichery, 1988). This toxin immobilizes the prey, which is then broken down physically by the cuttlefish's beak, radula and salivary gland secretions. Crab exoskeletons are not consumed.

The first attempts of juvenile cuttlefish to attack a crab are often unsuccessful and frequently result in the cuttlefish being pinched (Boulet unpublished results, as cited in Wells, 1962a). These pinches are not neutral to the cuttlefish because cephalopods have delicate skin that is slow to heal, and broken skin could lead to infection. Cuttlefish avoid the crabs' claws by eventually acquiring the highly successful attack method of swimming up above the crab and circling around to attack it from behind. How this predatory behavior is acquired is not known. Wells (1962b) suggested that cuttlefish may learn this tactic, perhaps via trial-and-error. In laboratory experiments, cuttlefish quickly learned to avoid attacking a shrimp behind glass; the pain incurred when their tentacles struck the glass was sufficient punishment to inhibit striking (Messenger, 1971, 1973).

Results of laboratory experiments using octopuses suggest that cephalopods can learn predatory behaviors by watching conspecifics (Fiorito and Scotto, 1992; Biederman and Davey, 1993; Suboski et al., 1993). There is ample evidence that learning plays a role in the predatory behavior of cuttlefish (*Order Sepioidea*; e.g. Boycott and Young, 1955; Wells, 1962a; Messenger, 1977; Dickel et al., 1997). Observational learning could allow cuttlefish to avoid the low predation success and injuries associated with inexperience during predation.

Our intent was to document trial-and-error learning in cuttlefish predation on crabs, and to test the hypothesis that this learning process could be shortened through observational learning. It has already been established that newly hatched cuttlefish respond immediately to their first sight of mysid shrimps, a common prey item for hatchlings (Wells, 1958, 1962a). Our naive cuttlefish immediately recognized crabs as potential prey in preliminary trials for this study; consequently, we expected that observing an experienced cuttlefish attack a crab from the rear would affect the observer's subsequent positioning and seizure of crabs but would not affect attention. We did not attempt to address learning mechanisms (i.e. observational conditioning, imitation, etc.; Heyes, 1994) in this initial experiment. Because of concerns about inadvertent experimenter cueing in cephalopod learning experiments (Bitterman, 1966, 1975; Boal, 1996) and the possibility that chemical cues are more important than has been recognized previously (e.g. Boal and Golden, 1999), trials were viewed remotely using a video camera, and dye tests were conducted to verify water flow patterns.

2. Materials and methods

2.1. Animal husbandry

Sepia officinalis Linnaeus 1758 were reared from eggs at the Marine Resources Center of the Marine Biological Laboratory. Newly-hatched cuttlefish were fed various crustaceans (amphipods, *Artemia nauplii* and *Mysis*). Juveniles were fed shrimp (*Palaemonetes* and later, *Crangon*), guppies and small *Fundulus*. When live food was limited, they accepted frozen food, such as shrimp, squid, and various teleosts.

Experimental cuttlefish were large enough to eat adult green crabs (*Carcinus maenas*) 17–18 weeks post-hatching and about 10–14 cm in mantle length (ML). At this point, the cuttlefish had never encountered crabs, either directly as food items, or indirectly as odors on laboratory implements. Preliminary trials were conducted to determine the largest size range of crabs that would be challenging, but could still be captured and paralyzed by the cuttlefish. Crab claw length and carapace width were measured since both factors appeared to affect whether or not a cuttlefish was successful. These trials showed that 10–14 cm ML cuttlefish could successfully capture crabs that had carapaces 4.5–6.0-cm wide and claws 2.5–3.0-cm long. In experiments, smaller cuttlefish were given crabs from the lower end of the size range, and larger cuttlefish were given crabs from the higher end of the size range.

Natural sea water was supplied to all housing and experimental tanks. The Experimental Tank was supplied with filtered sea water at approximately 20°C. Water used in cuttlefish housing was partially re-circulated; all the other water (ambient, crab housing and experimental tank) was discharged after use. A complete description of methods used for culturing cuttlefish can be found elsewhere (Forsythe et al., 1994; Hanley et al., 1998).

2.2. Experimental apparatus

Experiments were conducted in a circular fiberglass tank (91 cm high × 183 cm diameter). Partitions were placed to create a central arena and six side chambers (Fig. 1). By selecting either clear or opaque plastic doors, the experimenter could control whether any particular cuttlefish in one of the side chambers could see the central arena. In preliminary trials, when a crab was introduced into the central arena, the observer cuttlefish in the side chambers rammed vigorously against the clear doors in an effort to reach the crab. This was distracting to the demonstrator cuttlefish in the center; consequently, a 72.4-cm diameter clear Lexan® cylinder was added within the central arena to increase the separation distance between the observers and the demonstrators.

