Burying behaviour in the bobtail squid *Sepiola atlantica* (Cephalopoda: Sepiolidae)

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Abstract

The burying behavioural pattern of the small Atlantic bobtail squid (*Sepiola atlantica*) in natural substrate is described, quantitatively differentiating the movements in different phases and the chromatic changes associated with this behaviour. All specimens showed the same two-part sequence of burying, which triggered a display of colour changes peculiar to this species. Our results suggested that this was a consistent behaviour during burial. The mean latency time in the alert posture was 9.55 ± 5.49 s. The mean duration of the first phase was 12.2 ± 4.37 s. The mean duration of the second phase was 10.2 ± 2.95 s. The average time spent completing the behavioural pattern was 21.9 ± 4.93 s. Burying time was not related to size. Differences observed between individuals were associated with the number of movements in each phase. However, these movements were not significantly related to the size of the animals. A comparison of the burying pattern of this species with other members of the family Sepiolidae is made.

Keywords: *Burying,* Sepiola atlantica*, crypsis, cephalopod*

Introduction

In several bobtail squid species (Family Sepiolidae), the burying behaviour is relatively fixed and comprises a two-part sequence (Boletzky & Boletzky 1970; Boletzky 1996). In the first phase, burying consists of a series of flushing movements by which the animals blow up sand from underneath their bodies with vigorous funnel jets, until the animals are almost totally covered except for the dorsal surface of the head (Boletzky &

Boletzky 1970). In the second phase, the animal stretches out its dorsolateral arms and gathers sand to cover the head and neck areas, with a series of sweeping movements. In contrast to the first phase, the second tends to increase with age. Apparently, there is no visual or tactile feedback indicating to the animal when the sand cover is complete, and afterwards, the animals remain virtually motionless (Boletzky & Boletzky 1970).

Biology and ecology of the small bobtail squid *Sepiola atlantica* d'Orbigny, 1840, is poorly known (Guerra 1986, 1992; Yau & Boyle 1996; Reid & Jereb 2005) and its burying behaviour has not been investigated. In the present study, we describe the burying behaviour pattern of this species in confinement, quantitatively differentiating the movements at the different phases, and the chromatic changes associated with this behaviour.

Materials and methods

Twenty specimens were obtained in several dives from the Ría of Vigo (NW Iberian Peninsula; 42º14' N; 8º47' W) in October 2008. Depth of collection ranged from 3 to 6 m. The seabed was sandy without seaweed and with the presence of ripplemarks.

Specimens were transported to the Marine Station of the University of Vigo in Toralla Island. Each individual was placed in a 10.2-l tank (30 cm long × 18 cm wide × 19 cm deep) with an open seawater circulation system. Water temperature in the tanks ranged between 12 and 15°C. The system received a natural photoperiod of roughly 12:12 LD. The bottom of each tank was covered with a 2–3 cm layer of fine sand (grain size between 0.25 and 0.125 mm) taken from the same location where the specimens were collected. Bobtail squids were fed daily *ad libtum* with mysid shrimp (*Siriella armata*).

All observations were made after 48 h to ensure acclimatization to captivity as in Sinn and Moltschaniwskyj (2005). Due to the burying behaviour being performed by several sepiolid species mainly during the day (Anderson & Mather 1996; Hanlon & Messenger 1996), our observations were undertaken during daylight.

One observation of the burying process for each specimen was carried out and filmed (Sony PD- 170 with 4 × zoom). The duration of each burying activity was recorded for each phase, and the number of movements they made was quantified. These movements were the number of contractions and expansions of the mantle in the first phase and the number of arm stretches in the second one. Human interaction with bobtail squid was minimized through the use of visual barriers surrounding the experimental system during acclimatization and experimentation.

After observations, the dorsal mantle length (ML) of each specimen was obtained with a stereoscopic microscope (Nikon SMZ-1500) following the methodology for live animals employed by Sinn and Moltschaniwskyj (2005).

Burial duration and number of movements (expansions, contractions and sweeps) were plotted against ML and best-fit regression lines were calculated to determine any relationships.

Results

Pre-burying behaviour

When *Sepiola atlantica* settled onto the substrate, all individuals assumed an 'alert posture' (as defined by Anderson et al. 2004). In this position, the bobtail squids settled down on the tank bottom with their posterior mantle region, all arms were directed forward and raised off the sand (Figure 1A). After the alert posture, the bobtail squids quickly began the first burial phase (mean latency 9.55 ± 5.49 s).

Burying – Phase 1

This phase consisted of a series of flushing movements directed forwards and backwards, coupled with contractions and expansions (Figure 1B,C). The first funnel jet was directed forward. The mean number of contractions and expansions were 3.00 ± 1.03 and 3.30 ± 1.03, respectively, and no significant ($F = 0.934$; $r^2 = 0.049$; $p > 0.05$) relationship with ML was found, although a weak negative trend in the number of movements was observed in larger animals. The mean duration of the first phase was 12.2 ± 4.37 s. Contractions and expansions of the mantle were observed in synchronisation with fin movements.

