The Azorean squid *Loigol forbesi* (Cephalopoda: Loliginidae) in captivity: reproductive behaviour

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Abstract

Reproductive behaviour of *L. forbesi* in captivity is described. Male-male agonistic interactions increased when a female is introduced between males. Mate pair formation is quickly established with a consequent hierarchy of dominance between males. During such behaviour the male constrained the female against the tank wall, isolating her from the other male present. Exchange of the partners involved in courtship was few times observed. A chromatic pattern associated with courtship was displayed continuously by the dominant male. Three females laid eggs on the tank PVC walls. One of them survived 12 days after spawning. The reproductive organs of post-spawning females had mature, maturing and immature oocyte. Spawning behaviour and a brief description of the spawn is presented. An update of the components of behaviour is provided.

Introduction

Squid are schooling social invertebrates with a high developed system of communication and camouflage. They are able to exhibit many ritualised behavioural patterns that can be described as a combination of chromatic, postural and movement components (Packard & Sanders, 1971; Packard and Hochberg, 1977; Hanlon, 1982). Chromatic components, made up by selective nervous excitation of units of chromatophores and iridophores (Packard, 1982) are the core of the visual communication among cephalopods. Body patterns are associated with specific behaviour as feeding, predator avoidance, courtship, etc. and they can alter during the ontogeny of one species (e.g., Hanlon & Woltering, 1989). The accurate description of the components of patterning is required for several biological approaches as quantitative ethology, behavioural taxonomy and phylogenetic analysis (see Hanlon, 1983 and Hanlon et al. 1994). However, the description of the behaviour is somewhat complicate due to the multiplicity and ambivalence of the signals combined with the dependence of their expression on external environment (Moynihan & Rodaniche, 1982). Obviously, observations in the field are the ideal when this is possible, but when squid grounds are unknown or not accessible, studies in captivity are an alternative. Also, the proximity to the squid and the confined space can turn some behavioural patterns conspicuous.

Octopods and sepiids were the cephalopods preferred for behavioural studies, both *in situ* and in laboratory. Nevertheless, the behaviours of some myopsid were extensively investigated (in field e.g., Moynihan & Rodaniche, 1982; Sauer & Smale, 1993; Hanlon et al. 1994 and in captivity e.g., Hanlon 1982, Arnold, 1990). *Loigol forbesi* is the only loliginid species living around the Azores islands. It inhabits inshore water from the surface. at night.
down to 400 meters during daytime. A daylight squid jigging fishery was known since the last century (Drouet, 1858). Mainly maturing and mature squid are caught (Martins, 1982). However, the spawning grounds of this population and spawning migrations have never been detected. Natural laid eggs have been found only once (Porteiro & Martins, 1982) in the Azores. The Azorean L. forbesi proved to be efficient for culture experiments, at least for maintenance and rearing (sensus Boletzky & Hanlon, 1983). Squid embryos (laid by captive females) were transported to Marine Biomedical Institute, Texas and two successful rearing trials were carried out (Forsythe et al., 1989; Hanlon et al., 1989). During maintenance preliminary studies of adult squid behaviour were conducted (Porteiro et al., 1990). There, 17 chromatic, 6 postural and 5 movement components were described as well as feeding and agonistic behaviour. However, reproductive behaviour was not extensively emphasised. Presently, we have a closed sea-water system designed specifically for squid culture (see Gonçalves et al., 1995) which allow to maintain simultaneously a significant number of large squid. In this study we present additional qualitative information of the behaviour of captive squid mainly related with courtship and spawning.

Material and Methods

Several squid were maintained during two sets of experiments (16 October to 27 December 1994 and 2 February to 18 April). The squid size ranged was between 275 to 659 mm DML. Squid were caught by jigging, transported ashore and kept in a close sea water system. Basically this arrangement consist of three circular tanks (3.6 m Ø and 0.90 m high; ca 3000 l of natural sea water) painted with vertical dark stripes on the walls and ca. 4cm of gravel on bottom. Light was kept at dim during all the experiments. temperature was recorded daily and several water parameters controlled regularly (see Gonçalves et al. 1995, for a complete description of the system). The number of squid inside each tank was variable (1 to 7) and different combinations of sexes were maintained (e.g., 1 female and two males, 2 female and 2 males, 1 female and 4 males, etc.). Individual squid were distinguished through natural skin marks or artificial tags.