The experimental tank was filled with water to approximately 46-cm in depth. Water flowed from valves set above the water level on the outer wall of each side chamber, circulated around and beneath the loose fitting doors, and then exited through a drain in the center of the floor of the central arena (Fig. 1). Cephalopods, including

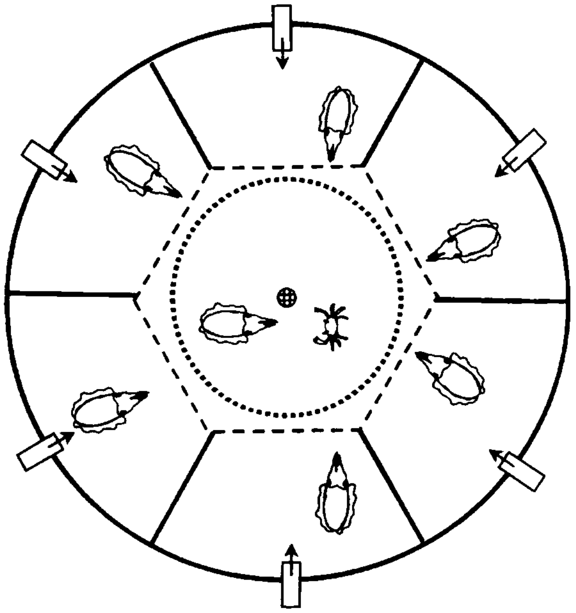

Fig. 1. Schematic showing the experimental tank (1.8-m diameter). A single demonstrator cuttlefish is shown in the center with six observers, one in each of the side chambers. The central arena and the side chambers were separated by removable transparent or opaque doors (dashed lines). A clear plastic cylinder (dotted lines) forced a minimum separation distance between observing cuttlefish and the crab and cuttlefish in the center. Water flowed from valves located on the outside wall of each side chamber to a drain in the floor of the central arena.

cuttlefish, can detect food odors (Budelmann et al., 1997; Boal and Golden, 1999) and the role of distance chemoreception ('smell') in cuttlefish predatory behavior is not yet understood. Consequently, our tank design was chosen to minimize water circulation from the central arena to the side chambers and water-flow patterns were verified using a dye test (see below).

A video camera mounted to the ceiling above the tank and attached to a nearby monitor and video recorder permitted experimenters to view trials while remaining out of sight of the cuttlefish. All trials were videotaped for later analysis.

Experimental trials were conducted in the central arena. Crabs were introduced using a set of tongs that had a 1-m long handle.

2.3. Procedure

Six cuttlefish were used at a time, with one placed into each side chamber of the experimental tank. They were given at least 12 h (overnight) to acclimate before experimental trials. Cuttlefish were divided into four groups as follows.

Group 0. Cuttlefish ($N = 12$) received no exposure to crabs before being tested. They were presented with crabs seven times, one crab per day, in the central arena with the opaque doors in place (in visual isolation). Predation behaviors were scored as described below.

Group 1. Cuttlefish ($N = 12$) had the opportunity to view seven 'crab-experienced' demonstrators attack crabs in the central arena before receiving a single opportunity to prey on a crab themselves. Observations and trials occurred in a single day.

Group 2. Cuttlefish ($N = 12$) received the same treatment as group 1 except that the demonstrators were satiated and no predation occurred.

Group 3. Cuttlefish ($N = 6$) received seven exposures to crab odor prior to testing. For each exposure, a crab was introduced into the central arena and allowed 15 min to wander freely before being removed. Opaque doors were not lifted, so cuttlefish could not see the crabs but could potentially smell them. The cuttlefish then received one opportunity to prey on a crab. Smell exposures and predation trials occurred in a single day.

Demonstrators were drawn from group 0. To insure that the demonstrators would attack crabs during demonstration trials, they received two additional trials. These trials were given in the central arena. The opaque doors were lifted and only the transparent doors were left in place. This procedure allowed the demonstrators to experience attacking a crab while in sight of six observers (the other demonstrators, in this case).

When demonstrating, a single demonstrator was transferred to the central arena and allowed 15 min to acclimate. A crab was then introduced; in all cases, the demonstrator successfully preyed on the crab. The demonstrator and crab were removed 15 min after paralysis of the crab had occurred. The demonstration procedure was repeated six times, one right after the other, with a new demonstrator for each trial.

In experimental trials (groups 1–3), a door was lifted and a cuttlefish was herded with a net from its side chamber into the central arena. The opaque door was lowered and the cuttlefish was allowed 15 min to acclimate (all opaque doors in place; cuttlefish in visual isolation). A crab was then introduced on the side of the compartment opposite where the cuttlefish was located, with its claws facing the cuttlefish. The trial continued until the crab was completely paralyzed by the cuttlefish. Sometimes a cuttlefish attacked the crab but was not successful in capturing it. In this case, 10 min after the crab was released (or, for cases with more than one attempt, 10 min after no further contact with the crab occurred), the crab was removed from the experimental tank and the trial ended. In rare occasions, the cuttlefish did not attend to, or even avoided, the crab; in these circumstances, the crab was removed and the trial ended after 5 min had elapsed. Whatever the outcome, at the end of the trial the cuttlefish was herded back into its side chamber and the opaque door was replaced. If the cuttlefish was successful in capturing the crab, it was allowed to eat it; if not, the cuttlefish was fed a single live fish (*Fundulus* sp.).

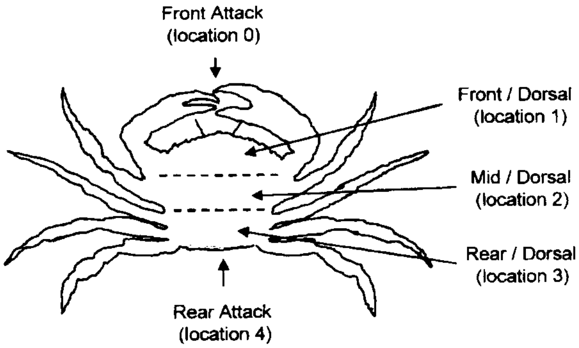

Fig. 2. Diagram of crab (prey) with attack locations indicated.

