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Social Behaviour of Individual Oval Squids (Cephalopoda, Teuthoidea, Loliginidae, *Sepioteuthis lessoniana*) within a Captive School

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Abstract

The social behaviour of individually identified squids was observed over a period of 4 mo. Squids were reared in captivity and maintained in a shoal of up to 18 animals. Distances between animals averaged 1.6 body lengths, similar to the 1.2 body lengths of wild teleost fish schools. Smaller animals schooled less frequently and were found at the periphery, and familiar and unfamiliar animals shoaled together. Eighty-six apparent reproductive events were observed. This species of squid, *Sepioteuthis lessoniana,* is not sexually dimorphic to the human eye and male squids copulated with both males and females but they directed their attention disproportionately towards females. Most copulations occurred more than a month before spawning, lasted only a few seconds and, unlike field observations on spawning grounds, were not followed by mate-guarding. Two body patterns are described that were used consistently in social interactions by both males and females; these patterns were both associated with reproductive behaviour but their exact function was unclear. A sister species, *Sepioteuthis sepioidea,* has been described as showing complex social behaviour; in *S. lessoniana,* no evidence for such behaviour was seen.

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Introduction

Field observations of *Sepioteuthis sepioidea* suggest that at least some squids show cooperative social behaviour as complex as that of flocking birds or herding mammals (MOYNIHAN & RODANICHE 1977, 1982; HANLON & FORSYTHE as cited by HANLON & MESSENGER 1996). The possibility of this degree of evolutionary convergence in social behaviour across such disparate taxa merits closer investigation.

Complex social behaviour, including reciprocal altruism, depends upon relationships between recognized individuals. Within animal aggregations, such relationships can be revealed by enduring group membership and preferential associations among individuals. In birds and mammals, social relationships between uniquely recognized individuals in a large group are common, although by no means universal (COLGAN 1983; ZAYAN 1994). Among other taxa, the case for individual relationships within aggregations is not so clear. In fish, for example, individual preferences for position within a school have been

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documented (HEALEY & PRIESTON 1973; PITCHER et al. 1982) and leader/follower individuals have been identified (PITCHER 1979; MUSCIALWICZ & CULLEN unpublished data as cited by PITCHER & PARRISH 1993), but preferential associations between individuals have been found only in herring (PARRISH unpubl. data, as cited by PITCHER & PARRISH 1993).

Many cephalopods are solitary or semi-solitary, making complex social behaviour unlikely. Squids form shoals of just a few to thousands of individuals. Here, we will use the term 'shoal' for groups that remain together for such social reasons as foraging, spawning or feeding (PITCHER 1983) and 'school' for shoals that exhibit synchronization and polarized swimming (PITCHER & PARRISH 1993); squids both shoal and school (HANLON & MESSENGER 1996).

There is no evidence for long-lasting social relationships in squids (HANLON & MESSENGER 1996). However, some researchers have observed what they describe as sentinel behaviour, wherein some animals play a socially defined role of alerting others to danger (MOYNIHAN & RODANICHE 1982; HANLON & FORSYTHE unpublished data as cited by HANLON & MESSENGER 1996). Also, it has been hypothesized that squids use their remarkably flexible body patterning for purposes of complex communication (MOYNIHAN 1985; HANLON & MESSENGER 1996). If these claims are correct, these squids show more complex social behaviour than schooling fishes and provide an important case of evolutionary convergence between invertebrates and mammals and birds.

To evaluate these claims, we studied the behaviour of individual squids within a captive shoal. While both the social behaviour and schooling behaviour of squids have been the subject of previous work (LAROE 1970; HURLEY 1978; MATHER & O'DOR 1984; HANLON & MESSENGER 1996), the social behaviour of individuals has not been studied previously. Two principal difficulties have stood in the way. Firstly, it has been difficult to keep squids alive and in good health for more than a few weeks in captivity. This difficulty has been reduced by advances in mariculture techniques (LAROE 1971; SEGAWA 1984; HANLON 1990; LEE et al. 1994). Secondly, it is difficult to distinguish individual squids. Tagging these soft-bodied, fragile and fast-moving animals is difficult at best (HURLEY & DAWE 1980). In this study we succeeded in identifying individuals when they became uniquely scarred, but were otherwise unharmed, by a failed attempt to tag them.

In this study, our subjects were captive-reared *Sepioteuthis lessoniana*, a loligonid squid common in coastal waters of the Indo–West Pacific (SEGAWA 1987). Many squids are cannibalistic (HANLON & MESSENGER 1996), with larger animals attacking and consuming smaller conspecifics. Unlike these species, *S. lessoniana* schools with mixed size classes and rarely consumes smaller animals, even in captivity (ibid). If the different size classes are also different ages (this is not known), mixed size groups could provide the opportunity for individuals to learn from more experienced conspecifics. *S. lessoniana* is considered closely related to *S. sepioidea*, a species noted for its complex social behaviour (MOYNIHAN & RODANICHE 1982; MOYNIHAN 1985; HANLON & FORSYTHE as cited in HANLON & MESSENGER 1996). *S. lessoniana* is also routinely cultured in captivity for up to six generations (P. G. LEE, pers. comm.) at the National Resource Center for Cephalopods in Galveston, Texas, so that it was readily available, in good health, and could be observed for a prolonged period. For all these reasons, *S. lessoniana* seemed an ideal species for our study.