Observations were performed during day time, from the top of the tanks and lasted from 10 minutes to ca. 2 hours. While observing, the black plastic cover was partially elevated and the exterior light held at minimum to avoid major disturbance. The water influx was also suspended. Squid were fed once or twice a day. Trachurus picturatus, Pagellus bogaraveo and Scomber japonicus were the main food items. Feeding took place in the morning and at noon.

The hierarchical organisation of behaviour as described by Packard & Sanders (1971), Packard & Hochberg (1977), and Hanlon (1982) and the behavioural components terminology previously established for loliginids (Hanlon, 1982, 1988; Porteiro et al., 1990; Sauer et al., 1992, 1993; Hanlon et al., 1994) were adopted.

Results

An average of 7.6 squid were maintained simultaneously in captivity and the average survival was 17.7 days (up to 46 days) in the first experiment. During the second trial the average survival was longer (22.8 days) mainly due to the preferential maintenance of immature, maturing squid and sexual segregation in some tanks.

Behaviour

Male-male interactions

Male-male agonistic behaviour was observed many times. In the tanks a hierarchy of dominance was established. Normally, the weaker male die with major damaged areas after few days in captivity.
and successively all squid but the dominant male die. The most obvious damaged areas were usually the same: the latero anterior part of the mantle, funnel and head. Injuries on the fins and on the posterior tip of mantle were caused by abrasion and collision on the tank walls, respectively. Lesions on the anterior dorsal tip of the mantle were commonly observed but could not be related with any type of contact. Agonistic interactions occurred between male of markedly different sizes are maintained together and between males of the same size or of different size when competing for reproductive dominance.

Fig. 1 - Dorsal view of courtship body pattern of adult male squid *Loligo forbesi* in captivity. Composed by the chromatic components, *White arm tip, Accentuated testis* and *Accentuated digestive gland.*

All the movement components previous described for the species (*Parallel positioning*, *Fin beating*, *Forward rush*, *Chase* and *Flee*) were observed. Some variation on *Forward rush* was recorded: the aggressor charges the subordinate male by jetting against him the tentacles resulting often in contact. This component was preceded by *Chase* (by the dominant male) and culminate with *Flee* (by the subordinate male). *All dark* is the common body pattern in these interactions (*Dark flashing* was also observed several times).

*Perpendicular positioning*, a new movement component, was recorded during low stress situations. This is characterised by the presence of several males close together moving on different vertical levels maintaining between them perpendicular positions. The chronic chromatic pattern was predominantly observed during such behaviour (*Clear. Dorsal stripe*).

Male-female courtship behaviour (mate pair formation)

Mature male-female interactive behaviour expressed by captive squid was characterised by *Pair formation*. When a female was in a tank with males, one of the latter would shortly afterwards change his behaviour. The male exhibits his dominance isolating the female from the other male squid. He constrained her against the tank wall limiting her swimming space. A short distance was maintained continuously between the two and prolonged fin to fin contact occurred. A circular motion around the tank wall was prevalent, in opposition to the normal forward backward radial trajectory. It was always the male who enforced the *pair formation* behaviour. However, the preservation of the couple depends of the male aptitude to avoid the evasive action of the female (unhealthy males are unable to maintain a pair with a health female). Sometimes male sexual dominance exchange was observed (*i.e.*, the dominant male is replaced by another). Also, the dominant male may change his partner preference (observed when a new female was introduced in the tank). Most of the pairs were stable for several days.