2.4. Analysis

Predation behaviors were scored by viewing the videotapes. For predation behavior to be scored, the cuttlefish had to attack the crab at least once. Some group 0 cuttlefish did not attack during one, or sometimes two, of their trials. As a result, these cuttlefish had fewer total scored trials; to accommodate this problem, statistical analyses were preformed using only the first five trials completed for each cuttlefish.

Six attack variables were scored. Three of these described the cuttlefish's approach to the crab (approach variables). Circling referred to whether or not the cuttlefish circled towards the posterior end of the crab before attacking (0/1). Angling referred to the degree to which the cuttlefish angled, head-down, to attack the crab, and height referred to the degree to which the cuttlefish elevated itself in the water column immediately prior to attacking (both variables, 0 = none; 1 = low; 2 = high). Scores on these three variables were affected by the crab's behavior; trials were excluded from the analysis if the crab faced away from the cuttlefish because no circling was required in these cases.

A fourth variable, attack location, was scored at the moment the cuttlefish attacked the crab and numerically described the attack's location on the crab's carapace. Attack locations were categorized as follows, location 0, head-on frontal attack; location 1, front/dorsal, anterior third of the dorsal carapace; location 2, mid/dorsal, median third of the dorsal carapace; location 3, rear/dorsal, posterior third of the dorsal carapace; location 4, direct rear attack (Fig. 2). When cuttlefish attacked diagonally, overlapping two locations, the attack location was assigned an intermediate rank; for example, 2.5 if an attack overlapped locations 2 and 3.

The fifth and sixth variables described events during the manipulation of the crab after the attack. A pinch was scored whenever the cuttlefish was pinched by the legs or front claws of the crab. A success was scored whenever the cuttlefish successfully paralyzed the crab. Both these variables were scored dichotomously (0/1). Handling time, the time from capture to paralysis (tremors of the crab's legs), was also noted.

An attack was considered unsuccessful if the cuttlefish released the crab before paralysis. Any further attacks were recorded in the same manner as the initial attack; however, only scores from the first attack were included in statistical analyses since this attack represented the subject's attack proficiency at the onset of each trial.

2.5. Dye tests

A dye test was conducted to determine if odors from crabs in the central compartment were likely to have reached the side chambers despite the arrangement of side inflows and central drain. Rhodamine was chosen because it is inert and its particle size (479 g/mol) is similar to the size of amino acids and other potential chemical signals.

Rhodamine dye (50 mg) was dissolved in 1 l of sea water (0.005% solution) and was released, by gravity, within 1–2 min using a flexible tube (1.5-m long, 0.5-cm ID) connected to a Pasteur capillary pipette placed just above the floor of the central compartment. For most of the dye introduction (approximately 90% of the time), the tube was placed near the door of one of the side chambers ('closest'). During the remaining 10% of the time, the tube was moved in a circular motion about 15 cm from the walls of the central arena. Dye was released in this manner because, during the average trial, a crab walked around the central arena wall until it was attacked and subsequently paralyzed. Fifteen minutes after dye introduction, three water samples were taken from near the bottom of the experimental tank —

one from the center of the central arena, one from the chamber closest to the most concentrated dye introduction, and one from the chamber opposite ('farthest') the most concentrated dye introduction. For the two samples from side chambers, samples were taken about 15 cm from the door because observer cuttlefish were usually located less than 15 cm from their respective doors. Sampling was repeated every 15 min for 1 h; each sample was analyzed using a Beckman DU-6 Spectrophotometer.

During the dye test, dye not only reached the chamber closest to the most concentrated dye introduction, but it also reached the chamber farthest away. As expected, dye was more concentrated in the closest than in the farthest chamber. The dye reached its greatest concentration at all three sampling locations 15 min after dye introduction. Thereafter, the dye became progressively diluted since new water was continuously flowing in from the outer wall of each chamber and exiting through the drain in the center of the central arena (Fig. 1). By 60 min, no dye remained in the central arena or in the farthest chamber. Some dye still remained in the closest chamber. Thus, our water inflow and drain arrangement probably reduced the amount of crab odor reaching the cuttlefish in the side chambers but clearly it did not eliminate it.

2.6. Statistical analyses

2.6.1. Variable correlation

While reviewing the data, it became clear that variables pinch and success were strongly correlated with one another. To evaluate the independence of our dependent variables, we computed confidence intervals for the Kendall tau-b statistic (τ_b, Agresti, 1984). To compare handling time between different attack locations, a Student's t-test was computed.

2.6.2. Trial-and-error learning

For group 0, Page tests for ordered alternatives (L) were used to evaluate the overall pattern of change across the five trials (Siegel and Castellan, 1988). Trial 1 was compared with trial 2 to evaluate any immediate changes in performance, and trial 1 was compared with trial 5 to evaluate any changes that occurred with prolonged experience. These analyses were accomplished using randomization techniques (Manly, 1991) in which the paired randomizations were based on the McNemar test, and the unpaired randomizations were based on the Fisher's exact test (Everitt, 1977). Total number of randomizations was calculated as the number of paired randomizations (Nr) multiplied by the number of unpaired randomizations. For each randomization, the odds ratio statistic (Or) was computed (Agresti, 1984). To evaluate the effect of pinches, sign tests were used (Siegel and Castellan, 1988).

