MATING BEHAVIOUR OF THE ATLANTIC BOBTAIL SQUID *SEPIOLA ATLANTICA* (CEPHALOPODA: SEPIOLIDAE)

M. RODRIGUES[1], M. E.GARCI[2], A. GUERRA[2] & J. S. TRONCOSO[1]*

[1]Departamento de Ecología y Biología Animal, Universidad de Vigo and Estación de Ciencias Marinas de Toralla (ECIMAT - UVIGO), 36331, Vigo, Pontevedra. Spain.

[2]Instituto de Investigaciones Marinas (CSIC, Vigo).

**Corresponding author: marcelorodrigues@uvigo.es*

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ABSTRACT. — The mating behavioural pattern of the bobtail squid (*Sepiola atlantica*) was observed and described for the first time in laboratory conditions. A total of five matings were recorded. No type of courtship was noted in any of the mating events. In all the cases, the male moved quickly toward the female holding her with his arms by the middle of the ventral region of the mantle. The male, situated below the female, introduced his pair of dorsal arms (the left dorsal arm is hectocotylized and passes the spermatophores) in the mantle cavity of the female, while grasping her by the ventral region with his laterodorsal pair of arms and by the neck with his lateroventral pair, sometimes introducing it into the female's mantle cavity. The male showed the same pattern of coloration during all the entire mating process, whereas the female changed slowly and successively her chromatic pattern. The duration of the mating varied between 68 and 80 minutes. A comparison of the mating pattern of this species with other species of the family is described.

INTRODUCTION

Mating in all cephalopods species consists of the male passing spermatophores to the female. The mating position, the mode of transfer of spermatophores by the male to the female and chromatic changes varies significant from species to species (Mangold 1987, Hanlon & Messenger 1996). Knowledge of the mating behaviour in members of the family Sepiolidae is scarce, perhaps due to the nocturnal activity habits of many members of this family (Hanlon & Messenger 1996). Studying individual animals in small tanks has its limitations for investigations of intraspecific relationships, both for the difficulty of reproducing natural conditions in the laboratory, hindering us from determining the "normal" mating behaviour (Hanlon *et al.* 1997), and also for within-species differences, like those found in *Octopus joubini* (Mather 1978).

In the bobtail squid, copulation was first reported by Racovitza (1894) for *Sepiola rondeletii* and *Rossia macrosoma*. Later on it was described for other species of *Sepiola, Sepietta* and *Euprymna* (Mangold-Wirz 1963, Bergström & Summers 1983, Boletzky 1983, Moynihan 1983, Singley 1983, Hanlon *et al*. 1997, Nabhitabhata *et al*. 2005) but never for *Sepiola atlantica* (d'Orbigny, 1839-1842). According to these authors, mating in sepiolids is rather violent, is completed in a short period of time, and without an initial courtship. However, Norman (2000) suggested the existence of courtship behaviour in wild *Euprymna tasmanica*.

The copulatory position observed in species of the genera *Euprymna* and *Sepiola*, "male to female neck", seems be a shared strategy in sepiolids (Moynihan 1983, Hanlon & Messenger 1996, Nabhitabhata *et al*. 2005), perhaps linked to the position of the hectocotylus and the arrangement of the bursa copulatrix (Norman 2000, Hoving *et al*. 2008).

Until now, within-species differences in the way of copulating observed in other cephalopod groups (Mather 1978, Hanlon & Messenger 1996 for a review) have not been noticed in any sepiolid species where this behaviour has been studied, at least under laboratory conditions.

The duration of mating in sepiolids varies greatly. For example, Moynihan (1983), Singley (1983) and Hanlon *et al*. (1997) reported 25 minutes, 45-80 minutes and between 30-50 minutes for *Euprymna scolopes* respectively. Racovitza (1894) reported 8 minutes in *S. rondeletii*, which is the only known mating time for this genus.

Cephalopods have highly variable and complex life history traits related to reproduction (Hanlon & Messenger 1996). Knowledge about the process and behaviour of bobtail squid species, with comparisons to other studied species, provides information to understand the evolution of mating behaviour. Biology and ecology of the small bobtail squid *S. atlantica* is poorly known, and its reproductive behaviour has not been investigated (Guerra 1986, 1992, Yau & Boyle 1996, Reid & Jereb 2005). The aim of the present study was to describe for the first time the mating pattern for this species.

MATERIAL AND METHODS

In March 2007, ten adult specimens were obtained in two dives from the Ría of Vigo (NW Iberian Peninsula): eight individuals off Area Milla (42º14` N; 8º47` W) and two individuals off Toralla Island (42º 12` N; 8º 47` W). Depth of collection ranged from 5 to 6 meters and the seabed was sandy without seaweed.