We monitored the behaviour of each individual squid within the shoal over a period of 4 mo. This squid has a laboratory life span of about 6–8 mo (LEE et al. 1994); thus, this study followed animals for over half their lives, from the time they first formed midwater shoals as juveniles through to post-reproductive senescence. On a daily basis, we recorded the location within the shoal where individuals were found and all the social interactions we observed. Results were analyzed for evidence of complexity in social organization such as preferential associations between individuals and highly ordered spatial structure within the shoal; we also looked for evidence for the use of body patterns as communication signals, as distinct from crypsis.

Methods

Subjects

Subjects were *Sepioteuthis lessoniana* LESSON (1830), cultured from eggs collected in Kin Bay near Ishikawa on Okinawa, Japan. A significant Japanese literature exists on the fisheries and biology of this species complex (reviewed by SEGAWA 1987; LARCOMBE & RUSSELL 1971; LEE et al. 1994). Previous authors have reported females to be smaller than males (SEGAWA 1987; SEGAWA et al. 1993a). This was not true of the *S. lessoniana* cultured in Galveston; indeed, our animals were not at all sexually dimorphic. Although the parallel mating position of our squids matched that described by SEGAWA (1987), the body patterning photographed in SEGAWA's report (SEGAWA et al. 1993a) did not match that of our animals. Several authors suggest that *S. lessoniana* may be several distinct species (SEGAWA et al. 1993b; IZUKA et al. 1994); thus, our animals may not be the same as those described by SEGAWA (1987).

Squids were hatched in the laboratory and reared in small tanks (HANLON et al. 1991; LEE et al. 1994). They were removed into the experimental tank as juveniles of approximately 5 cm mantle length (ML). Once they grew to approximately 10 cm ML, we caught them with a dip net and tagged them with two fingerling tags of distinct colours, inserted one through each posterior fin, close to the body. At the time of tagging, we also measured each animal's mantle length. We then released the animal immediately back into the experimental tank. All squids promptly pulled out the tags (within 24 h); fortunately, all remained healthy. We found that we could still identify individuals by their unique tagging scars; therefore, we did not attempt to re-tag animals. After death, all squids were re-measured, weighed and autopsied to determine sex because we could not determine sex using gross external characteristics.

Apparatus

The experiment took place indoors in a 5.4 m diameter, 1.3 m deep fibreglass tank lined with a matte-black plastic liner. Techniques for rearing and maintaining this species have been described elsewhere (HANLON 1990; LEE et al. 1994). Natural lighting was supplemented by artificial light sources, controlled by a seasonally adjusting timer. Squids were fed 2–3 times daily with live fish. Uneaten fish were not removed and the tank contained numerous live fish at all times.

It was difficult to observe the squids through a side window because they were acutely responsive to any motion by the observer. The squids did not appear to be disturbed by a person above the tank, however, so observations were made from a platform suspended 0.5 m above the tank. The observer could easily view the entire tank from this vantage point (Fig. 1).

Procedure

Observations extended from Oct. 1995 to Feb. 1996 and were performed by a single observer once each day. Observations were performed between 0700 and 2400 h, with most observations between 0800 and 1100 h or between 1500 and 2100 h because social interactions were rarely seen during the middle of the day.

After climbing out onto the platform, the observer waited until swimming behaviour was calm and steady, or for 10 min, whichever was longer, before commencing. Each observation period began with sequential 1 min observations of each individual. We recorded the location of each squid on a diagram of the tank and recorded whether the squid was swimming in parallel with its neighbours (yes/no). Next, the entire school was observed for 30–45 min. We noted all feeding behaviour, social interactions including mating and aggression, and any unusual behaviour. At the conclusion of the group observation period, we again recorded individual behaviour for 1 min periods, as described above. The spatial arrangement of individuals normally changed very little during the 1

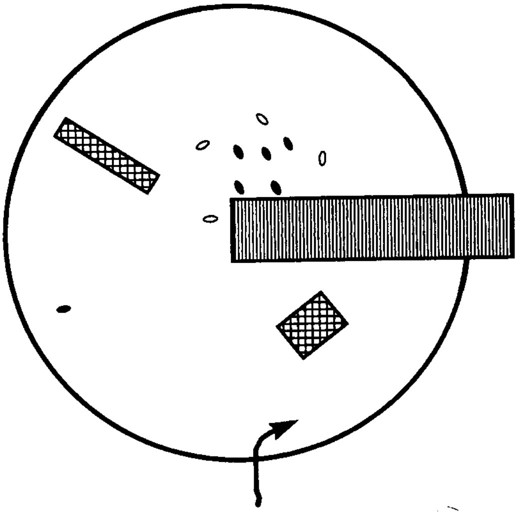

Fig. 1: Schematic of the experimental tank, drawn approximately to scale. Water flowed into the tank as indicated by the arrow and flowed out through a drain in the centre of the floor; filtration was provided externally. A catwalk suspended above the tank served as an observation platform. Two artificial reefs were placed within the tank so that spawning females could attach their eggs; the square one to the left of the platform was used during phase 2. A small number of squids are drawn to represent the general configuration of the shoal; white ovals represent smaller animals and black ovals represent medium and large animals (see text for details)

min series of observations and the shoal was usually located approximately as shown in Fig. 1.

