Male reproductive system and spermatophores production and storage in *Histioteuthis bonnellii* (Cephalopoda: Histioteuthidae): A look into deep-sea squids' reproductive strategy

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ABSTRACT

Coleoid cephalopods go through a single breeding period in their life cycle, i.e., they are semelparous, although a great flexibility has been observed in their reproductive strategies, which range from simultaneous terminal spawning over a short period at the end of the animal's life to continuous spawning over a long period of the animal's life. So far, the information available on deep-sea species reproductive strategies is still poor and most of our knowledge about squid reproduction relates to females. In particular, not much is known on what strategy male squids have evolved to store sperm into spermatophores and adapt to semelparity. In this study an investigation of male reproductive strategy of the deep-sea umbrella squid *Histioteuthis bonnellii* (Férussac, 1835) is presented. The reproductive system was examined in 119 males caught in the Sardinian waters (Central Western Mediterranean) and is described for the first time. Results indicate that this species produces and stores spermatophores over a considerable period of time. The total number of spermatophores found in the reproductive system ranged between 12 and 3097 and the size of spermatophores stored by a single individual varied greatly, up to over 300%. Spermatophore length (SpL) gradually decreased towards the distal end of the reproductive system, so that spermatophores found in the proximal part of Needham's Sac were larger than those found in the terminal organ. Body size and SpL of spermatophores from the proximal part of Needham's Sac were positively correlated. Both indices of the sperm mass and of the ejaculatory apparatus decreased with the increase of SpL, while the cement body index increased, indicating that larger spermatophores contain less sperm and are equipped with larger cement bodies. Up to 64 spermatangia were found, exclusively in the terminal organ. The large size range of mature males (ML: 60.0–198.0 mm; TW: 113.50–2409.00 g) and the variation in spermatophore number and size indicate that in *H. bonnellii* males the allocation and storage of sperm start early in the individual life and extends in time, while animals continue to grow and produce spermatophores presumably more successful in attaching to female tissues. This pattern enlarges the time window available for reproduction and likely maximizes the percentage of mating success as the animals grow older and chances of mating events become comparatively lower, due to the basic low density of specimens in the deep-sea environment. Both aspects are potentially indicative of adaptation to the deep sea.

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1. Introduction

Maximizing the production of viable offspring is the outcome of sexual and natural selection, reached through complex adaptations encoded in the genetic heritage of each species. Also called "reproductive strategies" (Stearns, 1992), these adaptations consist of a broad variety of physiological and behavioral mechanisms, which allow individuals to reach their reproductive goal and make the best use of the environment they live in.

Given the successful colonization by cephalopods of virtually all marine habitats, from very shallow waters to the deep-sea environment, it is reasonable to expect that their reproductive strategies may be variable. Indeed, though a general consensus exists on the fact that coleoid cephalopods are semelparous (Boyle and Rodhouse, 2005; Nesis, 1987), there is accumulating evidence that very different timing, mode and behavior of mating, maturing

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and spawning also exist (Boyle and Rodhouse, 2005; Hanlon and Messenger, 1996; Nesis, 1995; Rocha et al., 2001).

Due to the increased importance of cephalopods as fisheries resources in the past decades (Jereb and Roper 2005, 2010; Jereb et al., 2014) and to the key role of many cephalopod species in the marine environment (Clarke, 1996; Piatkowski et al., 2001; Sinclair and Valdimarsson, 2003), understanding cephalopods' reproductive strategies has become increasingly important for the understanding of the marine system and to attempt managing important fisheries.

While much of the available knowledge on squid reproductive strategies relates to females and to species inhabiting neritic environments, less information exists on males and deep-sea species, for which recent investigations have evidenced peculiar reproductive tactics and adaptations (Hoving et al., 2008a, 2008b, 2009, 2012; Laptikhovsky et al., 2007; Seibel et al., 2005).

Histioteuthid squids are deep-sea species known for their importance in the oceanic realm, where they are actively predated upon by cetaceans and other top predators (e.g., Clarke, 1996). The umbrella squid *Histioteuthis bonnellii* (Férussac, 1834), in particular, turned out to play a major role in the marine food chain both as predator and as prey (Bello, 1996; Blanco et al., 2006; Quetglas et al., 1999). In addition, this species has been shown to represent an intermediate paratenic host in the transmission of Anisakidae (Culurgioni et al., 2010; Hochberg, 1983; Oshima, 1972), which draws increasing attention on its ecological role, due to man's potential utilization of fish.

