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RESEARCH NOTES

Agonistic and reproductive behaviours of the cuttlefish *Sepia officinalis* in a semi-natural environment

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During the last decade, the cuttlefish *Sepia officinalis* has become a model system for behavioural[1] and neuroethological[2] studies. Unfortunately, adult behaviour in the field remains undescribed,[3] and therefore the external validity of these studies is uncertain. The problem is troubling because: a) Boal *et al.*[1] have shown that the size of the holding tank changes the expression of some cuttlefish behaviours and, b) a field study[4] on adult cuttlefish has found that a related cuttlefish, *Sepia latimanus*, does not occur in the high densities in which *S. officinalis* are kept, and that they express some behaviours, such as courtship, which are not observed in *S. officinalis*. This discrepancy may reflect a species difference, but it may also indicate that some of the published literature on the behaviour of *S. officinalis* is an artefact of studying them in a small space. Unfortunately, two of the behaviours of most interest to biologists, reproduction and agonistic behaviours, are also the ones that are likely to be altered when animals are confined. To examine their behaviour when they are less confined, we observed *S. officinalis* in one of the largest indoor marine tanks in North America, giving the cuttlefish 16 times greater tank volume/cuttlefish than the maximum used in previous studies (117.7 m^3/cuttlefish in our study, compared to 0.18 m^3/cuttlefish–7.3 m^3/cuttlefish.[1])

Cuttlefish (*S. officinalis*) were obtained from the Marine Resources Center, Marine Biological Laboratories, Woods Hole, MA. These animals have been cultured for several generations. Cuttlefish were fed frozen shrimp, fresh shrimp (*Cragnon* spp.), fresh or frozen fish (*Fundulus* spp.) daily. Animals were fed at approximately the same time each day. The water temperature was maintained between 17 and 22°C. Two months prior to the experiment, animals were placed in separate chambers (either 59.7 × 54.6 × 40.6 cm or 55.2 × 50.0 × 58.7) in which they were visually, but not chemically, isolated from one another. All animals were approximately the same age. The light cycle was 14L:10D when animals were housed separately and 13.5:9.5 L:D in the 15 m tank.

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Sexually mature cuttlefish were transferred to a 15 m circular tank (water depth, 1.5–2 m). The tank contained floor markings at 2 m intervals. 4 boxes of sand (38.1 × 24.8 × 10.2 cm) were placed equidistant from each other and 1 m from the edge of the tank. A rope suspended from the boom hung down to the bottom of the centre of the tank, where the outflow for the tank water was located. The females laid their eggs onto this rope. After 7 days, a line of 7 buckets (diameter: 27 cm, height: 37.8 cm), a large plexiglass oval tube (74.9 × 79.7 × 97.5 cm) and a large white lucite barrier (length: 203.2 cm, height: 47.6 cm) were added to the tank. The objects were added to give the environment more vertical relief, as would be found in the field.

A videocamera, attached to a time-lapse VCR, was installed overhead to monitor the movement of the cuttlefish in the tank 24h/day. The camera had approximately 1/2 of the tank in its field of view. Observers also filmed cuttlefish social interactions using a handheld Hi 8 mm videocamera. Animals were observed 12 hours/day starting 2 h prior to the onset of photophase to 2 h after photophase (6 am to 10 am, AST), 6 h after the onset of photophase to 10 h after the onset of photophase (2 pm to 6 pm), and 1.5 h prior to the onset of scotophase to 2.5 h after the onset of scotophase (8 pm to 12 am). Observations were made for 10 consecutive days.

One female was placed in the tank on Day 1 (mantle length, 13.8 cm). 1 day later, 2 males were added (mantle lengths, 15.6 cm and 16.8 cm). The female could be identified by her smaller size. The males were identified by unique markings on their mantle.[5] After three days, and for the next 5 days, males had telemetry tags inserted and removed and one male was replaced. The female died on Day 8 and was replaced with a new female (mantle length, 9.8 cm). Unless otherwise stated, behavioural data for this paper were collected during the first 4 days of the trial, before the animals were disturbed for telemetry tag placement.

The pattern of agonistic and reproductive behaviour was similar to that observed in small tanks. For example, we did not see any behaviours that we could classify as male courtship, as has been observed in field studies on two related cuttlefish species.[4,6] Males approached females and attempted to grasp them.[5,7,8] There was some evidence of female precopulatory behaviour (see discussion in Hanlon et al.[8]), but it was rare. Once in 12 matings, the female remained stationary and adopted an unusual grey coloration as the male approached. In most cases, however, the female blew water at the male (3/12 mating events), jetted away from the male (11/12), or inked (8/12). Female inking was effective in all cases in misdirecting the males, at least temporarily. It is possible that inking masks chemosensory cues[7] (but see Boal and Marsh[9]), as well as obscuring the male's vision. Female inking during attempted copulation showed an interesting pattern. The female either created a cloud of ink around her and then slowly swam away ($n = 3$), leaving the males in the ink, or the female would send the ink in one direction and jet in another direction ($m = 5$). Most copulation attempts were unsuccessful (3.8:1; unsuccessful:successful ($n = 12$)), attesting to the effectiveness of these techniques.

Mating interactions between male and female could result in damage to the female. We saw evidence of holes in the female's fin, ripped fins and damage to the skin of the mantle after mating or after an unsuccessful mating attempt. Nevertheless, females made little attempt to hide from males (i.e. remain out of their line of sight), even in the more complex tank environment.