Phase 1 of this study included only the animals that we had attempted to tag. This initial phase lasted from Oct. 26, 1995 to Jan. 30, 1996, and included a total of 39 h of observation. Initially, there were 18 squid, 13 males and 5 females. The exact number of animals in the tank changed with time: two animals died in Oct., two in Nov., seven in Dec. and three in Jan. Excluding Dec., this level of mortality is normal for squids in captivity at our facility; we do not know why we suddenly lost seven animals over a 5 d period in Dec. (we suspect tainted food because the entire laboratory sustained significant mortality during this time). Because changes in group size could have a substantial impact on behaviour, we focused our analyses on 33 observation periods (Nov. 13–Dec. 21) in which data were complete for 14 individuals (details below).

Phase 2 began at the end of Jan. when mortality had reduced the group from the original 18 to just four animals. We introduced five new squid: a group of four from a tank containing about 45 squids and a single animal from a tank where it had been alone for more than 3 wk. One squid from the introduced group of four died shortly after transfer; therefore, observations reported include those for a total group size of eight. Before releasing the new animals, we measured and recorded their mantle lengths. We were able to identify these new animals by using relative size and pre-existing scarring. This second phase lasted from Jan. 30 to Feb. 7, 1996, and included a total of 16.5 h of observation. We observed the composite group as in phase 1 except that observations were made twice per day instead of just once.

Reproduction

Altogether (phases 1 and 2), we observed 86 apparent reproductive events. Based upon the behaviour we saw, we labelled the animals involved as either the actor or the recipient of the action. Observed behaviours fell onto four ordered classes: 1. 'Pre-mating Behaviour' consisted of rapid back-and-forth swimming of an actor adjacent to a recipient; 2. In a 'Flip', the actor turned over so that it was swimming ventral-side up, above or beside the recipient; 3. In an 'Attempt', the actor jetted forward, upside down, and attempted to make contact with its arms to the recipient's head or arms presumably to deposit a spermatophore; and 4. In a 'Contact', such physical contact was achieved and, presumably, a spermatophore was transferred to the recipient although this could not be seen.

The entire sequence, from Pre-mating Behaviour through Contact, took approximately 2–3 s to complete. Events that included a Contact usually concluded with the actor jetting rapidly away. Reproductive events that did not include a Contact were frequently followed by the recipient moving away, although whether this movement was an active termination of the event or simply routine swimming by the recipient was not clear. The recipient did not perform any other noticeable behaviours during this time.

Each class of behaviour was almost always preceded by each of the others of lesser rank, in order. For purposes of analysis, we scored each incident once as the behaviour that came closest to Contact (the highest rank, above) unless otherwise specified. Thirty-four per cent of all reproductive events were scored as Pre-mating Behaviour, 21 % as Flip, 17 % as Attempt and 28 % as Contact.

For each reproductive event, we recorded the confidence of the experimenter that the participants had been accurately identified. In 91 % of events, the experimenter was confident of the identification of the recipient; in 84.3 % of events, the experimenter was confident of the identification of the actor. For each analysis, we included only those events in which the experimenter felt confident in the identifications of the animals involved; sample sizes vary slightly, therefore. Sixteen squids were present during at least some of the reproductive events, four females and 12 males.

In all but one of the observed events, the actor was later determined (by autopsy) to be male. Assuming there were four female and 12 male squids in the tank, our error rate in actor identifications was about 3.2 %. Because the entire sequence took no more than 2–3 s to complete and could occur anywhere in the tank at any time during observations, we consider this error rate acceptable. Our error rate in identifying recipients was probably much lower because recipients remained dorsal-side up and generally swam calmly and slowly throughout the event, whereas actors moved very rapidly and darted off immediately after making contact with the recipient.

Spawning was not observed until the end of Jan., during phase 2.

Body Patterning

Two types of body patterns were observed with great enough stereotypy and frequency that we noted their occurrences. Firstly, animals sometimes accentuated the visibility of their gonads ('Accentuated Gonads') so that their gonads appeared bright white through their mantles, a behaviour described as a "chromatic signal" by HANLON & MESSENGER (1996, p. 125). Accentuated Gonads was usually, although not always, reciprocated by an adjacent animal (see below). We were not able to distinguish male from female Accentuated Gonads.

Secondly, squids sometimes darkened their mantle and spread their arms widely ('Spread Arms'), at times in a slightly head-down and arms-up, V-position. Spread Arms was often shown while following or chasing another animal. This behaviour seems not unlike the Zebra Display of *Sepioteuthis sepioidea* (MOYNIHAN et al. 1982; MOYNIHAN 1985; HANLON & MESSENGER 1996) but was less dramatic in body coloration.

Analyses

Because squids continued to grow throughout the study, we used relative size rather than absolute size in our analyses. Squids were grouped into three size classes: small, medium and large (see below). Class membership remained stable throughout the experiment.

