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Effect of Early Feeding Experience on Subsequent Prey Preference by Cuttlefish, *Sepia officinalis*

ABSTRACT: *Food preferences were investigated in cuttlefish during the first 3 months' posthatching, using choice tests between crabs, shrimps, and young fish. The results showed that without previous feeding experience, cuttlefish preferred shrimps on Day 3. This suggests an innate food preference; however, it was possible to induce a preference for an originally nonpreferred prey item in 3-day-old and naïve cuttlefish, demonstrating the flexibility of this initial behavioral preference in response to previous individual experience. This preference suggests a learning process involving a form of long-term memory, demonstrated for the first time in juvenile cuttlefish. Until Day 30, juvenile cuttlefish fed exclusively shrimps chose shrimps. This preference probably depends on their previous feeding experience. Finally, it appears that from Day 60, cuttlefish reared on the same restricted diet have a tendency to switch their preference to novel prey items, which diversify their diet. © 2004 Wiley Periodicals, Inc.* Dev Psychobiol 45: 239–244, 2004.

Keywords: *feeding preference; development; learning; long-term memory trace; cephalopods*

The opinion that food preferences may be established early in the life of animals has been implied by Hess (1962). This contention has been supported by many subsequent studies on precocial animals such as the snapping turtle, *Chelydra serpentina*, the chick, *Gallus gallus*, the garter snake, *Thamnophis sirtalis*, the zebra finch, *Taeniopygia castanotis* (reviewed in Stasiak, 2002), and the lynx spider, *Oxyopes salticus* (Punzo, 2002). All of these studies have shown that feeding experiences during early development exerted particularly potent influences on subsequent food selection. The strategy developed by some of these animals in response to their respective prior experience was to prefer the food first experienced; this is called the primacy effect (Burghardt, 1967; Burghardt & Hess, 1966; Punzo, 2002). No evidence of such an effect has been reported in cephalopods. Other studies have shown that young animals also can select food by choosing what not to eat. This is the case in avoidance learning illustrated, for example, in *Octopus cyanea* during the first week after settlement (Wells & Wells, 1970). These authors observed that when a young octopus, feeding mainly on crabs, tried to catch a xanthid crab, the latter was able to grip the mesh floor of the aquarium so firmly that the octopus was unable to turn it over, kill it, and carry it home. As a consequence after a few unsuccessful attempts at capture, these crabs stayed alive and were not attacked again. Avoidance learning also was reported in the young chick (Hogan, 1973a, b, c) that quickly learns to reject foods with bad tastes or other irritating properties.

In the cuttlefish, *Sepia officinalis*, there is no planktonic or larval stage. Hatchlings emerge as miniature replicas of adults, assume a necto-benthic mode of life, and use the same prey-capture strategies: tentacle strikes for prey capable of a rapid escape (mysids, shrimps, fishes) or a ‘‘jump’’ strategy for less mobile prey such as crabs (Chichery & Chichery, 1991; Messenger, 1968). From

Received 8 April 2004; Accepted 5 August 2004
Correspondence to: L. Dickel
Contract grant sponsor: French Ministère de la Recherche et de la Technologie and Université Paris XIII
Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/dev.20034