2.6.3. Observational learning

Performances of the four groups were compared using contingency tables and χ^2-tests of independence (Fisher's exact test if expected values were small; Everitt, 1977).

3. Results

3.1. Predation tactics

Cuttlefish used different approaches to capture the crabs. Success varied by approach (see Fig. 2) and included rear attacks (location 4) and six variations of dorsal attacks (forward- and backward-oriented attacks at locations 1–3). Frontal attacks (location 1) were never successful. The rear attack position (location 4) was the usual position in which the cuttlefish inserted toxin to paralyze the crab. If the cuttlefish attacked from a position other than the rear, it manipulated the crab until it was in location 4.

Handling time was separated into two components, manipulation time and toxin time. Manipulation time, the time required to manipulate the crab into the toxin-insert position, was highly variable for attack locations 1–3 (mean = 16.6 s, S.E. = 3.79); manipulation time was zero for attack location 4. Toxin time ranged from 13 to 572 s (mean = 62.1 s, S.E. = 11 s); no relationship with experimental variables was detected. Handling time (manipulation time + toxin time) was significantly greater for attack locations 2 and 3 than for location 4 (Student's $t = 1.86$, $P < 0.05$).

3.2. Correlations between variables

Variables pinch and success were almost perfectly negatively correlated with one another (Fig. 3a and b; $\tau_b \sim -0.96$; 95% confidence interval -1.00 to -0.79); only success was used in remaining statistical analyses.

Attack location was significantly related to all three approach variables, attacks near the posterior end of the crab (location 4) often involved circling, elevating, and angling downward, whereas attacks near the anterior end (locations 0–1.5) never involved circling, increasing height, or angling (Kendall τ_b, circle, 0.78; height, 0.41; angle, 0.47; $P < 0.05$ for all).

Attack location was also associated with success. Front attacks (locations 0–0.5) almost always failed (straight front attacks (location 0)