In phase 1, most analyses were performed on a subset of the data. Unless otherwise specified, analyses were performed using 33 observation periods (Nov. 13–Dec. 21) in which data were complete for 14 squids, three females and 11 males. Of these squids, four animals were classified as small (average ML = 10.1 cm), five as medium (average ML = 12.7 cm), and five as large (average ML = 16.0 cm). All three females were large (average ML = 15.6 cm). Average mantle length for males was only 12.4 cm, but the largest animal in the group was male.

In phase 2, the resident group was composed of two large females, a medium male and a small male. The introduced group was composed of three large females and a large male. The introduced single squid was a large female.

Schooling distances are commonly computed using total body length rather than mantle length. Total body length is difficult to measure in squids because their arms can extend or contract, giving a different apparent size. In general, mantle length is about 60 % of total body length, giving an estimate of about 22 cm body length (13.1 cm ML) at death, on average, for our squids.

Use of space was computed using approximations from planar geometry, for simplicity's sake.

Spatial organization was evaluated statistically using analyses of variance (SOKAL & ROHLF 1969) or Kruskal–Wallis analysis of variance by ranks (SIEGEL & CASTELLAN 1988). Statistical significance for social behaviour variables was determined using chi-square goodness of fit (ibid). Expected values were never smaller than 5; therefore, corrections for small sample size were not required. The significance of relationships between body patterning and other behaviour were determined using Spearman rank-order correlations (ibid). Results from analyses with an associated probability level >0.20 are not normally included, for the sake of brevity.

Results

Phase 1: Single Group

Spatial organization

The squids formed a single shoaling group. In 96 % of observations, nearest neighbour (NN) distance was less than 1 m; occasionally an animal would swim off by itself (Fig. 1). Among nearest neighbour observations of less than 1 m, mean NN distance was 35.8 cm (SD = 15.3 cm, n = 14), about 1.6 body lengths. Eighty-one per cent of observed distances were within 1 SD of the mean, indicating that squids were evenly dispersed within the shoal, rarely closer than 20.5 cm (1 body length) or more than 51.2 cm apart (2.3 body lengths). The mean of the 14 observations greater than 1 m was 144.4 cm (SD = 46.1 cm). Distance to nearest neighbour (d) was not related to size class (small, d = 36.1 cm, SD = 17.0 cm; medium, d = 32.6 cm, SD = 13.6 cm; large, d = 38.8 cm, SD = 15.4 cm; KW = 3.02, k = 3, $p > 0.10$) or to sex (females, d = 41.3 cm, SD = 14.5 cm; males, d = 34.2 cm, SD = 15.1 cm; KW = 2.18, k = 2, $p > 0.10$).

Location within the shoal was evaluated using individual's distance to the group centroid. Individual squids were not located in a predictable location with respect to the centre of the shoal (analysis of variance, $F_{13,32} = 1.75$, $p > 0.10$). Squid size class was a good predictor of location in the shoal, however. The four smallest squids were significantly farther from the centre of the shoal than either the five medium-sized or the five largest squids ($F_{2,32} = 7.20$, $p < 0.005$). No difference in location was found between the medium and the large squids. Location within the shoal was not related to sex. The mean ranked distance to the centre of the shoal for medium and large squids only (there were no small females) was 5.0 for the three females and 5.7 for the seven males.

All squids spent most of their time either in mid-water or on the bottom. (Young squids, prior to shoaling and prior to this experiment, were normally found above larger animals, near the surface.) To evaluate vertical distribution, we analysed the locations of seven squids, two small, two medium, and three large, for which we had complete data for a full 3 mo of observations. For these animals, vertical location (bottom, middle, and top thirds of the tank) differed significantly between individuals (analysis of variance, $F_{6,12} = 18.26, p < 0.001$) and across time blocks (analysis of variance, $F_{2,12} = 7.07, p < 0.05$). Squids were less likely to be located on the bottom during the third month of observations than during the first or second months. We could not discern any pattern in vertical location based upon either size class or sex.

The fraction of squids swimming in parallel with each other at any one time ranged from 0 % to 100 %, with a median of exactly 50 %. If any squids were swimming in parallel (excluding zeros) then, on average, half were doing so. Females were equally likely to be swimming in parallel as males (42 % of the time, both). The four smallest squids were significantly less likely to be swimming in parallel than the 10 medium and large squids (means 26 % and 48 %; analysis of variance, $F_{1,12} = 8.50, p < 0.05$).

Social recognition

Our maps of the spatial arrangements of individuals revealed no evidence for any long-term preferential associations over this 2 mo period. Analyses were performed within size classes to correct for differences in locations for the size classes within the shoal. We found no consistent distances between particular pairs across time (analyses of variance, small with small, $F_{5,32} = 0.69$; medium or large with medium or large, $F_{44,42} = 0.25$; small with medium or large, $F_{39,32} = 0.13$; $p > 0.50$ in all cases).

To investigate shorter-term associations, we examined five-observation (2.5 d) running averages between all possible pairs of squids. An average of less than 36 cm (mean NN distance, see above) occurred only once, between a small male and a medium male. Averages of less than 51 cm (mean NN plus SD) were found for only eight pairs of squids. It is doubtful that this tiny number of close associations (0.2 % of 3640 running averages computed) is biologically meaningful.