Broadly distributed in the north temperate hemisphere (Voss et al., 1998), *H. bonnellii* was once considered poorly represented in the Mediterranean Sea, being seldom caught by trawlers. However, increasing evidence has been gathered with time that this species is rather common throughout the Mediterranean basin (see Bello, 2000 and Quetglas et al., 2010 for reviews), where it usually occurs between 500 and 1500 m, only occasionally extending beyond 2000 m (Cuccu et al., 2007b; Jereb and Roper, 2010; Quetglas et al., 2010; Voss et al., 1998).

In spite of its ecological importance, the biology of *H. bonnellii* is still poorly known. Preliminary investigations on reproductive aspects of this species in the western Mediterranean have been carried out (Quetglas et al., 2010), and additional scattered information exists for the Italian waters (Cuccu et al., 2007b; Capua et al., 2009; Lazzaretti et al., 1995). However, no mature females have been caught, and no mature, mated specimens have been examined, only a few spent females have been found floating in the Ligurian Sea (Quetglas et al., 2010). Indeed, the capture of fully mature females of this species seems a very rare event, and only two records exist to date: one refers to a 330 mm ML specimen caught off west Greenland (Kristensen, 1980), and the other to a 225 mm ML female caught in the North Atlantic at 37°N 56°W (Stephen, 1982). This lacking of mature females to examine hampers females' reproductive strategy investigation.

As for males, observations on *H. bonnellii* spermatophores revealed intraspecific variations that "could prove valuable in identifying genetically separate populations" (Voss et al., 1998), but studies on sperm allocation and spermatophore production and storage have not been carried out for this species so far.

In this paper we offer a contribution to improve our knowledge of male deep-sea squids' reproductive strategies by studying *H. bonnellii* from Sardinian waters (central western Mediterranean Sea). To this goal, male reproductive system has been investigated and spermatophore production, morphometrics and storage are described.

2. Materials and methods

A total of 119 males were collected during commercial bottom fishing carried out in the Sardinian waters (western Mediterranean)

from 2005 to 2012, at depth ranging between 400 and 700 m. Hauls were performed by daylight and a bottom otter trawl with a 20 mm cod-end stretched mesh size was used.

Dorsal mantle length (ML, to the nearest mm) and total weight (TW, to the nearest 0.1 g) were recorded on fresh specimens. Maturity stages were assigned using a four-stage scale (I, immature; II, maturing; III, mature; IV, spent); specimens were considered mature when spermatophores were packed in Needham's sac.

Size at maturity (ML$_{50\%}$) was estimated by fitting the mantle length−frequency distribution of the proportion (P_i) of mature and spent specimens (stages: III and IV), grouped in 20 mm ML classes, to the logistic model:

$$P_i = 1/[1+\exp(-\alpha+\beta MLi)]$$

where ML$_{50\%} = -\alpha/\beta$.

Total weight at maturity (TW$_{50\%}$) was also estimated following the same procedure, grouped in 100 g TW classes.

The following measurements were recorded on reproductive systems: total gonad weight (GW), testis weight (TeW) and spermatophoric complex weight (SpCW) to the nearest 0.01 g and terminal organ length (TOL) to the nearest mm. Gonadosomatic (GSI= GW$*$100/TW) and Hayashi [HI=SpCW/(SpCW+TeW)] indices also were computed.

A sample of 18 mature specimens was selected and the reproductive systems were removed and preserved in 5% saline formalin for further examination. The total number of spermatophores and spermatangia (when present) were recorded and total length of both structures was measured to the nearest 0.01 mm (i.e., SpL and SgL, respectively).