*Jockeying* and *Parrying* (*sensus* Hanlon et al. 1994) were observed several times and often culminated in violent male-male
agonistic behaviour (see above). Stress also increased in the tank also when a male interfered with the trajectory of the pair. Subordinate squid tend to avoid such.

interactions which is difficult given the confined space of the tanks. The courtship behaviour is characterised by a specific chromatic pattern. That pattern is more obvious in the male. At least, three dorsal chromatic components are expressed to form the Courtship body pattern (Fig. 1): Accentuated testis, Accentuated digestive gland and White arm tips. Strong green iridescence on the dorsal mantle areas was exhibited. During all watching periods the dominant male frequently showed this pattern (it can persist during a long time). However, it was more conspicuous when another male was in the proximity of the pair. The female display was of a less obvious chromatic pattern, but also some distinct components were consistently present (e.g., Dorsal stripe, Mantle spots, Dark fin line). White arm tips (as in males), Accentuated ovary, Accentuated nidamental glands and Asymmetric ocular spot (on the male side) were also observed during more intense interactions. Asymmetry of the chromatic components was shown (stronger on the adjacent squid side) by the two partners.

Spawning behaviour

Three spawns were laid by captive females during the period of observation (Table1.). All squid initiated spawning during afternoon and continued through the night. Two of the females (A1, B2) that started egg-laying, finished one day later and then died. One female (B1) lived for 12 days after spawning. During that period she ate 4 small meals (average daily feeding rate of 2.1% against 6.5% before spawning). The female A1 had paired with a dominant male (two male squid were in the tank) and B1 formed a stable pair with a sole male in the tank. The squid B2 were all the time alone.

Since light seemed to influence behaviour of the squid, observations of the spawning process were greatly limited by the dark condition in the tank. However, some postural and movement components were observed. The female approached the egg mass (previously laid) with conic arms and when in contact with them she splayed the arm tips and attached a new strand (Egg holding). This activity took around 20 seconds or less. During this operation the squid undulated the fins vigorously in a forward movement. The body was kept parallel to the substratum.

Figure 2. Top plan of the tank and detail of the area selected by squid to lay eggs (see text).
Frequently, the female swam fast to the egg strands handled them and jetted water violently toward the spawn in backward movement. This behaviour was observed many times. Male also made such behaviour, but less frequently. The male was always swimming close to the female, showing the courtship body pattern with high iridescence (Mate guarding).

At least the first strands laid by the squid were fixed close to the surface on the tank wall, over a PVC liner, (fig.2). The squid B2 laid eggs on the same place where the previous strands were laid by B1. Water flow was at a maximum at spawn location (oxygen level was close to saturation uniformly in the tanks). Later some capsules were disperse and others were in a bunch, throughout the gravel bottom. Few of them were cemented on the basalt gravel. It was not clear to what extent these strings were displaced from the PVC wall by the jetting and manipulation behaviour of the female.

Table 1. Reference of L. forbesi female squid that spawn in captivity. DML i - Dorsal mantle length when captured; BW i - Total body weight when captured; OVW f - Ovary weight after spawning; ODPW f - Proximal oviduct weight, after spawning.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Date of capture</th>
<th>Days captive before spawning</th>
<th>Days from spawning to dead</th>
<th>DML i (mm)</th>
<th>BW i (g)</th>
<th>OVW f (g)</th>
<th>ODPW f (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>05.11.94</td>
<td>16</td>
<td>1</td>
<td>31</td>
<td>993.0</td>
<td>33.7</td>
<td>55.4</td>
</tr>
<tr>
<td>B1</td>
<td>09.02.95</td>
<td>8</td>
<td>12</td>
<td>330</td>
<td>885.0</td>
<td>16.8</td>
<td>6.0</td>
</tr>
<tr>
<td>B2</td>
<td>03.03.95</td>
<td>7</td>
<td>1</td>
<td>279</td>
<td>607.9</td>
<td>56.9</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Reproductive organs of post-spawning females**

Post-spawning females still had mature, maturing and immature eggs in the proximal oviduct and ovary (see Table 1). All had mated but few sperm were in the bursa copulatrix. The ovary of the female B2 had a high quantity of oocyte close to maturation but less few eggs in the oviducal complex. The female B1 had a few hundreds mature eggs in the oviduct and oocyte in various developmental stages in the ovary.