The specimens were transported to the Marine Station of the University of Vigo in Toralla Island Each individual was placed in a 10.2 litre tank (30 cm long x 18 cm wide x 19 cm deep) with an open sea water circulation system. Afterward acclimation, specimens were coupled together and transferred to 20.2 litre tanks (30 cm long x 27 cm wide x 25 cm deep).circulation on the same open sea water system.Water temperature ranged between 14-

17ºC. The system received natural photoperiod of 12:12 LD. The bottom of the tank were covered with a 2-3 cm layer of fine sand taken from the same location where the specimens were collected. Squids were fed daily *ad libtum* with mysid shrimp *Siriella armata*.

The five couples were formed after consideration of small differences observed between sexes, mainly the relative size and position and coloration of the gonad when the chromatophores are contracted, which can be observed through the musculature of the mantle. In females, the gonad is translucent orange and visible in the half dorsal area of mantle, whereas the male gonad is smaller and opaque-white in the dorsal posterior region of mantle. In addition, the posterior area of the female's mantle is broadly rounded while it is more angular in the male (adapted from Singley 1983). The mating process was photographed (Nikon D200 camera, lens Mikkor 60 mm), and filmed (Sony PD-170 with increase, 4x). The duration of the mating was defined as the time passed from the first moment in which the male and the female were contacted until they separated. During observations, human interaction was minimized through the use of visual barriers on the experimental system.

After mating occurred, the specimens were separated and maintained in captivity until their natural death, which occurred within a period of 2 to 30 days. The sex was confirmed and each specimen was measured and weighted post-mortem

RESULTS

Table I, shows measurements and sex of the ten specimens used in the study. All males showed the hectocotylus on the dorsal left arm. All females showed a bursa copulatrix within the mantle cavity on their left region.

The copulatory position "male to female neck", was consistent in the five mating events observed. The subsequent response of the male specimens was immediate, during all five mating episodes in the laboratory, four occured during daylight and the one at night. No type of courtship was noted in any of the mating events.

In all the cases, mating seemed to be initiated by the male. The male left the bottom of the tank and moved quickly toward the female. When approached, the female showed a general pale and translucent colouration, having only small points (non expanded chromatophores) dark-brown in colour, while the male showed a cream-yellowish background with strong dark coloration produced by a mosaic of expanded brown chromatophores. The male initially grasped the female using all arms on the middle of the ventral region of the mantle (Fig. 1a), then shifted his grasp to the female's neck. During the process, the male showed a different chromatic pattern than the female, with the chromatophores being more expanded than those of the female, which showed a general pale colour (Fig. 1a). As a general rule, it can be said that the male kept same colour during all the mating process, whereas the female slowly and successively changed her chromatophoric pattern.

The male then pulled the female down to the bottom of the tank, where copulation took place. The male, situated below the female and on the bottom of the tank, introduced his dorsal arms carrying spermatophores in the mantle cavity of the female, while grasping her by the ventral region with his laterodorsal pair of arms and by the neck with his lateroventral pair, introducing it sometimes in the mantle cavity of the female. During this process the female remained with her arms placed on the bottom of the tank while the apex of her body was directed up and elevated from the bottom (Fig. 1c). The ventral arms were not used during mating. During the passing of the spermatophores, a similar pattern of dark colouration was observed in both sexes (Fig. 1b, c) with both specimens lying down on the bottom (Fig. 1c, d). However, in the female, this colour pattern changed,and she was observed with contracted chromatophores on her head, prominent iridophors (green metallic colour) around the eye-balls and the arms with a pink background where some red non expanded chromatophores are visible (Fig. 1e).

The male was situated in a horizontal position at the bottom before they become separated. During this phase, the female maintained her vertical position, with the top of the mantle towards the surface of the tank, still being griped by the male's arms (Fig. 1f). The female displayed a general white colouration with very few expanded chromatophores, while the pattern colour of the male did not change significantly in relation to previous phases (Fig. 1g).

The mean time duration of the mating was 73.8 minutes ± 4.60 (Table I). After the couple separated, if the male tried to approach the female again and she swam away from him.

DISCUSSION

Males of *Euprymna hyllebergi* always started the mating leaving out the bottom as observed in *S. atlantica* (Nabhitabhata *et al*. 2005, present paper). Nabhitabhata *et al*. (2005) also observed that *E. hyllebergi* males always held females using laterodorsal, lateroventral and ventral arms. However, in our observations *S. atlantica* ventral arms were only used in the first moments of grasping the female and not during the mating. Stages of mating behaviour in *E. hyllebergi* were divided by the authors in five stages: a) female hovers by, male attention; b) male approaches female from below; c) male grasps female by mantle; d) male-grasp moves to female's neck; e) male pulls female down to substrate (Nabhitabhata *et al.* 2005). These five stages also apply in *S. atlantica*. However, as mentioned above, mating in *S. atlantica* was different than in *E*. *hyllebergi* because the ventral arms of former species were not functional in the mating act.