In our scan samples, we noted pairs of squids that appeared to be swimming together, in parallel, and slightly apart from the shoal. There were 51 such occurrences, dispersed evenly across the weeks of observation. Of the 91 possible pairs of individual squids, 27 pairs were observed to be swimming together in this way at some point. Sixteen of the 51 occurrences were of a single pair, however: the largest female (no. 16) and the smaller of the two large males (no. 18). (This male died in Dec. from unknown causes.) No other pair was seen together more than four times.

Reproduction

Seventy-eight reproductive events were observed between Nov. 13 and Dec. 21; in 66 of these, both the actor and the recipient were identified with confidence. Eighty-two per cent of these occurred in the first 3 wk of December.

Variation in reproductive behaviour between males was substantial. Four of the males (three medium and one large, no. 18) initiated 61 of the 69 reproductive events in which the male was identified with confidence. Male mating behaviour differed both within and between size classes. The four smallest males together initiated only three events, five medium-sized males together initiated 45 events, and the two large males initiated 21 events. One large male, one medium male and two small males never initiated any reproductive events.

Males directed 'reproductive' behaviour towards each other as well as towards females. In 31 of the 63 observed events in which the recipient was identified with confidence, the recipient was female, while in 32 cases the recipient was male. If events were independent and recipients were determined by chance, 3 out of every 13 events would have been directed towards females; reproductive events were significantly biased towards female recipients ($\chi^2 = 24.23$, $p \ll 0.001$). Interestingly, the bias in favour of female recipients was evident only in incompleted reproductive events (24F:14M, $\chi^2 = 34.39$, $p < 0.001$). Contacts were randomly distributed (3F:18M, $\chi^2 = 0.91$, $p > 0.30$).

Males varied in their behaviour; lumping all events together can hide information. If we look at individual males, male no. 18 directed his behaviour towards females significantly more often than would be expected by chance alone (11F:5M, $\chi^2 = 18.80$, $p < 0.001$). Unlike no. 18, however, each of the other reproductively active males directed most of their reproductive behaviour towards other males. Both separately and together, the distribution of their recipients was not statistically different from chance (15F:28M, $\chi^2 = 3.38$, $p > 0.05$). Too few contacts were made by any particular male to be able to examine whether individual males also failed to direct most of their contacts to female recipients.

Individual squids were not equally likely to be recipients. Among females, the smallest female was approached once and Contact was made (no. 15), the middle-sized female was approached four times with no Contacts (no. 14), and the largest (no. 16) was approached 26 times with just two Contacts. Approaches to males were more evenly distributed, ranging from zero to five per male with zero to two of these resulting in Contact.

The likelihood that a reproductive event would reach Contact was related to the sex of the recipient. Of the 32 approaches to male recipients, 13 ended with Contact; for female recipients, 3 of the 31 approaches ended with Contact. Assuming independent events, this difference by sex in the outcome of approaches is statistically significant ($\chi^2 = 4.83$, df = 1, $p < 0.05$). It is possible that events were not independent. If we look instead only at the first reproductive event between any particular pair of squids, 18 of 32 events that males directed at other males ended with Contact while 3 of 27 events that males directed at females ended with Contact. This difference is again significant ($\chi^2 = 4.31$, df = 1, $p < 0.05$). Interactions with females were more likely to be terminated before apparent spermatophore transfer.

Body patterning

Accentuated Gonads was observed on 68 occasions. In 56 of these occasions, the initiator was clearly interacting with one or several squids. Most of the time, we did not notice any reaction from the other squid. Twelve times, the other animal responded with its own Accentuated Gonads and four times, the other squid swam away immediately.

In 22 cases, there was no clear social context for the observed Accentuated Gonads; for example, the initiator was off away from other squids or the initiator appeared to be showing Accentuated Gonads more or less continuously throughout the observation period, regardless of what other squids were around. Small males rarely showed Accentuated Gonads and never without a clear social context; there was no clear pattern amongst the other individuals.

Most Spread Arms occurred between medium and large males. Only 3 of 36 recipients responded with Spread Arms; the rest responded by swimming away. Females were involved in Spread Arms encounters only twice; once the largest female (no. 16) showed Spread Arms at the smallest female (no. 15) and once a large male (no. 18) showed Spread Arms at the largest female (no. 16).

Accentuated Gonads and Spread Arms correlated positively with each other ($r_s = 0.61$, $p < 0.05$). Male and female squids that showed Accentuated Gonads more frequently were more likely to be the recipients of reproductive events than those that showed Accentuated Gonads less frequently ($r_s = 0.68$, $p < 0.01$). Among males, both body patterns correlated positively with the total number of mating events in which they were actors (Accentuated Gonads: $r_s = 0.84$, $p < 0.001$; Spread Arms: $r_s = 0.82$, $p < 0.01$).

Other behaviour

Observed food consumption ranged from 0.04 fish/h to 0.47 fish/h (n = 103 feeding events observed). We did not find any patterns relating feeding to either sex or size class.

Clear bouts of agonistic behaviour were observed on only six occasions. Twice, one squid directly attacked another, causing it to flee with no apparent injury. Four times, chases were seen. No pattern was detectable for individuals or size classes involved, but in all cases the apparent initiator was male.