**Discussion**

Agonistic behaviour between males was the main reason for mortality both by collision and abrasion on the tank walls or by direct charge. This behaviour was observed in several species kept in captivity but bodily contact has only been rarely observed (see Arnold, 1990; Hanlon et al. 1983). Probably, the confined space in the tanks combined with the big size of the squid aggravate agonistic interactions compared to animals in the wild. In the wild the
subordinate squid can run away before being touched during an attack. These interactions are more evident when different sizes male squid are maintained together or when males compete for females (see Hanlon, 1990). No cannibalism was recorded probably due to small differences between the size of captive squid.

Pair formation was observed for the first time for *L. forbesi*. However, it has been described for several other squid held in captivity (e.g., *L. opalescens* by Hurley, 1977; *L. pealei* by Arnold, 1990; *L. (D.) plet* by Hanlon, 1981) as well as in the natural spawning grounds (e.g., *Sepioteuthis sepioidea* by Moynihan & Rodaniche, 1982; *L. v. reynaudii* by Sauer & Smale, 1993). During our observations, captive pairs seem to be quite stable and male dominant, except when a weak male is not able to contain the evasive behaviour of the female. The latter behaviour can support the assumption by Hanlon (1994) that female choice and evaluation of the partner is an important reproductive strategy between squid. However, male dominance has been described by several authors based on the displacement of an intruder male by the paired male and the maintenance of the original pair. Pair stability on field is not known but it probably split during night (see Sauer & Smale, 1993). The long duration of the pairs observed in this study can be an artefact due to the captive condition.

Although, we did not observe copulation, we know that sperm is stored only in the *bursa copulatrix* (Porteiro & Martins, 1994) which indicate that just head-to-head mating occurs. Very little sperm was found in the *bursa copulatrix* of post-spawning females. This suggests that the amount of sperm in this organ is not enough for fertilisation if a prolonged spawning period occur or if one intensive spawning is made. We agree with Hanlon (1994) that squid reproductive tactics would be better understood if analysed in a sperm competition contest. The existence of small mature ("sneaker") males within this species (Martins, 1982; Boyle & Pierce, 1994) as well as *Jockeying* and *Parrying* movement components can indicate an alternative reproductive strategy, as observed for *L. v. reynaudii* (Hanlon et al. 1994). The mechanisms of sperm competition are not known for the majority of cephalopods.

Arnold (1990) suggest that *L. pealei pair formation* and consequently reproductive dominance by the male is established by a visual stimulus (i.e., the presence of egg masses in the aquarium). Hurley (1977) found some evidence that egg deposition is visually stimulated by the presence of previous spawns but he could not relate the male reproductive dominance to presence of eggs. In our case pair formation occurred without any visual external stimulus, as it was established as soon as a female was introduced among males.

Male chromatic components related to reproductive dominance behaviour have not been described previously for this species. *White arm tips* could be associated to the importance of the arms in mating and egg mass handling. The *Accentuated testis* is a component very common among loliginids and interpreted as a sign of mature condition. The component *Accentuated digestive gland* is described here for the first time and it could be a sign related to the condition of the squid, since this organ is used as storage reserve. Similar courtship body pattern is not found in the literature.

Is accepted that acute body patterns (expressed during few seconds or minutes) are more useful for behavioural taxonomy and phylogenetic approaches than chronic patterns (which can be displayed during long periods). However, courtship body pattern (as a chronic pattern) is stereotyped, species-specific and one which provide more information about the species characteristics (Hanlon, 1988). It would have ethological interest to compare the behaviour between Azorean and European populations of *L. forbesi* since they are genetically separated (Brierley et al. 1993; Pierce et al., 1994) and body patterns can be used probably as a character which shows variations at the population or sub-
species level (Hanlon, 1988; Hanlon et al. 1994). One example of different reproductive behaviour between these two populations is show in the number of eggs within each strand. Lum-Kong (1993), reported a mean value of 109 eggs per string against less than 90 found by us in several spawns. Also, the percentage of mated immature females in Azores seems to be higher than L. forbesi from elsewhere (Porteiro & Martins, 1994). In order to provide basic information for further ethological comparison it is presented an update of body components recorded for the Azorean population of L. forbesi (Table 3).

Table 3. Update of the behavioural components recorded for Loligo forbesi (based on Hanlon, 1988; Porteiro et al., 1990; this study; and unpublished observations).