The only evidences of courtship in the family Sepiolidae were recorded by Norman (2000) in wild *E. tasmanica*. The lack of female courtship behaviour found in the present study could be a function of the limited space for females to ‘choose’ their partners due to relatively small size of the experimental tanks, as observed in *Sepia officinalis* (Adamo *et al*. 2000). However, absence of courtship, copulatory position, duration of mating and spermatophore placement of *S. atlantica* matches with the observed in the sepiolid *E. scolopes* (Moynihan 1983, Singley 1983, Hanlon *et al*. 1997). The mating pattern also coincides with that observed in *S. rondeletii* (Racovitza 1894), *Sepiola robusta* (Boletzky 1983) and *E. hyllebergi* (Nabhitabhata *et al*. 2005) except for the mating duration, which was much longer in *S. atlantica* than in the other three species (68-80 minutes *versus* 7-10 minutes). *R. macrosoma* also showed a male to female neck position during mating (Mangold-Wirz 1963, Racovitza 1894). A more detailed comparison with *S. atlantica* is not possible because of the lack of information on mating duration and spermatophore placement in *R. macrosoma*. Instead of a male to female neck arrangement during mating, *Rossia pacifica* showed a male parallel position (Brocco 1971, Summers 1985). Additionally, in the other sepiolid on which mating is known, *Sepietta oweniana*, the mating position was “head to head” and its duration was shorter than in *S. atlantica* (Bergstrom & Summers 1983, present paper).

Nowadays, there is still little evidence for sperm competition in bobtail squids although some observations strongly support its occurrence. Thus, the long mating times observed in *E. scolopes* and in *S. atlantica* combined with the presence of an internal seminal receptacle, strongly suggest the possibility of sperm competition behaviour among males (Hanlon *et al*. 1997, present paper). The short duration of mating observed in several species of bobtail squids seems to be in disagreement with the existence of sperm competition that seems to need one mating of long duration. Nevertheless, sperm competition is possible even when copulation duration is short; and the long mating duration could be linked with other process like mating guarding or capacity sperm transfer (Linn *et al*. 2007).

Moynihan (1983) is the only paper that describes the chromatic changes that occur during mating in the family *Sepiolidae* and looks at the Hawaiian bobtail squid *E. scolopes*. Based only on the coloration pattern of *Sepia latimanus* during mating, this author inferred that the *E. scolopes* individual at the bottom was the female, while the one located on top was the male. However, this assumption was never investigated further. According to this author, the first individual showed a fine reticulation of dark brown marks on a cream or light yellow background, throughout the whole of the copulation and even beyond. The one on top slowly and successively changed chromatic pattern. Considering these descriptions, the observation on the sex of the mating specimens of *E. scolopes* by Moynihan (1983) is wrong as showed by Singley (1983) and Hanlon *et al*. (1997), where the mating position of both male and female of the Hawaiian bobtail squid and the Atlantic bobtail squid is the same ('male to female neck'), and the chromatic pattern similar. Nabhitabhata *et al*. (2005) reported the male colour pattern during mating in *E. hyllebergi*, was a dark brown colour, while the female showed a pale brown colouration during the mating bouts.

Due to possibility of sperm storage of a previous mating (Boletzky 1983, Hanlon & Messenger 1996) we can not be certain that the spermatophores found in the bursa copulatrix were transferred by male to the female in the mating bouts, However it was noted that males transfer spermatophores with the hectocotylized arm, and hence, genuine mating occurred in all cases.

In conclusion, the information we obtained allow us described for the first time the mating behaviour of *S. atlantica.* In general, our observations coincide with other sepiolid species, and consisted in five stages. Each stage triggered a display of colour change unique to this species. However, further data are needed to make conclusions about the function of the long mating duration observed in *S. atlantica*.