Males attempted to remain close (less than 3 mantle lengths away) to the female after copulation and chased away the other male if he approached. This behaviour has also been observed in cuttlefish held in small tanks.[3] The female frequently attempted to jet away from the male during this time. Males sometimes exhibited the typical male-male agonistic display, the Intense Zebra Display,[10] to the female during these events. Males have been observed to express the Intense Zebra Display to females before,[1] although not in this context. However, females ($n = 2$) tolerated a male's presence during egg-laying and did not jet away. The dominant male remained less than 3 mantle lengths away during this time ($n = 6$ bouts of egg-laying). Both females and males periodically inspected the eggs, as has been observed previously.[5]

Males formed a dominance hierarchy in the large tank (Fig. 1), just as they do in smaller tanks.[5,11] The male that was subordinate spent significantly less time close to the female ($L = 36$, $p = 0.05$, $n = 5$ different subordinate-dominant pairs; Fig. 1) and made only unsuccessful copulation attempts. Agonistic contests were frequent with a maximum rate of one agonistic encounter every 3.8 min (measured over a 4 h period). In small tanks, males also spent most of their time expressing agonistic behaviour.[5] All the time periods showed at least some agonistic interactions. Few escalated to physical contact (maximum: 3 escalated contests/63 agonistic interactions/4 hr period). When contact occurred, however, injury could result, with both males suffering from abrasions of the skin and the subordinate could also suffer from a ripped fin. Subordinate males occasionally inked during attacks by the dominant ($n = 3$). Nevertheless, some agonistic interactions were initiated by the subordinate. We observed 3 reversals of dominance ($n = 2$ different dominant cuttlefish). In 2 of the 3 changes, the previously dominant male turned a very deep copper colour, a pattern not previously observed.

When males were not interacting with each other (i.e. producing Intense Zebra Displays) and were not within 3 mantle lengths of the female, they tended to maximise the distance between each other. These results are consistent with Boal et al.'s[1] hypothesis that *S. officinalis* males tend to maximise the distance between conspecifics. This raises the question of the ecological validity of dominance hierarchies in *S. officinalis*. Although they can be formed in the lab,[5] they may not exist in the field. Subordinate males may simply move away.[5]

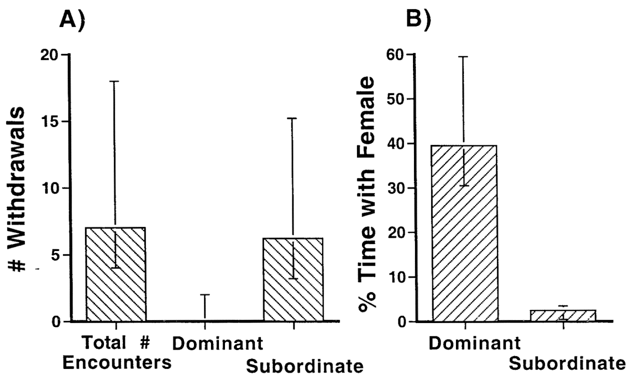

Figure 1. Dominant males spent more time with females than did subordinate males. The first bar represents the total number of male-male encounters per time period. **A.** Subordinate males consistently withdrew from dominant males during agonistic encounters. This behavioural response was used to identify subordinates. **B.** Dominant males spent more time with females. The y-axis records the percentage of the female's total time on tape in which she was within 3 mantle lengths of each male. The bars in **A** and **B** denote medians and errors are 1st and 3rd quartiles. Values are averaged over different time periods ($n = 14$) and 3 different males.

Males expressed agonistic behaviour during all time periods (6 am–10 am: 23 ± 26 (s.d.) agonistic encounter; 2pm–6pm: 34 ± 24; 8pm–midnight: 20 ± 23, n = first 3 time periods). Leaving out data from the first day males were placed together, agonistic encounters still occurred during all time periods. Agonistic behaviour was most frequent when males were initially introduced (day 1: 55 ± 10 agonistic encounters/4h; day 3: 10 ± 8 agonistic encounters/4h).

Prior to the addition of the males, the female spent much of her time settled on the bottom midway between the edge and centre of the tank. After the addition of the males, the female was never seen pausing in those areas. The female spent most of her time close to the tank wall, a distribution pattern similar to that observed in small tanks with mixed sex groups,[5] unless she was egg-laying. The female did not jet until after the males were added, and then the female jetted 7.5 min ± 4.6 min/day or 16% of the time the female was visible on the 8 mm tape.

Both the subordinate male and the female attempted to jet out of the tank, often crashing into the walls and injuring themselves. This observation suggests that females and subordinate males would leave the area of the dominant male, if they could. If this is true, then one of the largest indoor salt water tanks in North America is probably too small to allow *S. officinalis* to demonstrate typical mating and agonistic behaviour.

Although the behaviours observed in the large tank were similar to those reported for small tanks, we did find some differences. We found that dominant males were the only ones to copulate with the female in contrast to Boal.[5] This may be a function of our smaller sample size, the brevity of our study, or that with lower densities, it is easier for males to monopolise females and/or females to avoid subordinate males. We also observed that females can elude males by using different inking strategies. These strategies are probably not effective and/or possible in small tanks. We also noted that females were invariably accompanied by males during egg-laying. This is similar to what is observed in *S. latimanus* in the field[4] and may indicate that the mating system in *S. officinalis* is similar. These results also point to the necessity of field studies. Without them, it will remain difficult to understand cuttlefish mating and communication systems.[1,5,8]

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