Phase 2: Combining Groups

Spatial organization

The two groups of squids formed a single shoal, although their shoaling behaviour differed. Average nearest-neighbour (NN) distances for the introduced group were significantly less than those for the resident group (mean of 56 versus 92 cm; $F_{1,11} = 26.85$, $p < 0.001$). Both distances are substantially less than the 215 cm possible had the squids spread out maximally, although both are larger than the NN distances observed in phase 1. To verify that the two groups formed a single shoal, we calculated centroids for the two groups separately. As expected, the distances of the introduced group to their centroid were substantially less than the distances of the resident group to their centroid. Interestingly, members of the resident group were, on average, as close to the centroid of the introduced group as they were to their own centroid (149 cm versus 156 cm), indicating that, despite differences in shoaling behaviour, the two groups formed a single shoal.

The introduced group spent 52 % of the time swimming in parallel, a mean similar to that observed in phase 1. Individuals in the resident group swam in parallel only 8.2 % of the time, on average.

The single animal introduced into the group never shoaled or schooled with other squids.

Social recognition

Squids did not associate preferentially with familiar squids. We examined NN identities for the three squids from the introduced group and two squids from the resident group which had all maintained similar NN distances. Both groups were more likely to have a nearest neighbour from the introduced group than would be expected by chance (introduced group, $\chi^2 = 9.36$; resident group, $\chi^2 = 10.69$; $df = 1$, $p < 0.05$ for each). Because this bias was similar for both groups ($\chi^2 = 3.26$, $df = 1$, $p > 0.05$), it indicates that proximity to members of the introduced group was a result of that group's central location within the shoal.

When spawning, females (n = 3) did not have a consistent nearest male neighbour. One of the three males (the one from the introduced group, 'L2') stayed closer than the other males to the artificial reef on which the females attached their eggs (closest 67 % of the time). This male was the closest male to the three females in 58 %, 42 % and 67 % of the observations.

Reproduction

Only two copulations were observed during phase 2. All animals were from the introduced group. Both included the male L2; two different females were involved.

Spawning by three females in all was observed: two females from the resident group (no. 14: 5 d; no. 16: 1 d) and one female from the introduced group (1 d).

Handling of the eggs occurred on all 5 d in which eggs were present. Individuals seen handling the eggs included the single introduced male L2 and the solitary introduced female, as well as the spawning females.

Body patterning

The pattern of occurrence of Accentuated Gonads did not appear to be different from that during phase 1; because of the relative paucity of data (14 events), it was not analysed.

One of the introduced males, L2, showed Spread Arms with great frequency (30 times, total) and directed his displays at four of the five females present. Twenty-one of his Spread Arms were directed at the female doing most of the spawning (no. 14). The female to which he did not direct any Spread Arms (no. 15) was one of the two females not seen to spawn. None of the males ever directed a Spread Arms at other males during phase 2. The solitary introduced female performed a Spread Arms once at male L2. No other instances of Spread Arms were seen during phase 2.

Discussion

Ours was an unusually long-term study of individual behaviour within a group (over half their expected life span). The squids' behaviour may have been atypical because the animals were reared in the laboratory. For example, stable dominance interactions and leader–follower roles might never develop with ample food and no predators present. As another example, groups of *Sepioteuthis sepiodea*, a related species, sometimes form long lines with all individuals facing the same direction (perpendicular to the line itself). They will also form small clusters. With the constraints of a round tank, the linear arrangement would be impossible and all one would see would be clusters. Our group size was also quite small; one male and one female were responsible for nearly a third of all reproductive behaviour observed. Our inability to match our species with one for which field data exists further complicates interpretation of our results. Our results should be treated with caution, therefore.

Despite this limitation, we think our results are valuable. First, little is known about squid behaviour, and nothing at all is known about the social behaviour of individual squids. A field study would be far preferable, but the practical problems associated with such a study are formidable. At the very least, our partial information can provide a guide for future, more focused studies.

Unlike most squid behaviour studies, our squids were reared in the laboratory, a clearly impoverished environment. However, the squids were not traumatized by capture, which is often highly stressful and frequently results in high mortality, nor were they in the process of acclimatizing to the captive environment. *Sepioteuthis lessoniana* reproduces successfully in the laboratory and has been cultured for multiple generations, indicating that laboratory conditions were not so aversive that behaviours fundamental to survival and reproduction were totally compromised. All non-reproductive behaviours seen in the laboratory (the reproductive behaviour of different species varies markedly) have also been reported in wild populations of *S. sepiodea* (MOYNIHAN & RODANICHE 1982; personal observation). And in a study of the effect of crowding on the behaviour of *Sepia officinalis*, increasing crowding increased the frequency of behaviours associated with aggression, but all behaviours observed under normal mariculture densities were also observed when space per animal was increased tenfold (BOAL et al. unpubl. data). We believe that the behaviours we observed in this experiment were not strongly dissimilar from natural behaviour, therefore.

Spatial Organization

Spatial organization is a crude but preliminary way of investigating social organization. For these squids, social organization did not appear to be particularly complex, at least by this measure.

The squids clearly functioned as a group, shoaling together 96 % of the time in phase 1. Inter-individual distances were regular, indicating that individual animals moved with respect to others in the group. Mean inter-animal distances (36 cm) were much smaller than had the animals spaced themselves so as to maximize their use of space (163 cm). All areas of the tank were occupied at least some of the time and we saw no evidence that squids avoided proximity to the tank wall. Clearly, the squids were swimming more closely together than required by physical necessity.