hatching, cuttlefish are completely independent of parental care or attention. As a consequence, the neonates are directly confronted with predators and foraging exigencies. Contrary to Wells' suggestions (1958), active feeding can begin before the yolk reserves contained in the inner yolk sac are entirely consumed (Boletzky, 1975; Dickel, Chichery, & Chichery, 1997). For the majority of cephalopod species, the behavioral mechanisms responsible for prey selection under natural conditions are still unknown. Most of our knowledge of prey selection has come from rearing experiments in the laboratory (reviewed in Boletzky & Hanlon, 1983) or from gut-contents analyses (Blanc, Pinczon du Sel, & Daguzan, 1998, 1999; Guerra, Nixon, & Castro, 1988; Henry & Boucaud-Camou, 1991; Najai & Ktari, 1974; Pinczon du Sel, Blanc, & Daguzan, 2000). Adult *S. officinalis* are considered to be generalist predators, feeding mainly on shrimps, crabs, and fishes; however, laboratory experiments have revealed that in adults, the preferred prey item is the crab (Boletzky & Hanlon, 1983). Moreover, we recently showed (Darmaillacq, Dickel, Chichery, Agin, & Chichery, in press) that adult cuttlefish from the same fishing ground did not all display the same food preference, with some preferring shrimps and others preferring crabs. Little is known about the diet of young animals, although this is of critical importance if we are to better understand the cuttlefish's predatory behavior. Boycott (1958) and Wells (1962) suggested that juveniles of *S. officinalis* innately recognize and detect prey shaped like mysids, and later as they grow, learn which prey to feed on and which to avoid. During rearing, hatchlings or young cuttlefish were usually fed shrimps, prawns, mysids, young fish, or any other prey which have an elongated shape moving along their long axis; however, several authors have shown that juveniles also are able to feed on crabs (Blanc et al., 1998; Boucher-Rodoni, Boucaud-Camou, & Mangold, 1987; Henry & Boucaud-Camou, 1991).

Furthermore, previous studies failed to demonstrate long-term memory in very young cuttlefish with the ''prey behind a glass tube'' protocol (Dickel, Chichery, & Chichery, 1998; Messenger, 1973). Dickel et al. (1998) recorded good performances during the short-term retention tests (resting interval of 5 min) in juveniles from 8 days of age, but poor 60-min retention performances were observed until 15 days of age. This result confirms that of Messenger (1973), who found very low 24-hr retention performance in juvenile cuttlefish.

In this work, we were first interested in determining whether early juveniles showed any preference for one of their potential types of prey (crabs, shrimps, or young fish). Second, we wondered whether it would be possible to change this eventual preference by restricting the diet to only one type of prey in very young juveniles. Despite the results obtained by Messenger (1973) and Dickel et al. (1998) concerning poor long-term memory performances in juveniles, we finally wondered to what extent they would be able to store this induced preference.

METHODS

Animals

Cuttlefish (*Sepia officinalis*) used in these experiments ($N = 133$) all came from eggs laid in the Laboratory of the ''Centre de Recherches en Environnement Côtier'' (Luc sur Mer, France) by females originally trawled in the English Channel, and maintained under controlled conditions (water temperature $= 15 \pm 1°C$) until hatching occurred. So that we did not include prematurely hatched cuttlefish in the experiments, they were collected among those hatched on the day with the highest hatching proportion. We collected hatchlings at 8 a.m. from eggs that had hatched during the night (i.e., within the previous 10 hr). From the day of hatching (Day 0), cuttlefish were gradually habituated to a water temperature of $20 \pm 1°C$ over 1 hr, and then reared in isolation in separate opaque black plastic tanks ($7.5 \times 8 \times 7$ cm) supplied with running oxygenated seawater ($20 \pm 1°C$); they were maintained under daylight conditions. Each juvenile was used only once.

Experiment 1: Food Preferences during the First Three Months' Posthatching

Cuttlefish were tested for their initial food preference on Day 3 [mean dorsal mantle length (DML) $= 8.9 \pm 0.1$ mm; $n = 30$] and thereafter on Day 6 (DML $= 10.3 \pm 0.5$ mm; $n = 19$), Day 30 (DML $= 15.9 \pm 0.2$ mm; $n = 24$), Day 60 (DML $= 29.7 \pm 0.2$ mm; $n = 8$), and Day 90 (DML $= 42.6 \pm 1.0$ mm; $n = 12$). The first choice test was conducted on Day 3 since Wells (1958) and later Dickel et al. (1997) reported that most of the first attacks occurred at this age. As a consequence, cuttlefish were not fed prior to Day 3. Juvenile cuttlefish can endure such a period of fasting as they still have inner yolk reserves (Boletzky, 1975; Dickel et al., 1997). Cuttlefish from the other groups were fed daily ad lib with shrimps of suitable size, except for a period of 2 days prior to the day of testing to ensure that their feeding motivation was high. Pilot observations showed that the juveniles were most active in the morning, and as a consequence, all subjects were individually tested between 9:00 a.m. and 12:00 a.m.