A subsample of 13 mature specimens was used to investigate spermatophore length distribution within the reproductive system; Needham's sac was subdivided into 3 sections of approximately equal volume (Sections 1, 2 and 3; Fig. 3a) followed by a fourth section made up by the terminal organ (Section 4; Fig. 3a). SpL was measured on samples of spermatophores ($N=5-20$; usually 20) extracted from each section; spermatophore length index also was computed (SpLI= SpL$*$100/ML), accordingly. The non-parametric Kruskal–Wallis test and the Mann–Whittney pairwise comparisons were performed to investigate differences in spermatophores size within the four sections and between the proximal (1) and distal (4) sections, respectively.

A subsample of 8 reproductive systems was used to investigate spermatophores structure; 10 spermatophores from each of the 4 selected sections of above, for a total of 320 spermatophores, were extracted and the following measures were taken: ejaculatory apparatus length (EAL), cement body length (CBL) and sperm mass length (SML) (Fig. 3b and 3c). Corresponding relation of each spermatophore section with spermatophore total length was computed as a percentage index (SpL; EALI, CBLI, SMLI, respectively).

Oral sperm mass length (OSML) and trailing end length (TEL) were also recorded (Fig. 3d) on spermatangia. Mean SpL of the spermatophores from each section of Needham's sac (SpLS1, SpLS2, SpLS3 and SpLS4) was plotted against body size (ML and TW) to investigate SpL relationship with the animal growth.

3. Results

3.1. Male maturity condition and reproductive morphology

Data on maturity stages and size of the analyzed sample are reported in Table 1. The majority of the examined specimens was mature (i.e., 62 over the total 119 males analyzed; 52%), 34 were maturing, 18 were immature and 5 were spent. Both GSI and HI mean values increased from immature (0.10% and 0.49, respectively) to mature males (0.85% and 0.76, respectively) and decreased again

Table 1
Histioteuthis bonnellii: number of specimens, dorsal mantle length, total weight, gonadosomatic (GSI) and Hayashi (HI) indices of the sample analyzed at each maturity stage.

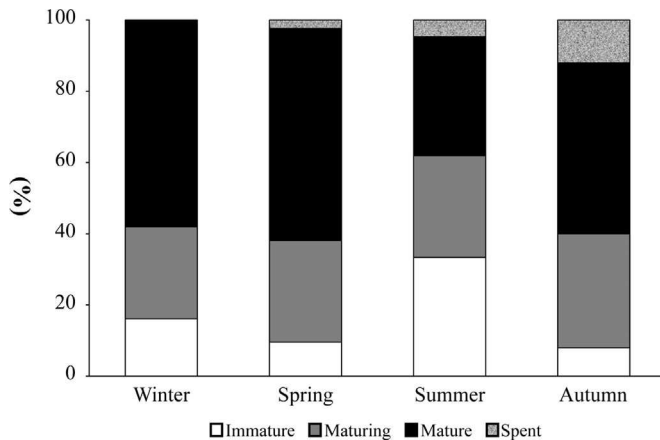| No. of specimens	Maturity stage	Mantle length (mm)	Total weight (g)	GSI	HI
18	Immature	24.5–92.0	11.62–284.00	0.03–0.23 (0.10 + 0.07)	0.38–0.56 (0.49 + 0.05)
34	Maturing	40.0–107.5	46.90–500.00	0.21–0.81 (0.47 + 0.17)	0.41–0.63 (0.51 + 0.06)
62	Mature	60.0–198.0	113.50–2409.00	0.58–1.45 (0.85 + 0.20)	0.61–0.94 (0.76 + 0.11)
5	Spent	114.0–150.0	699.00–994.00	0.19–0.62 (0.48 + 0.17)	0.57–0.71 (0.66 + 0.10)

In brackets: mean + standard deviation.

Fig. 1. *Histioteuthis bonnellii*: seasonal occurrence of maturity stages in male specimens.

in spent individuals (0.48% and 0.66, respectively; Table 1). Mature as well as immature and maturing animals were present all year round, while spent specimens were caught in spring, summer and autumn (Fig. 1). Mature individuals represented the bulk of the sampled specimens in each season.