We cannot determine elective group size from this study except to note that up to 18 individuals preferred to shoal together under these circumstances rather than split into two or more groups. Field observations of *Sepioteuthis* include groups ranging in size anywhere from 5 to 200, but usually under 40 (HANLON & MESSENGER 1996; personal observation), so our group was not extraordinarily large or small.

The average distance between nearest neighbours (phase 1) was about 1.6 times approximate total body length, with most distances falling between 1.0 and 2.5 body lengths. Although our hand recording method was crude, this result compares favourably with results for other captive squids. In groups of six similarly sized *Loligo opalescens*, HURELY (1978) found mean separation distances of 0.7 to 2.5 body lengths; in juvenile

Illex illecebrosus, MATHER & O'DOR (1984) found mean nearest-neighbour distance to be about 1.0 body lengths for a group of 38, 1.5 body lengths for a group of 20, and 2.0 body lengths for a group of four. On spawning grounds, the density of *Sepioteuthis lessoniana* (probably not the same species) was reported to be about 4 animals/m^2 (SEGAWA et al. 1993a) for squids of 30–50 cm ML, equivalent to about 1.0 body length between individuals. Similarly, the density of schooling fish is generally about one fish per cubed body length (PITCHER & PARRISH 1993), or about 1.2 body lengths between individuals. These similarities of animal spacing within schooling squids and fish lend further support for non-hydrodynamic explanations for spatial positioning in schools (PARTRIDGE & PITCHER 1979; MATHER & O'DOR 1984; review, PITCHER & PARRISH 1993).

In phase 1, small squids swam in parallel less often than larger squids and were located at the periphery of the shoal. Very young squids do not school, but these animals were all large enough that they chose to remain with the group. HURLEY (1978) also found less parallel swimming among smaller squids in her study of groups of similarly sized *Loligo opalescens*. Our squid's nearest-neighbour distances did not differ by size class, suggesting that smaller squids were equally motivated to shoal, so it is not clear why the smaller squids were at the periphery. Size-based segregation has been reported in shoals and schools of fish; however, most reasons given for such segregation (VAN OLST & HUNTER 1970; HAMILTON 1971; PITCHER et al. 1982, 1985, 1986; PITCHER & PARRISH 1993; but see PARRISH 1989; PARRISH et al. 1989) have been previously disproved for squids (HURLEY 1978). We did not see evidence for enforcement of spatial segregation by aggression from larger animals or dominance in other activities such as feeding rates or food access. The explanation for size-based segregation in shoaling and schooling squids remains uncertain, therefore.

In phase 2, two-dimensional shoaling distances displayed by the squids (resident group, 2.9 m^2; introduced group, 0.4 m^2; solitary introduced squid, 3.1 m^2) coincided with historical conditions (resident group, 7.3 m^2; introduced group, 0.3 m^2; solitary introduced squid, 22.3 m^2) rather than possible space available (all, 3.2 m^2). The solitary squid introduced from a third tank never shoaled or schooled (no parallel swimming observed). This squid's behaviour suggests that the closer shoaling of the introduced group may have resulted from historical reasons rather than stress from relocation. The variation in preferences for distance to nearest neighbour between the individuals from the introduced group and those from the resident group provide adequate explanation for the introduced group's central location within the mixed-group shoal; similar shoal structure has been documented in fish (HEALY & PRIESTON 1973).

In both phase 1 and phase 2, peripheral squids swam in parallel less often than those in more central positions. It would be interesting to know whether peripheral squids rarely swim in parallel, in general.

Social Recognition

We found little evidence for social recognition in these squids. One pair of squids was seen together often in phase 1; this pair consisted of the most sexually attractive female (no. 16) and the most sexually active of the large males (no. 18). Perhaps this pair recognized each other; alternatively, they could have been simply assorting on the basis of sexual interest. No other significant long-term or short-term preferential associations between individuals were observed in phase 1, and no signs of group recognition or group fidelity were observed in phase 2.

In *Sepioteuthis sepioidea*, HANLON & FORSYTHE (unpublished field data as cited by HANLON & MESSENGER 1996) reported that small shoals of animals stayed together for several days, with a particular male and female maintaining close proximity; however, these animals were not tagged, so individual identification could not be confirmed. Our observations did not provide much indirect evidence in support for social recognition in these squids; however, our procedure did not explicitly test individual recognition, nor was there any pressing reason why we might have seen evidence for it.

Reproduction

Information about mating behaviour in squids is sparse and behaviour varies widely between species (HANLON & MESSENGER 1996). Most commonly, adult squids migrate inshore to spawn according to some seasonal schedule. Copulations of *Sepioteuthis lessoniana* observed on a spawning ground by SEGAWA and colleagues (1993a) lasted for only "a few" seconds, consistent with our observations. Unlike our observations, the male swam next to the female afterwards while she laid eggs. In *Loligo pealei*, females also mate on the spawning grounds; however, we know that the females arrive there already inseminated (DREW 1911; HANLON 1996). Thus, mating on the spawning grounds is only part of the story of reproduction in squids. Unlike most published field observations, most of the copulations we observed occurred well in advance of any spawning. Our observations may extend rather than conflict with previous reports, therefore.