Animals were offered a three-way choice between crabs *Carcinus* species, shrimps *Crangon crangon*, and young fish *Gobius minutus* of suitable size, i.e., the carapace width of the crabs nearly corresponded to half of the DML of the cuttlefish, and the total length of the shrimps and the young fish corresponded to the DML of the cuttlefish. The prey were enclosed in separate compartments behind glass partitions that allowed the cuttlefish to inspect visually and choose a prey item without being able to reach it (Figure 1). All six possible combinations of the three prey items were randomly presented to each age group. The relative positions of the three prey items were systematically

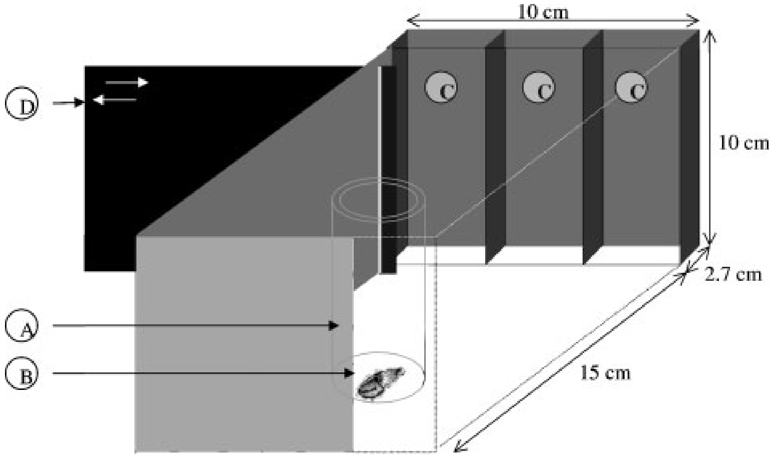

FIGURE 1 Schematic representation of the experimental apparatus. A = the glass cylinder, B = the cuttlefish, C = the compartments, and D = the sliding door. The apparatus is placed in a tank ($80 \times 54 \times 12$ cm) supplied with running oxygenated seawater (20 ± 1°C).

varied from one trial to the next to control for the possibility that cuttlefish were choosing based on position rather than prey type.

First, the cuttlefish was gently removed from its housing tank, positioned in a perspex cylinder, and left to acclimate for 5 min; the head of the animal faced the three prey compartments (Figure 1A and B). The prey were then placed into their respective compartments (Figure 1C), with 3 prey in each one, to ensure a constantly moving visual stimulus that optimizes cuttlefish attack behavior. In addition, preliminary observations revealed that at the end of the 5-min presentation, prey were still moving and, hence, were highly attractive to cuttlefish. At this time, cuttlefish were not yet able to see the prey, which were hidden behind a sliding PVC door (Figure 1D). The cylinder was nearly equidistant from all three types of prey (distance = 3 cm on Day 3, 3.5 cm on Day 6, 5 cm on Day 30, 9 cm on Day 60, and 12 cm on Day 90). Under such conditions and by taking into account the size of the prey relative to that of the cuttlefish, prey appeared equally in the cuttlefish's anterior visual field such that it was able to see all of them at the same time (Messenger, 1968). At the end of the 5-min acclimation period, the sliding door was opened rapidly so that all prey appeared at the same time. As soon as the cuttlefish detected a prey and tried to move towards it, the cylinder was carefully removed to ensure that all tested cuttlefish could see all three prey types from their point of departure. The first prey item chosen by the cuttlefish was recorded. The sliding door was closed after the first attack of the cuttlefish. If the cuttlefish failed to attack within the first 5 min of the test, the prey were hidden again and the cuttlefish was tested 5 min later.

Experiment 2: Effect of Early Feeding Experience on Subsequent Food Preference

Testing was carried out on Day 3, using two groups of 20 previously unfed cuttlefish (DML = 8.9 ± 0.1 mm). Juveniles of Group 1 were fed one crab (*Carcinus* species; carapace width from 2.5 to 3.5 mm); those of Group 2 were fed one shrimp *Crangon crangon* (total length from acron to telson from 8 to 9 mm). The animals were not fed from Day 3 to Day 6. On Day 7, each animal was examined in a two-way choice test between crabs and shrimps presented in the apparatus described earlier (Figure 1), using the same procedure to record their first choice of prey item.