The smallest mature male caught measured 60.0 mm ML (113.50 g TW) and 50% of the total sampled population was mature at 100.0 mm ML (400.00 g TW). Mature specimens did not bear a hectocotylus and no distal modification of the first pair of arms, as described by Voss et al. (1998), was observed in the examined specimens. With the exception of the terminal organ, the reproductive apparatus is covered by the same thin reddish membrane which covers the other organs, excluding the gills (Fig. 2a). In mature males, the testis is thin and characterized by a honeycomb structure. The dissection of the spermatophoric complex showed that this is composed by a large spermatophoric organ and a rather large Needham's sac (Fig. 3a). The latter is composed by a bag-like sac and a tubular terminal section (i.e., terminal organ), of about the same length, separated by one characteristic loop. The testis and the main fraction of the spermatophoric complex are attached to the digestive gland; only the terminal organ (TO) is free and opens near the left funnel-locking cartilage.

In the 18 mature reproductive systems (ML: 78.0–170.0 mm; TW: 251.40–1440.0 g) preserved the testis mass ranged between 0.59 and 2.62 g, reaching a maximum of 0.32% of total body weight and spermatophoric complex ranged between 1.20 and 13.3 g, reaching 1.29% of body weight. The total length of the terminal organ ranged from 23.3% to 34.4% of mantle length. In spent animals testis showed various degrees of tissue consistency reduction, till complete degeneration and few to none spermatophores were present in Needham's sac (Fig. 2d).

3.2. Spermatophores

Data on spermatophores number and size are reported in Table 2. The total number of spermatophores found in mature males varied between 12 and 3097 (mean + SD: 977 + 928). The

correlation between spermatophores number and male size (ML and TW) was not significant (ML: $R^2 = 0.0255$, $N = 18$, $P > 0.05$; TW: $R^2 = 0.0012$, $N = 18$, $P > 0.05$). No dysfunctional or tentative spermatophores were found in mature males.

Total spermatophore length (SpL) ranged between 2.32 and 15.45 mm (mean + SD: 7.11 + 3.49 mm) and gradually decreased towards the distal end of Needham's sac with significant differences in size within the four sections (Table 2) (Fig. 3). Spermatophores from the proximal part of Needham's sac, or Section 1 (Fig. 2c; spt1), were always larger than those found in the terminal organ or Section 4 (Fig. 2c; spt4), differences statistically confirmed by the Mann–Whittney pairwise comparisons (Table 2). This difference in size was conspicuous and in one specimen it ranged between 122.8% and 303.5% (mean + SD: 186.6 + 50.6%).

Mean SpL of spermatophores from Section 1 (SpLS1) ranged from 3.07 and 14.57 mm (Table 2) and was positively correlated with male body size (ML and TW) (Table 3).

On the contrary, the relationship between SpLS2, SpLS3, SpLS4 and body size (ML and TW) was weak or absent (Table 3).

Trends of spermatophores and spermatophores components actual length and indices are shown in Fig. 4a and b. Value ranges of actual length and corresponding indices of spermatophores and spermatophores components are reported in Table 4. Ranges and statistics of the correlation between SpL and spermatophores components indices are reported in Table 5. The relationships between spermatophores components and spermatophores size showed different trends depending on which parameters were considered, i.e., the actual measurements or the computed indices (Fig. 4a and b, respectively). In the first case the increase of the cement body length is the most striking aspect of the analysis, while the ejaculatory apparatus length increases only slightly with spermatophore length, and the sperm mass length seems rather stable after a first increasing phase. The relationships between spermatophores length and spermatophores components indices showed that sperm mass and ejaculatory apparatus indices decrease with increasing SpL, whereas cement body index increases and these relationships are significant (Table 5).

3.3. Spermatangia

Spermatangia were found in the terminal organ of about 2/3 of the examined mature reproductive systems (i.e., 72.22%); their number varied between 1 and 64 (mean + SD: 21 + 17).

A sample of 20 spermatangia was measured. Spermatangia total length (SpL) varied between 2.02 and 8.12 mm (mean + SD: 4.44 + 1.88 mm). Oral sperm mass (OSM) length represented between 20.96% and 39.73% of SgL (mean + SD: 31.99 + 5.40%), while the trailing end (TE) length ranged between 60.00% and 79.04% of SgL (Fig. 3d). The relationships between SpL and OSM and TE length were significant (Table 5).