Our most surprising result was the apparent failure of males to discriminate on the basis of sex when apparently passing spermatophores (Contacts). If the mating behaviour we observed in captivity is not abnormal, we are challenged to explain how such apparently maladaptive behaviour could persist.

We hypothesize that males do have a bias towards female recipients but were disproportionately rebuffed by them. This explanation seems plausible for four reasons. First, Spread Arms was used primarily by males, providing a good indicator of the sex of other squids. Second, among squids cultured in the laboratory, males usually mature sexually before females (personal observation). Medium and large males initiated most reproductive events, indicating that they were sexually mature. Females, on the other hand, did not spawn for at least another month after this particular subset of data was collected so females may have been sexually immature. Third, the most attractive recipient, a large female (no. 16), received a full 36 % of all approaches, suggesting that she was indeed particularly attractive to males. In only three cases did these approaches result in a Contact, suggesting that she was rejecting their advances, either by moving away or by some more subtle means that we failed to detect. Fourth, in phase 1, interactions with female recipients were more likely to be terminated before apparent spermatophore transfer than interactions with male recipients, suggesting that females were actively terminating interactions prior to Contact. In phase 2, when females were spawning, only two reproductive events occurred but both were directed at females and both ended with Contact. For these reasons, we believe that males do have a bias towards female recipients but that this bias was masked by circumstances.

Body Patterning

The two body patterns we observed appear to function primarily as reproductively related signals. The more sexually active individuals (more often involved in what appeared to be reproductive behaviour), both male and female, were more likely to show Accentuated Gonads than were the less sexually active individuals. Accentuated Gonads was observed in situations that were not clearly social, suggesting that this signal could simply indicate the sender's reproductive condition. In support for this hypothesis, the frequency of Accentuated Gonads was positively correlated with the frequency of receiving a reproductive event.

Spread Arms was primarily exhibited by males and was shown more frequently by the more sexually active males than by the less sexually active males. Spread Arms appears similar to the Zebra Display of *Sepioteuthis sepioidea,* the Intense Zebra Display (IZD) of *Sepia officinalis* and the Lateral Display of *Loligo plei,* all of which are agonistic displays seen primarily between pairs of sexually active males. In phase 1, most Spread Arms was seen between pairs of medium and large males. Like the IZD of *S. officinalis,* Spread Arms was also used by males when near a mate or potential mate (phase 2). In *S. officinalis,* the IZD appears to function as a signal of reproductively related arousal and is normally initiated by males (TINBERGEN 1939). It is also performed by males or occasionally females rebuffing a displaying male (personal observation), and in male–male dominance contests (TINBERGEN 1939; ADAMO & HANLON 1996). Our observations of Spread Arms in *Sepioteuthis lessoniana* fit this pattern, suggesting it could be a similar signal for *S. lessoniana.*

Complex Social Behaviour

In a small observational study such as this one, we cannot draw any firm conclusions about the possibility of such complex social behaviour as sentinel behaviour and complex communication (MOYNIHAN & RODANICHE 1982; MOYNIHAN 1985; HANLON & FORSYTHE as cited in HANLON & MESSENGER 1996); however our small amount of evidence does not support such hypotheses.

Sentinels often incur the cost of an increased risk of predation. Currently, we have no evidence for individual recognition (BOAL 1996), many squids lay their eggs together and hatchlings are planktonic (BOYLE 1987) so are unlikely to remain with kin, and indirect evidence from this study does not support shoal fidelity. Until we have evidence for at least one of these three, hypotheses for altruistic social behaviour are unlikely (PITCHER & PARRISH 1993). Alternate explanations for the responsiveness of central animals to the alarm of peripheral animals have been proposed for fish schools (e.g. the Trafalgar effect, WEBB 1980) and merit consideration for squid schools.

There is no single criterion for what constitutes 'complex communication', but it usually implies, at the very least, that signals are used with some degree of context sensitivity. Communication that indicates information solely about the internal state of the sender is not normally considered complex. Accentuated Gonads and Spread Arms were shown primarily by the more sexually active squids, and were used at least sometimes by both males and females interacting with both males and females. Accentuated Gonads was sometimes shown when no other animal was near by. According to this preliminary information, these signals do not meet the basic criteria for complex communication. They do suggest that an examination of the role of hormones in cephalopod reproductive behaviour and body patterning could prove fruitful.

Conclusions

Sepioteuthis lessoniana schools and shoals in ways that are similar to many schooling fishes, including nearest-neighbour distances of between one and two body lengths, size-based segregation, and absence of preferential associations or group fidelity. Our results support previous studies finding convergences in behaviour between the schooling fishes and squids (PACKARD 1972; but see O'DOR & WEBBER 1986).

The behaviour of different individuals within the shoal varied markedly, with a minority of squids participating in most of the social interactions. Three hypotheses based upon observations of untagged individuals were not supported: 1. Preferential associations between particular pairs of squids were not found; 2. Males did not reliably discriminate on the basis of sex; and 3. The two most obvious body patterns used in social interactions were not used exclusively by one sex. These results leave us with more questions than answers. We wish for more information about the sequences of behaviours within particular social encounters, for example. We trust that our understanding of squid behaviour will develop at a more rapid pace as more studies of tagged individuals are conducted.

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