Burying – Phase 2

No pause was observed between phases 1 and 2. In the second phase, the animal stretched out its laterodorsal arms and with the distal ends of these arms collected and covered its head with sand (Figure 1D,E). Initially, the arms were spread far apart, and then they were swept forwards and backwards. The movements of gathering the sand particles were smooth and continuous and the arms always moved synchronously and in the same direction (mean number of sweeps 6.00 ± 0.79). No significant ($F = 0.413$; $r^2 = 0.022$; $p > 0.05$) relationship between the number of sweeps and ML was observed. However, in larger animals a weak positive trend was noted. The bobtail squid remained immobile when burying was complete (Figure 1F). The mean duration of the second phase was 10.2 ± 2.95 s. At the end of the second phase, all the studied specimens performed two to four strong water jets through their funnel accompanied by a sand geyser (as defined by Anderson et al. 2002).

Mean duration of the complete behavioural pattern was 21.9 ± 4.93 s. Total burial time was not significantly ($F = 0.718$; $r^2 = 0.119$; $p > 0.05$) related to the size of the animal.

Body coloration changes

Different chromatophoric patterns were observed during the burial behaviour. These chromatic changes are described in detail in Table I.

In general, when the specimens displayed the alert posture, they acquired a lightly coloured pattern showing a general background resemblance to the substrate. Afterwards, they began a serial of patterned colour changes from light to dark, synchronously related to the mantle contractions and expansions.

Discussion

The burying sequence of *Sepiola atlantica* is similar to others sepiolid species (see Anderson et al. 2004 for a review) differing only from *Euprymna hyllebergi* in that they performed the second phase with their ventrolateral arms (Nabhitabhata et al. 2005).

Another different aspect from other studied sepiolids was that the first funnel jet in *Euprymna scolopes* and *E. hyllebergi* went backwards (Anderson et al. 2002; Nabhitabhata et al. 2005) and in *S. atlantica* the jet went forwards. The main differences observed between individuals were related to the number of movements of each phase. Differences among individuals were also reported in this kind of crypsis to *E. scolopes* (Anderson et al. 2002) and *Rossia pacifica* (Anderson et al. 2004). Our data support the adaptability between individuals in the burial process.

Our data confirm the observations made by Boletzky and Boletzky (1970) and Boletzky (1996) about the time spent in the different phases. The first phase is longer than the second in all *Sepiola* species studied. However, although burying time was not significantly related to size, two interesting aspects were observed in our study: in the first phase, as ML increased, the number of contractions and expansions tended to decrease, and in the second phase, the number of sweeps tended to increase with the size of the animal.

The mean time to complete the burying behaviour in *S. atlantica* was 50% lower than *E. scolopes* (Anderson 1997; Anderson et al. 2002) and 10% lower than *R. pacifica* (Anderson et al. 2004).

Anderson et al. (2002) suggested that the dark pattern of *E. scolopes,* when in alert posture, may be a possible ecological advantage as a prelude to a potential escape response. In contrast, in the present study *S. atlantica* assumed a light pattern immediately upon settling to the substrate, resembling the seabed. Endler (1991) proposed that colour changes occur in the time at which the animal is most vulnerable to predation. Therefore, it seems that *S. atlantica* has developed a chromatic pattern that would serve against predation during this activity. However, further studies are needed to make conclusions about the function of the colour changes.

In conclusion, the burying behaviour of *S. atlantica* is partially stereotyped and coincides with some aspects of other studied sepiolid species. Each phase of this activity triggered a display of colour changes, unique to this species, related to the contractions and expansions of the mantle. Lastly, the burying pattern in the family

Sepiolidae is a good example of the high flexibility found in cephalopod defensive strategies.