4. Discussion

Giving the key role of reproduction in all living species, the understanding of reproductive patterns and strategies plays a
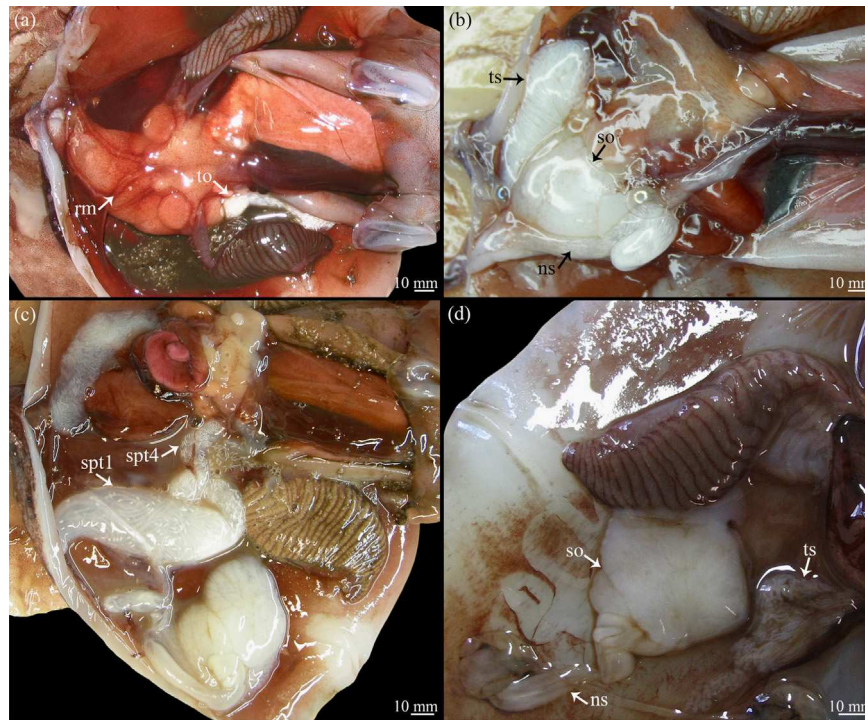
Fig. 2. *Histioteuthis bonnellii*: (a) ventral view of the opened mantle showing the internal organs covered by the reddish membrane; (b–c) reproductive system of a mature male: (b) view of the internal organs after membrane removal; (c) spermatophores in Sections 1 and 4 (spt1 and spt4, respectively), after the dissection of the spermatophoric complex, are indicated by the arrows; (d) reproductive system of a spent male; so, spermatophoric organ; ts, testis; to, terminal organ; ns, Needham's sac; rm, reddish membrane that covered the organs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
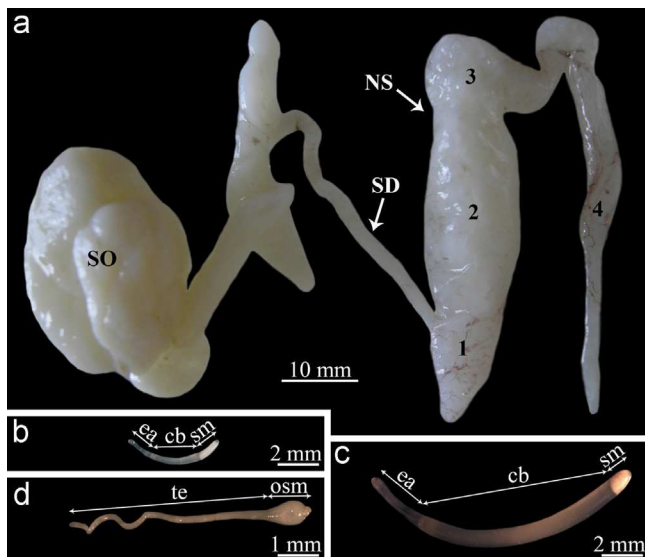

Fig. 3. *Histioteuthis bonnellii*: (a) spermatophoric complex dissected, showing the spermatophoric organ (SO) the Needham's sac (NS) and the spermatophoric duct (SD); numbers 1–2–3 indicate the sections of the Needham's sac selected to investigate spermatophores length and 4 the terminal organ section; (b) spermatophores found in Section 4 and (c) spermatophore found in Section 1 of the same specimen, a male of 155 mm ML; (d) spermatangium removed from Section 4 of a mature male of 150.0 mm ML; ea, ejaculatory apparatus; cb, cement body; sm, sperm mass; osm, oral sperm mass; te, trailing end.

major role in our understanding of living systems. Due to the short life cycle of the majority of cephalopods, the existence of an annual recruitment, i.e., a successful breeding, mating and eggs and hatchlings survival, is crucial to the population (Boyle, 1990). As such, the understanding of cephalopod reproductive strategies is essential, due to cephalopods' relevance to the fishery and to their role in the marine system.