Statistical Analysis

Prey preference for each of the eight groups was compared using chi-square tests of independence. The binomial test (with $p = 0.5$) was used to analyze the effect of early feeding experience on subsequent prey choice in the juveniles.

RESULTS

Experiment 1

First, Figure 2 shows that as early as 3 days of age, each of the three types of prey elicits attacks by juvenile cuttlefish. Prey preference, however, depends on the age of the cuttlefish. Up to Day 6, the juveniles showed a significant preference for shrimps compared to young fish and crabs, χ^2 (2, $n = 30$) = 16.8, $p < .001$, for the juveniles on Day 3 and χ^2 (2, $n = 19$) = 11.5, $p < .01$, for cuttlefish on Day 6 (Figure 2). Moreover, 3-day-old naïve juveniles significantly prefer shrimps to young fish, χ^2 (1, $n = 28$) = 5.1, $p < .05$. On Day 30, cuttlefish again showed a significant preference for shrimps over young fish and crabs, χ^2 (2, $n = 24$) = 7.75, $p < .05$ (Figure 2). However, on Days 60 and 90, cuttlefish showed no significant preference between the three types of prey, respectively, χ^2 (2, $n = 8$) = 3.25, n.s.; χ^2 (2, $n = 12$) = .5, n.s. (Figure 2); young fish seemed to be slightly preferred to the two others on Day 60.

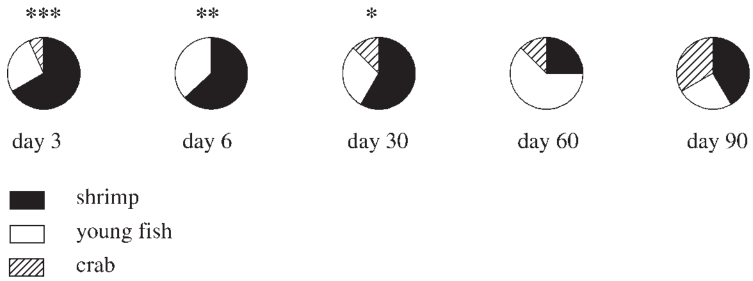

FIGURE 2 Prey preferences observed after choice tests between shrimps, crabs, and young fish in juvenile cuttlefish on Days 3, 6, 30, 60, and 90 of postembryonic development. Numbers in each age group: $n_{D3} = 30$, $n_{D6} = 19$, $n_{D30} = 24$, $n_{D60} = 8$, and $n_{D90} = 12$. Asterisks indicate significant preference for a particular prey item compared to the others, chi-square test, $^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$.

Experiment 2

Juveniles from Groups 1 and 2 were given one crab or one shrimp, respectively, on Day 3. In each case, once the prey was detected, it was promptly captured and consumed.

On Day 7, the results of the prey-preference experiment show a preference for the original diet, which was chosen by 31 of 39 juveniles, binomial test, $p < .001$. One cuttlefish was removed from the experiment because it seemed unhealthy (i.e., it floated at the surface and was unable to control its buoyancy at the beginning of the choice test). In Group 1, 16 of 20 juveniles that were fed one crab on Day 3 selected the prey which they had first experienced whereas 4 chose the novel prey (Table 1). In Group 2, 15 of the 20 juveniles originally fed with shrimps continued to choose them (Table 1).

DISCUSSION

To our knowledge, these experiments are the first laboratory study using live common prey to assess the dietary preference of juvenile cuttlefish. Our results show that cuttlefish choose prey according to its type. Furthermore, each of the three prey items (i.e., shrimps, crabs, or young fish) elicits predatory behavior by juvenile cuttlefish, as early as Day 3. Previously unfed 3-day-old cuttlefish spontaneously preferred shrimps. This initial preference remains prevalent throughout the first week of life. The preference seems then to widen to include young fish during the first 2 months of postembryonic development. This appears to agree with Wells' observations (1958, 1962), that naïve cuttlefish generally preferred elongated, moving prey compared to crabs, which were always chosen less often. In our choice experiment between two prey with the same elongated shape, moving in the direction of the longer axis, and both swimming, juveniles prefer shrimps. This suggests that their visual prey-recognition process involves higher order identification rather than the basic characteristics (elongated shape moving horizontally) used by toads and other animals (Ewert, 1985, 2004; Prete, 1999).