Chromatic components:

<table>
<thead>
<tr>
<th>Dark components:</th>
<th>Light components:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. All dark</td>
<td>1. Clear</td>
</tr>
<tr>
<td>2. Lateral mantle streaks</td>
<td>2. Dorsal mantle collar iridophores</td>
</tr>
<tr>
<td>3. Ring</td>
<td>3. Dorsal iridophores splotches</td>
</tr>
<tr>
<td>4. Fin and mantle spots</td>
<td>4. Gold iridescent sheen</td>
</tr>
<tr>
<td>5. Infraocular spot</td>
<td>5. Accentuated testis</td>
</tr>
<tr>
<td>6. Dark first or third arms</td>
<td>6. Accentuated nidamental gland</td>
</tr>
<tr>
<td>7. Dark arms</td>
<td>7. Accentuated ovary</td>
</tr>
<tr>
<td>8. Mid-ventral ridge</td>
<td>8. Accentuated digestive gland</td>
</tr>
<tr>
<td>10. Dark fin line</td>
<td></td>
</tr>
<tr>
<td>11. Mantle margin stripe</td>
<td></td>
</tr>
<tr>
<td>12. Dorsal stripe</td>
<td></td>
</tr>
<tr>
<td>13. Shaded eye</td>
<td></td>
</tr>
</tbody>
</table>

Postural components:

<table>
<thead>
<tr>
<th>Movement components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Parallel positioning</td>
</tr>
<tr>
<td>2. Fin beating</td>
</tr>
<tr>
<td>3. Forward rush</td>
</tr>
<tr>
<td>4. Chase</td>
</tr>
<tr>
<td>5. Flee</td>
</tr>
<tr>
<td>6. Inking</td>
</tr>
<tr>
<td>7. Jetting</td>
</tr>
<tr>
<td>8. Jocking and Parrying</td>
</tr>
<tr>
<td>9. Mate guarding</td>
</tr>
</tbody>
</table>

Fresh laid egg strings were similar to those described for L. pealei by Roper (1965) (long and soft). The duration of softness of the string tunics can presumably be related with the fertilisation process. This mechanism remains to be studied. Variation in the way that squid make their spawning bed is known (see Vechicne, 1988; Sauer et al. 1992). From our observations it is difficult to say how this species makes their spawns in nature. The location where the squid fix their eggs in the tanks is probably related to the water current or to the existence of a PVC fold (see Fig.2). The later supposition, if correct, can suggest that crevices or small caves are used for spawning as discussed before by Porteiro & Martins (1992). L.
vulgatis in Madeira spawn in such bottom structures (P. Wirtz, pers. comm.)
Death of captive squid after spawning has been recorded by several studies and
accepted as a natural behaviour. The presence of mature, maturing and
immature eggs in the reproductive system of post-spawning females and the survival
of one female for several days (12) after egg-laying could support the assumption
of a prolonged intermittent spawning
before exhaustion. Several questions arise
when this problem is examined: why does
the squid die before all the eggs are laid? is
this ecologically justified? have the wild
female the same behaviour or is it
influenced by captive condition? does she
need a high food input after spawning one
batch of eggs? This should be investigated
carefully to establish the reproductive
behaviour of this loliginid.

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References
genetic investigation of population structure of Loligo forbesi Steenstrup. 1856 from British Isles
and the Azores. Pp, 61-69, in: Recent Advances in Cephalopod Fisheries Biology, T. Okutani,
Drouet, H. 1858. Mollusques marins des Iles Açores. Mémoires de la Société, Académique de l'Aube,
(Cephalopoda: Loliginidae) in captivity: maintenance and transport. International Council for the
Exploitation of the Sea, C.M. 1995/F:14 Ref. K
International Society of Invertebrate Reproduction.1 p.
Hanlon, R. T., 1982. The functional organization of chromatophores and iridescant cells in the body
Hanlon, R. T., 1988. Behavioral and body patterning characters useful in taxonomy and field
influences of sperm competition behavior. Programme and Abstracts of the Napoli CIAC
Symposium "The Behaviour and Natural History of Cephalopods" (Vico Equense, 5-11 June,