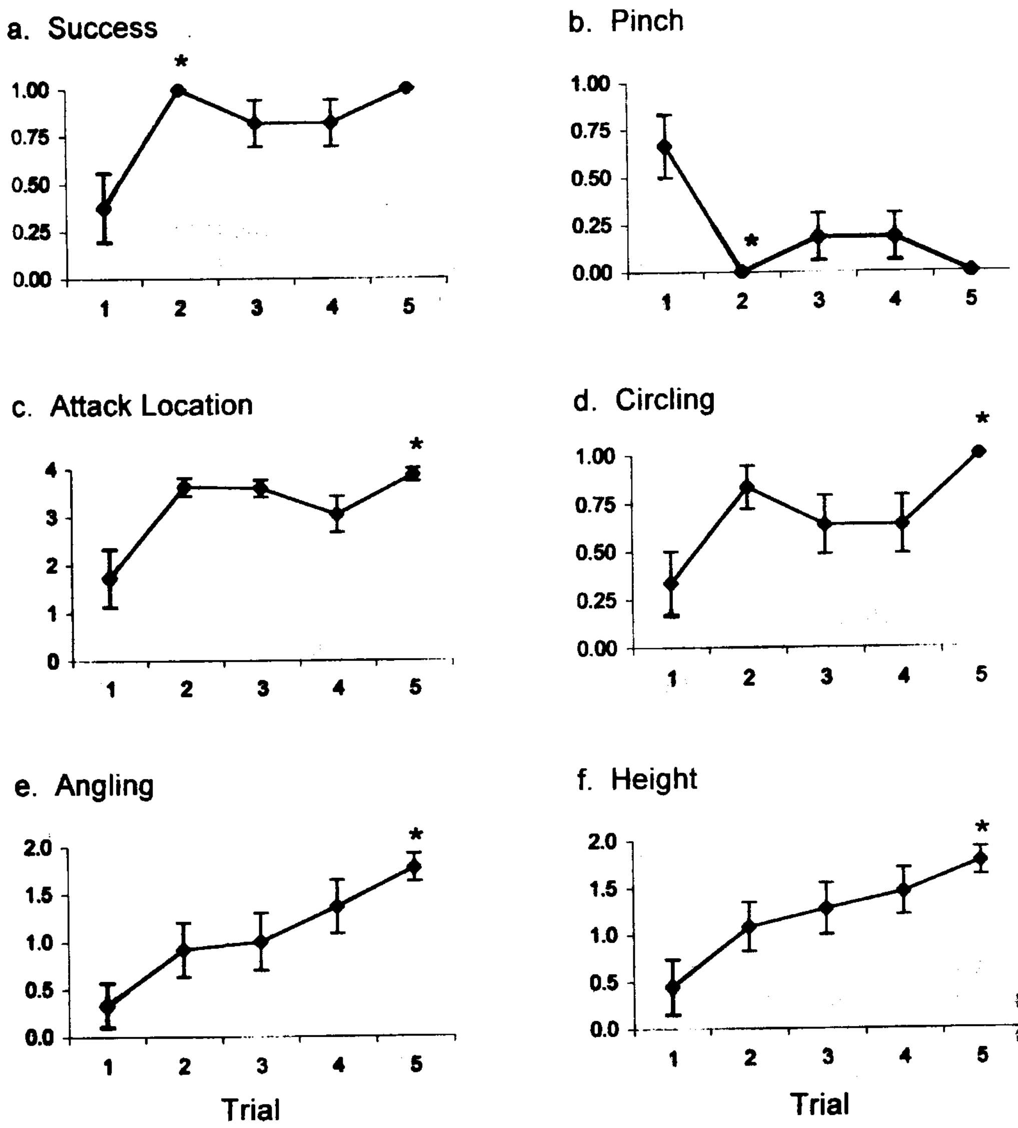

Fig. 3. Performances of cuttlefish (group 0; $N = 12$) on five sequential predation trials (mean ± S.E.). This group had no previous exposure to crabs. Variables are (a) success (1 = success; 0 = failure), (b) pinch (1 = pinch; 0 = no pinch), (c) attack location (see Fig. 2 for scoring), (d) circling (1 = yes; 0 = no), (e) angling (2 = high; 1 = low; 0 = none) and (f) height (2 = high; 1 = low; 0 = none). Improvement was statistically significant (*) between trials 1 and 2 for the variables success and pinch and across all five trials for attack location, circling, angling and height.

always failed). Mid/dorsal, rear/dorsal, and rear attacks (locations 2–4) resulted in high success rates (Kendall τ_b, 0.29; $P < 0.05$) even though handling time was significantly longer for locations 2 and 3 than for location 4 (see Section 3.1).

3.3. Trial-and-error learning

The performance of group 0 cuttlefish changed throughout the five predation trials (Fig. 3). The proportion of cuttlefish that succeeded increased significantly between trial 1 and trial 2 (Fig. 3a) and remained high throughout the next three trials (trial 1 vs. trial 2, Or = 28.85; Nr = 2240; $P = 0.0089$).

Attack location also changed with successive trials (Fig. 3c), with attacks directed more towards the posterior portion of the crab's carapace ($L = 576.5$; $K = 5$; $N = 12$; $P < 0.05$). A significantly higher proportion of cuttlefish attacked at locations 2–4 during trial 2 than during trial 1 (Or = 28.85; Nr = 2240; $P = 0.0089$), and a significantly greater proportion specifically attacked at location 4 during trial 5 than during trial 1 (Or = 4.85; Nr = 2240; $P = 0.036$).

A more gradual change with trial experience was found for approach variables (Fig. 3d–f). Significantly more cuttlefish circled, increased height, and angled with increasing experience (circling, $L = 571.5$; $K = 5$; $N = 12$; $P < 0.05$; height, $L = 578.5$; $K = 5$; $N = 12$; $P < 0.05$; angling, $L = 585.5$; $K = 5$; $N = 12$; $P < 0.01$).

Pinches were presumably a key negative impetus for improving predation tactics. Seven of the 12 cuttlefish in group 0 were pinched with a crab's front claws. Six were pinched in the first trial, and three of these were again pinched in either trial 3 or trial 4. No cuttlefish received pinches two trials in a row. Pinched cuttlefish were significantly more likely to circle and attack from a more posterior location on the trial that immediately followed (attack location, seven of eight changed to more posterior location; sign test, $P < 0.05$; circling, five of five added circling; sign test, $P < 0.05$). Trials with no pinches preceded by similarly pinchless trials showed no such change in behavior. Graphs of the performances of pinched and never pinched cuttlefish provide no evidence, however, that pinched cuttlefish learned more effectively (Fig. 4).

3.4. Observational learning

All predations by demonstrators were successful and no pinches occurred. The three experimental groups (groups 1–3) did not perform differently from one another (Fig. 5), approach variables, attack location and success were similar in all three groups (circling, $X^2 = 2.88$; df = 2; height, $X^2 = 2.80$, df = 4; angle, $X^2 = 4.17$, df = 4; attack location, $X^2 = 2.11$, df = 4; $P > 0.20$ for all; success, Fisher's exact test, $P = 0.35$). The three experimental groups were combined, therefore, for comparison with the first, second, and fifth trials of the baseline group.

Cuttlefish in the three experimental groups were significantly more likely to attack at more posterior locations (Fig. 6c; $X^2 = 25.58$, df = 12, $P < 0.01$) and to succeed in capturing the crab (Fig. 6a; $X^2 = 14.61$; df = 3; $P < 0.01$) than were cuttlefish in group 0 on trial 1. Approach behavior (circling, height and angling) did not differ between cuttlefish in the three experimental groups and cuttlefish in group 0 on trial 1 (Fig. 6d–f).

No significant differences were found between cuttlefish in group 0 on trial 2, and experimental cuttlefish, for any variables.

Experimental cuttlefish circled and angled significantly less frequently than did cuttlefish in group 0 on trial 5 (Fig. 6d, circling, $X^2 = 10.18$, df = 3, $P < 0.02$; Fig. 6e, angling, $X^2 = 10.33$, df = 3, $P < 0.02$), and they were less likely to use height (Fig. 6f, $X^2 = 4.98$, df = 1, $P < 0.05$) or attack at the rear-most location (Fig. 6c, $X^2 = 7.40$, df = 1, $P < 0.01$). Success did not differ between experimental and group 0 cuttlefish on trial 5 (Fig. 6a).