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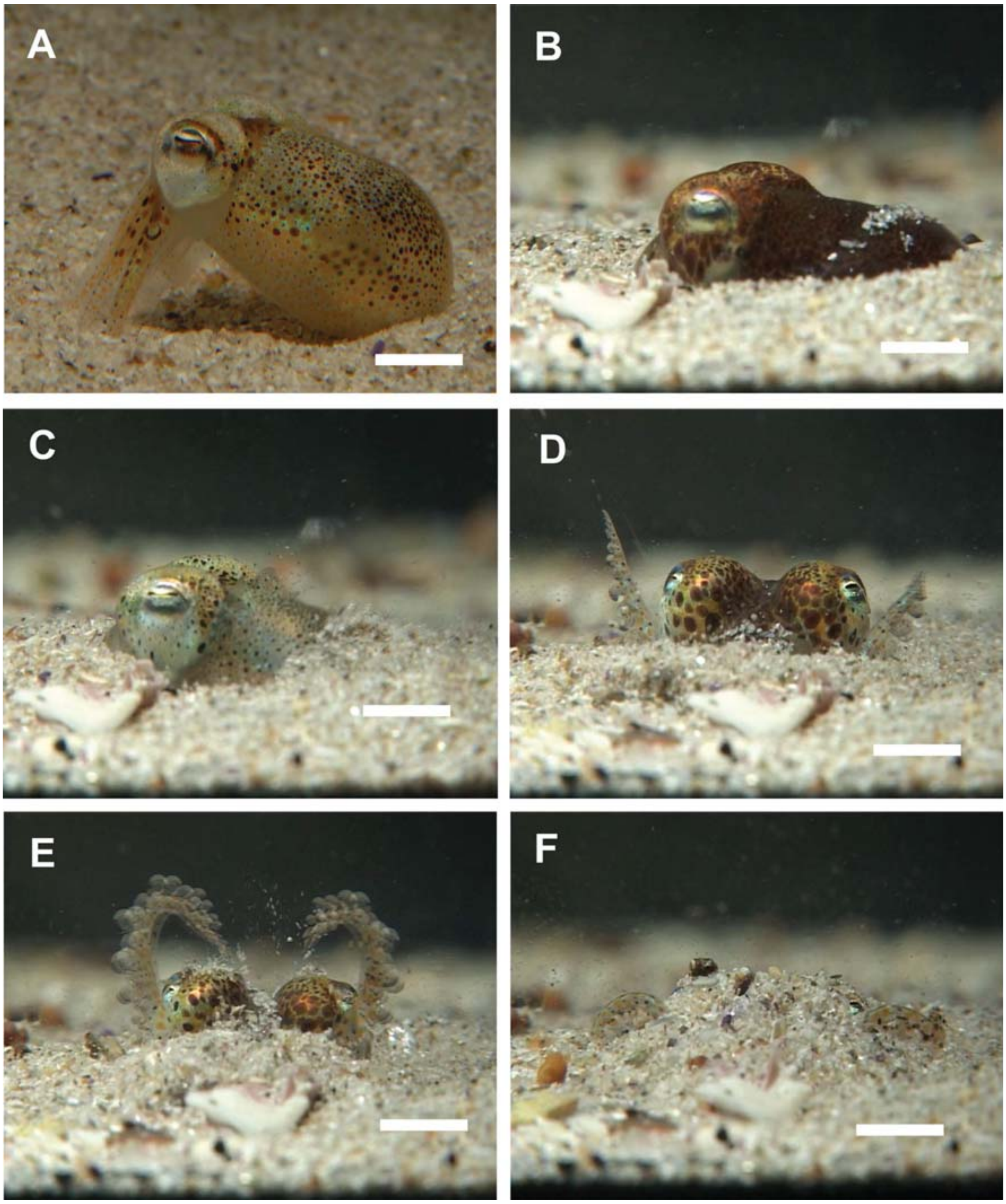

Figure 1. Burying pattern in *Sepiola atlantica*. **A**, The squid settling on the substrate and assuming alert posture. **B,C**, Afterwards the bobtail squid begin a series of contraction and expansion movements of the mantle until, except for the dorsal region of the head, it is entirely covered. **D,E**, The dorsolateral arms are stretched out over the surface of the substratum to gather sand particles in a circular area around the head. **F**, The animal is completely buried. Scale bars: A, 10 mm. B–F, 5 mm.

Table I. Colour changes during the burying episodes of the bobtail squid *Sepiola atlantica.*

Phases	Mantle	Head	Arms
Alert position	• Medium contracted chromatophores: brown, black and yellow. • Coloration reduces in intensity from dorsal to ventral region. • Iridophores: metallic blue and green in the dorsal and lateral region.	• Between the eyes: brown expanded chromatophores on a yellowish background • Eyeballs: silvering with small black and yellow dots. • Iridophores: metallic green in the eyeballs.	• Expanded chromatophores of brown, black and yellow colours near the head. The size and intensity of coloration towards the apex of the arms is reduced. Transparency in the apex.
Phase 1			
Contracted mantle	• Very contracted chromatophores: brown and black on a translucent background. • Iridophores: metallic blue and green throughout the mantle.	• Between the eyes: contracted chromatophores brown in a translucent background. • Eyeballs: silvering with brown and black dots of different sizes. • Iridophores: metallic blue and green in the eyeballs.	• Very contracted brown and black chromatophores. Metallic green iridophores between chromatophores.
Expanded	• Expanded chromatophores	• Expanded chromatophores brown on a dark	• Not observed.

mantle	acquired a dark brown coloration.	yellow background.	
Phase 2	• Not observed.	• When the arms are stretched, chromatophores are brown. • When contracted, iridophores of metallic green are predominant. When returning the arms, brown chromatophores increase and a mixture of yellow and metallic green iridophores were observed.	• Dorsolateral arms: translucent with small brown, black and yellow (yellow being bigger than the others) chromatophoric points. Metallic green and blue iridophores also observed.