

1 **MATING BEHAVIOUR OF THE ATLANTIC BOBTAIL SQUID**

2 ***SEPIOLA ATLANTICA* (CEPHALOPODA: SEPIOLIDAE)**

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11 Running title: Mating behaviour of *Sepiola atlantica*

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

26 **INTRODUCTION**

27

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

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

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

49 Until now, within-species differences in the way of copulating observed in other
50 cephalopod groups (Mather 1978, Hanlon & Messenger 1996 for a review) have not been

51 noticed in any sepiolid species where this behaviour has been studied, at least under laboratory
52 conditions.

53 The duration of mating in sepiolids varies greatly. For example, Moynihan (1983),
54 Singley (1983) and Hanlon *et al.* (1997) reported 25 minutes, 45-80 minutes and between 30-
55 50 minutes for *Euprymna scolopes* respectively. Racovitza (1894) reported 8 minutes in *S.*
56 *rondeletii*, which is the only known mating time for this genus.

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

64

65 MATERIAL AND METHODS

66

67 In March 2007, ten adult specimens were obtained in two dives from the Ría of Vigo
68 (NW Iberian Peninsula): eight individuals off Area Milla (42°14` N; 8°47` W) and two
69 individuals off Toralla Island (42° 12` N; 8° 47` W). Depth of collection ranged from 5 to 6
70 meters and the seabed was sandy without seaweed.

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

76 17°C. The system received natural photoperiod of 12:12 LD. The bottom of the tank were
77 covered with a 2-3 cm layer of fine sand taken from the same location where the specimens
78 were collected. Squids were fed daily *ad libitum* with mysid shrimp *Siriella armata*.

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

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

93

94 **RESULTS**

95

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

99 The copulatory position “male to female neck”, was consistent in the five mating events
100 observed. The subsequent response of the male specimens was immediate, during all five

101 mating episodes in the laboratory, four occurred during daylight and the one at night. No type of
102 courtship was noted in any of the mating events.

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

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

125 and the arms with a pink background where some red non expanded chromatophores are
126 visible (Fig. 1e).

127 The male was situated in a horizontal position at the bottom before they become
128 separated. During this phase, the female maintained her vertical position, with the top of the
129 mantle towards the surface of the tank, still being griped by the male's arms (Fig. 1f). The
130 female displayed a general white colouration with very few expanded chromatophores, while
131 the pattern colour of the male did not change significantly in relation to previous phases (Fig.
132 1g).

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

135

136 **DISCUSSION**

137

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

149 The only evidences of courtship in the family Sepiolidae were recorded by Norman
150 (2000) in wild *E. tasmanica*. The lack of female courtship behaviour found in the present study
151 could be a function of the limited space for females to ‘choose’ their partners due to relatively
152 small size of the experimental tanks, as observed in *Sepia officinalis* (Adamo *et al.* 2000).
153 However, absence of courtship, copulatory position, duration of mating and spermatophore
154 placement of *S. atlantica* matches with the observed in the sepiolid *E. scolopes* (Moynihan
155 1983, Singley 1983, Hanlon *et al.* 1997). The mating pattern also coincides with that observed
156 in *S. rondeletii* (Racovitza 1894), *Sepiolo robusta* (Boletzky 1983) and *E. hyllebergi*
157 (Nabhitabhata *et al.* 2005) except for the mating duration, which was much longer in *S.*
158 *atlantica* than in the other three species (68-80 minutes *versus* 7-10 minutes). *R. macrosoma*
159 also showed a male to female neck position during mating (Mangold-Wirz 1963, Racovitza
160 1894). A more detailed comparison with *S. atlantica* is not possible because of the lack of
161 information on mating duration and spermatophore placement in *R. macrosoma*. Instead of a
162 male to female neck arrangement during mating, *Rossia pacifica* showed a male parallel
163 position (Brocco 1971, Summers 1985). Additionally, in the other sepiolid on which mating is
164 known, *Sepietta oweniana*, the mating position was “head to head” and its duration was shorter
165 than in *S. atlantica* (Bergstrom & Summers 1983, present paper).

166 Nowadays, there is still little evidence for sperm competition in bobtail squids although
167 some observations strongly support its occurrence. Thus, the long mating times observed in *E.*
168 *scolopes* and in *S. atlantica* combined with the presence of an internal seminal receptacle,
169 strongly suggest the possibility of sperm competition behaviour among males (Hanlon *et al.*
170 1997, present paper). The short duration of mating observed in several species of bobtail squids
171 seems to be in disagreement with the existence of sperm competition that seems to need one
172 mating of long duration. Nevertheless, sperm competition is possible even when copulation

173 duration is short; and the long mating duration could be linked with other process like mating
174 guarding or capacity sperm transfer (Linn *et al.* 2007).

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

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

194 In conclusion, the information we obtained allow us described for the first time the
195 mating behaviour of *S. atlantica*. In general, our observations coincide with other sepiolid
196 species, and consisted in five stages. Each stage triggered a display of colour change unique to

197 this species. However, further data are needed to make conclusions about the function of the
198 long mating duration observed in *S. atlantica*.

199

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255 Table I. — *Sepiola atlantica*. Size of the ten specimens used in the study. Mantle dorsal length (ML)
 256 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

257

258 FIGURE CAPTION

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

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