4. Discussion

4.1. Learning

The immediate success of the predation observers (group 1) on their first and only trial suggested that observational learning had occurred. Had we

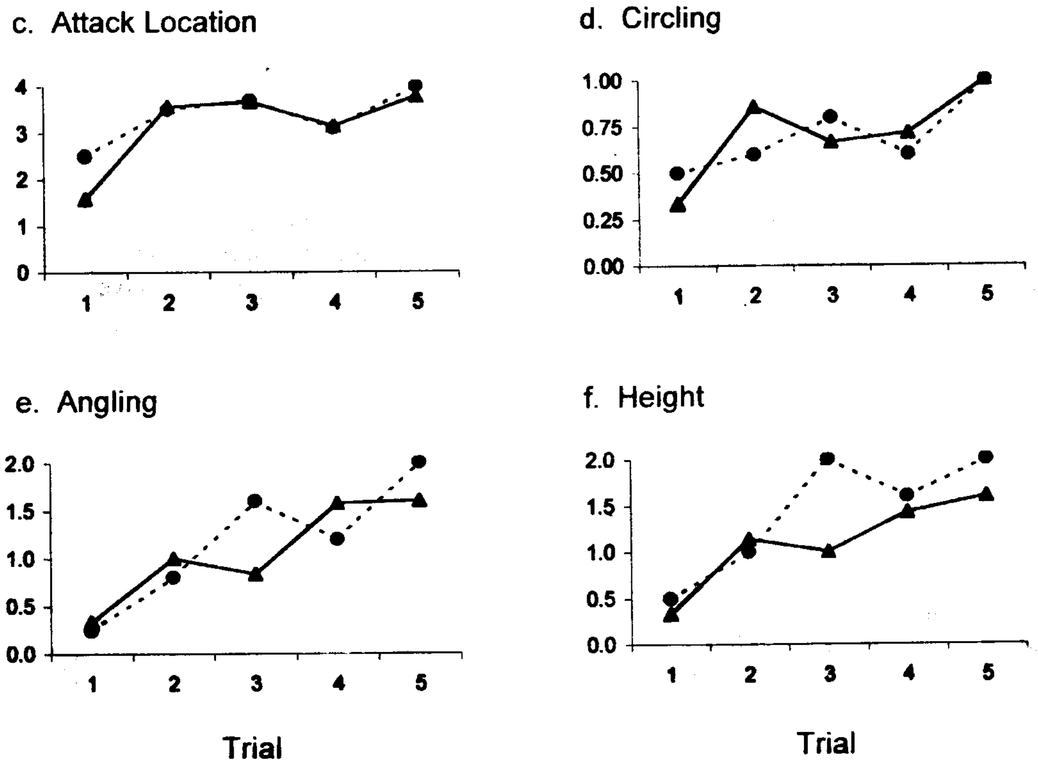

Fig. 4. Mean performances of the seven cuttlefish that were pinched at least once during training (solid lines) and the three cuttlefish that were never pinched (dotted lines). Variables are the same as those in Fig. 3. Performances of pinched and never-pinched cuttlefish did not differ.

not included group 2, the crab observers, and group 3, those exposed to crab odor, we would have concluded incorrectly that cuttlefish improved predation techniques through observational learning. Instead, our results provide no evidence that cuttlefish predation on crabs is influenced by observing a conspecific. At least one other factor, odor, is important in the development of cuttlefish predation tactics.

Are predation tactics important to cuttlefish? While crabs are an important and preferred prey species for cuttlefish (Boletzky and Hanlon, 1983; Hanlon and Messenger, 1996), preying on them brings risks; an aggressive crab is capable of breaking the skin and injuring the underlying tissues of cuttlefish with their strong claws. Cephalopod immune systems are not well-developed and skin injuries can lead to infection and even death (Hanlon and Forsythe, 1990a,b). In our study, the cuttlefish that got pinched with the front claws were never successful in preying on that crab (they always released the crab). The rear attack position is also advantageous because it is the most frequently used position for inserting toxin to paralyze the crab. With a rear attack, cuttlefish expend less energy manipulating the crab. Thus, successful predation tactics are important for cuttlefish.

Is crab predation behavior innate? Among group 0 cuttlefish, there was a gradual increase in circling, angling and increasing attack height between trials 1 and 5, as well as a reduction of attacks in any but the most posterior carapace location. This gradual improvement suggests trial-and-error learning. Although these improvements did not affect outcome, since all trials resulted in successful predations after trial 1, they did result in lower handling time, an indication of lower energy costs for the cuttlefish.