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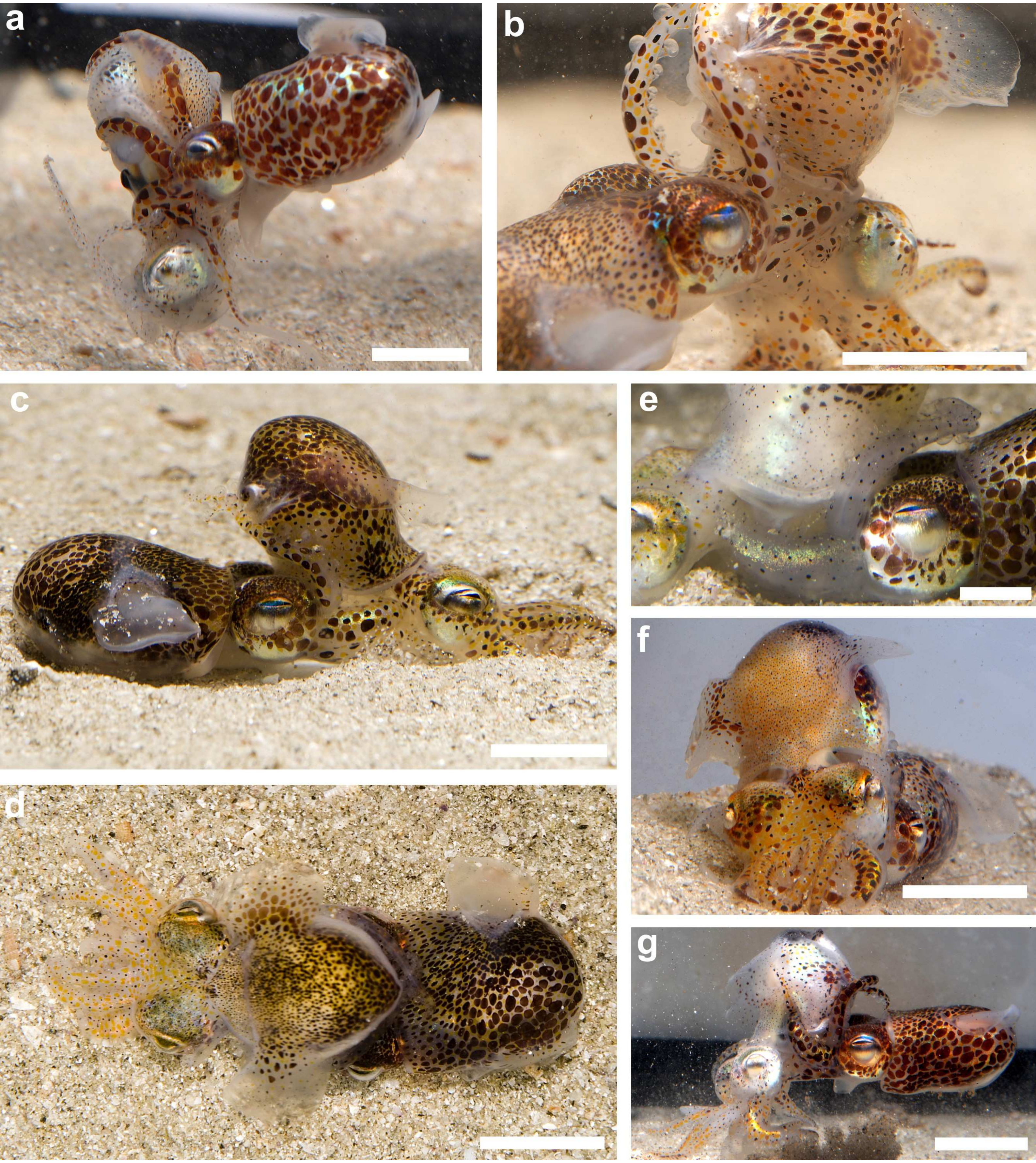
Table I. — *Sepiola atlantica.* Size of the ten specimens used in the study. Mantle dorsal length (ML) mm. Total length (TL) mm. Total Body Weight (BW) g. and Duration in minutes.

Couples	**Female**			**Male**			**Mating bouts**
	ML	TL	BW	ML	TL	BW	Duration
1	14.1	33.6	1.67	13.5	36.2	1.25	68
2	16.7	40.7	1.94	14.3	38.0	1.83	76
3	17.7	41.5	2.03	14.5	38.7	2.19	80
4	15.2	39.4	1.52	12.9	33.6	2.03	74
5	14.5	36.2	1.84	14.7	35.8	1.96	71

FIGURE CAPTION

Fig. 1. — Copulation pattern in *Sepiola atlantica*: a, The male moves quickly toward the female, then holds her with the arms by the middle of the ventral region of the mantle. b, The male, situated below of the female, introduces his pair of dorsal arms in the mantle cavity of the female. c, d, The male acquired a darker colour pattern than the female one during the fertilization process. e, The male has a constant dark colour pattern affecting the whole body; the female showing her head with contracted chromatophores and quite visible iridophors (green metallic colour) around the eye-balls and the arms with a pink background. f, Before separating, the male is in a horizontal position at the bottom, while the female maintains her vertical position with the top of the mantle towards the surface of the tank. She remains gripped by the male’s arms. g, The female displays a white colouration before they separate. Scale bars: a, b, c, d, f, g 10 mm; e 5mm.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