Table 1. Food Preference on Day 7 by Cuttlefish after One Meal (on Day 3) on Either One Crab or One Shrimp

			First Meal	Choice Test
Group	*n*	Prey	No. Prey Eaten	No. Preferring First-Fed Prey
1	20	crab	20	16
2	20	shrimp	20	15
Totals	40		40	31

Until Day 30, cuttlefish that were previously fed only shrimps still preferred shrimps. Their choice seems to be guided by both an initial preference for these prey items and, perhaps, early experience capturing them. From Day 60, cuttlefish tackled other prey, suggesting that their diet begins to diversify, regardless of their previous shrimp-only diet. At this point, there was no preference for the initially preferred prey, and early experience did not seem to be responsible for this; instead, cuttlefish tended to prefer novel prey items other than the familiar one. This result explains why cuttlefish become generalist predators during development. Moreover, it is consistent with previous observations of laboratory-reared animals. We often observed that it was difficult to maintain cuttlefish beyond the age of 30 days' posthatching when fed a single type of prey. As cuttlefish grow, the size of prey and the prey type change. Our results may support the hypothesis that cuttlefish go through a "sensitive" period during which they build a representation of which kinds of prey they are capable of eating to diversify their diet. Further investigations will allow us to test this hypothesis.

If early prey choice would have been "hard-wired," it is unlikely to be changed by a single exposure to a different prey type; however, Experiment 2 (in which a naturally nonpreferred prey item such as crabs was preferentially selected by cuttlefish that had been once fed on them; Figure 2) underlines a fundamental principle in the cephalopod life history: the ability to take advantage of a changing environment (and altering food choices) and the ability to override innate preferences. This result also confirms that found in the first experiment. During the course of the first week of postembryonic development, early experience is more important than the novelty effect in the feeding strategy.

Furthermore, this suggests the existence of a form of memory for food preference in juveniles. Dickel et al. (1998) and Messenger (1973) failed to demonstrate long-term memory in very young cuttlefish with the ‘‘prey behind a glass tube’’ protocol despite the session consisting of many trials. Our data show that giving a naturally nonpreferred prey item to cuttlefish just once on Day 3 induced a good long-term retention of this novel preference in 7-day-old animals. This would be the first demonstration of a form of long-term memory in cuttlefish during the first week of life. Given the evidence for avoidance learning in young animals (Hogan, 1973a, 1973b, 1973c; Wells & Wells, 1970) that allows them to know which food items not to choose, the question remains: Can young animals learn about the positive effects of foods? On Day 3 of our experiment, something akin to learning that occurred after only one trial led to the prey item acquiring a reward value. As a consequence, the nature of the reinforcement appears to be crucial in term of memory formation: In the case of a negative reinforcement (i.e., the possible pain incurred when the tentacles strike the glass in the ‘‘prey behind a glass tube’’ protocol), cuttlefish would need many trials to acquire the task, but would not have a long-term memory of it. Conversely, in the case of a positive reinforcement (i.e., a successful capture and the consumption of a prey), we have shown that a single sensory experience is sufficient to produce a long-lasting change in the food preference of a juvenile cuttlefish. In other words, we can assume that eating a prey item (even a nonpreferred prey item) would be more effective for long-term memory formation in juvenile cuttlefish than the inability to capture their naturally preferred prey. This event is especially important, as feeding is absolutely vital to hatchling survival.

NOTES

We are grateful to Dr. Adam Shohet for helping to correct the English. We also thank Dr. Nadav Shashar for stimulating conversation and helpful comments about the final version of this article. This research was supported by a Ph.D. grant from the French Ministère de la Recherche et de la Technologie and Université Paris XIII to A.-S. Darmaillacq. We also thank the staff of the Centre de Recherches en Environnement Côtier for their technical assistance.

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