Can learning account for improvement in performances between trials 1 and 2? Among cuttlefish in group 0, there was a significant decrease in pinch frequency, increase in predation success and change in attack location between trials 1 and 2 (Fig. 3a–c). This quick improvement could be a result of rapid learning. We suspect that crab odor could function as some kind of behavioral primer, initiating a state of food arousal (Tuersley and McCrohan, 1987), as has been demonstrated previously in other mollusks such as *Aplysia* (Kupfermann, 1974) and *Lymnaea* (Tuersley and McCrohan, 1987). Cuttlefish can detect food-related chemicals in the water; in particular, ventilation rates increase in response to either the sight or the smell of food (Boal and Ni, 1996; Boal and Golden, 1999). Our dye test confirmed that cuttlefish in the side chambers probably did have the opportunity to smell crabs in the central arena. Heightened food arousal could have caused the cuttlefish to be more attentive to the crabs (and to the crabs' claws), resulting in greater predation success and a lower incidence of pinches. Records of attack latency would have been very helpful in this analysis, but these results were not anticipated and videotape resolution was inadequate to determine the moment of attention. In support for

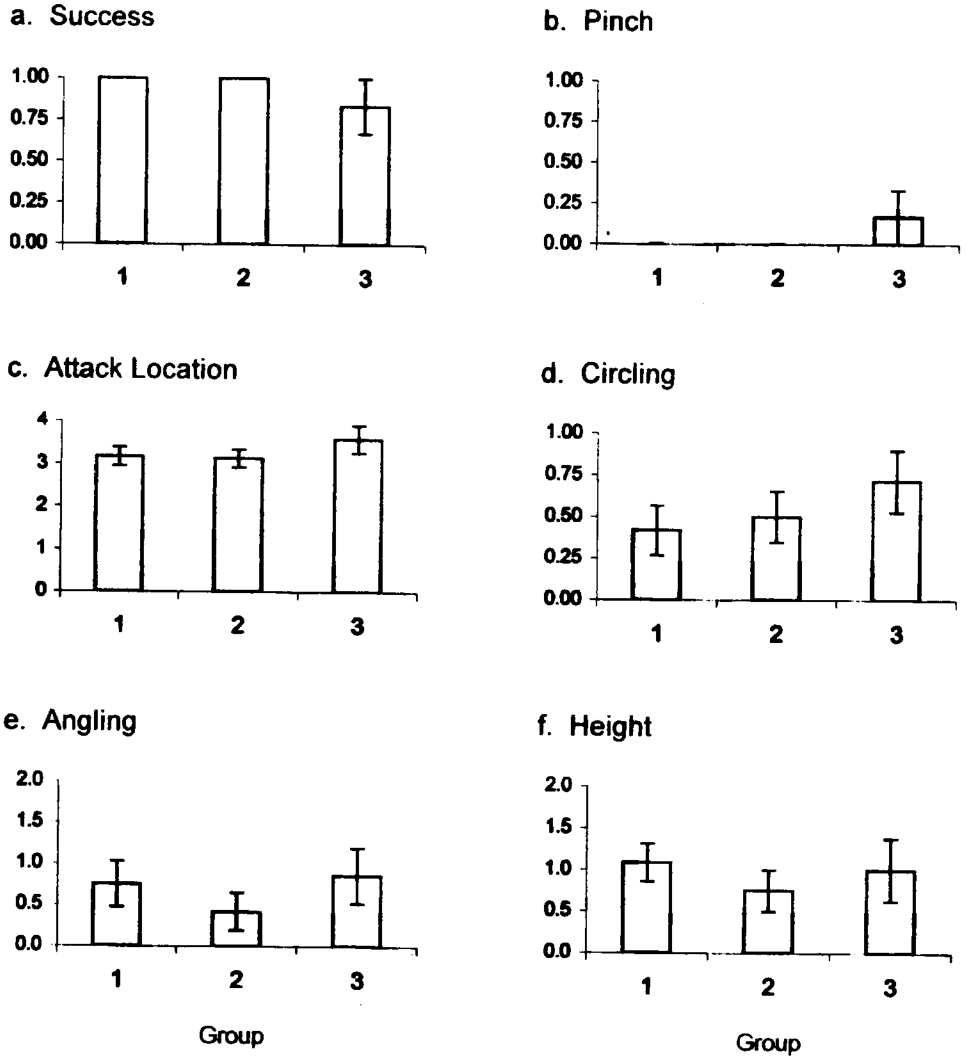

Fig. 5. A comparison of first attacks on crabs by the three experimental groups of cuttlefish (mean ± S.E.). Group 1 had previously observed experienced cuttlefish prey on crabs ($N = 12$), group 2 had previously observed non-attacking cuttlefish in the same tank with crabs ($N = 12$), and group 3 had previously been exposed to crab odor ($N = 6$). Variables are the same as those in Fig. 3. Performances of the three groups are not statistically different from each other.

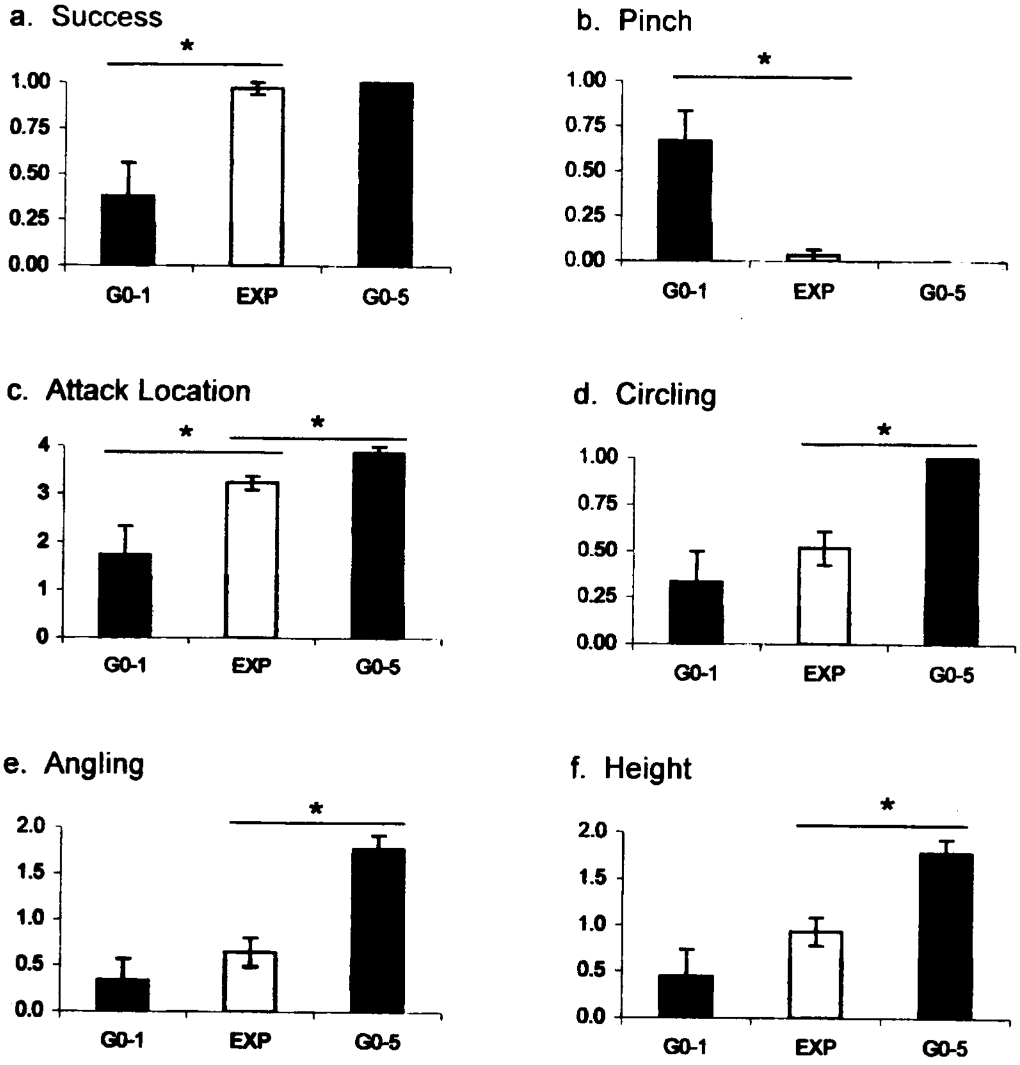

Fig. 6. A comparison of first attacks on crabs by group 0 cuttlefish on trials 1 and 5 (from Fig. 3; $N = 12$; solid bars) and experimental cuttlefish in groups 1–3 (from Fig. 5; $N = 30$; open bars). Variables are the same as those in Fig. 3. Performances of the experimental cuttlefish (groups 1–3; EXP) were significantly different (*) from the performances of the cuttlefish in group 0 on trial 1 (G0-1) for variables success, pinch and attack location, and from cuttlefish in group 0 on trial 5 (G0-5) for variables attack location, circling, angling and height.

an arousal hypothesis, cuttlefish in the side chambers sometimes rammed their opaque doors when a crab in the central arena approached their chamber (during testing trials), suggesting that crab odors had initiated a state of food arousal.

Was our failure to demonstrate observational learning because none of the observers saw a demonstrator get pinched, so none had the opportunity to learn from a bad example? Interestingly, observational learning does not depend upon viewing consequences of behaviors; octopuses that watched a conspecific choose one of two colored balls later chose the same colored ball themselves, despite the absence of any observable reward or punishment (Fiorito and Scotto, 1992). In our experiment, cuttlefish did view other cuttlefish that obtained a prized reward, and cuttlefish from group 0 that were never pinched improved their predation techniques as much as those that did get pinched. It is possible that our results would have been different had cuttlefish viewed other cuttlefish being pinched, but we do not find this explanation to be compelling.

We believe the most likely explanation for our results is that there is no evidence for non-reproductive social behavior in cuttlefish (Boal, 1996; Hanlon and Messenger, 1996); consequently, opportunities for social learning in this species are probably rare under natural conditions.

Does our failure to demonstrate learning from conspecifics indicate that cuttlefish do not learn from observing the world around them? Previous experiments have demonstrated that vision is extremely important to predatory behavior of cuttlefish (Messenger, 1968; Hanlon and Messenger, 1996). We did not test for the effect of seeing crabs without smelling them; consequently, we were unable to determine what role visual input alone might play in enhancing predatory behavior. Our experiment does indicate that odor may also be an important attribute of prey, perhaps by triggering food arousal (Tuersley and McCrohan, 1987), which could lead to improved approach and attack. Thus, an interesting and unexpected outcome of our experiment was to focus attention on how learning is at least as likely to be influenced by characteristics of the prey (e.g. appearance, odor, behavior) as by the behavior of conspecifics.

5. Conclusions

We found no evidence that cuttlefish learned from observing the predatory behavior of conspecifics. An odor stimulus from the crab prey was enough to explain the improvement in first approach and attack; refinements in approach and attack in subsequent trials appeared to be the result of trial-and-error learning.

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