While there is increasing evidence that deep-sea cephalopod species developed reproductive strategies which may differ greatly from those typical of neritic species (e.g., Seibel et al., 2005), deep-sea environments are difficult to investigate and our knowledge of deep-sea cephalopods is still limited, though recent technologies and increasing research efforts allowed important progress in this field in recent years, both in the Mediterranean Sea (Cuccu et al., 2007a, 2009, 2011a, 2013) and in other areas (Hoving et al., 2009, 2010; Laptikhovsky et al., 2007). One of the main difficulties in investigating deep-sea species is the collection of adequate number of mature specimens. As commented in Section 1, this is particularly true for *H. bonnellii* mature females, of which only 2 records occur to date, in spite of the wide distribution and abundance of the species worldwide and in the Mediterranean Sea (Capua et al., 2009; Cuccu et al., 2007b; Quetglas et al., 2010; Voss, 1969; Voss et al., 1998).

So far most studies on cephalopod reproduction have concentrated on females and only recently has much attention been directed to reproductive strategies of males (Cuccu et al., 2011b; Hoving et al., 2008a, 2008b, 2010; Laptikhovsky et al., 2007). Indeed, as commented by Hoving et al. (2010), males have to confront with the same challenging environments and life style constraints as females: they have to sustain the high costs of sperm/spermatophores production and maximize the short period of time available to mate and fertilize females. Therefore it is important to investigate which strategies male cephalopods developed to understand the species reproductive adaptations.

In the present case, observations carried out on the largest sample of *H. bonnellii* males examined to date ($N = 119$ specimens) allowed one to shoot a glance into the reproductive strategy of this histioteuthid in the Mediterranean Sea.

4.1. Male maturity condition and reproductive morphology

Males from Sardinian waters are mature at a size of 60.0 mm ML (113.50 g of TW) and are caught all year round, representing

Table 2

Histioteuthis bonnellii: size of mature specimens examined (ML: mantle length; TW: total weight), number of spermatophores (Sp), number of spermatangia (Sg), spermatophores size in the four sections considered, where the first three (Sections 1–3) are located in the bag-like portion of Needham's sac, the fourth one (Section 4) in the tubular terminal section (terminal organ), and results of the Kruskal–Wallis Test and of the Mann–Whittney pairwise; in brackets: mean $\pm$ SD.

ML (mm)	TW (g)	Sp (No.)	Sg (No.)	ML (mm)	TW (g)	Sp (No.)	Sg (No.)	Spermatophores total length (mm)				Kruskal–Wallis test	Mann–Whittney pairwise comparisons Sections 1–4
								Section 1	Section 2	Section 3	Section 4	χ^2 (P)	P
78.0	251.40	195	23	78.0	251.40	195	23	ND	ND	ND	ND	–	–
86.0	271.64	428	0	86.0	271.64	428	0	ND	ND	ND	ND	–	–
89.0	367.30	571	0	89.0	367.30	571	0	2.97–3.13 [3.07 $\pm$ 0.08]	2.70–2.98 [2.81 $\pm$ 0.09]	2.58–2.75 [2.65 $\pm$ 0.11]	2.44–2.58 [2.50 $\pm$ 0.10]	49.75 ($<$ 0.01)	$<$ 0.01
100.0	641.44	3097	34	100.0	641.44	3097	34	ND	ND	ND	ND	–	–
104.0	445.00	1353	14	104.0	445.00	1353	14	4.08–4.43 [4.27 $\pm$ 0.12]	3.61–4.03 [3.80 $\pm$ 0.11]	2.95–3.40 [3.15 $\pm$ 0.17]	2.59–2.88 [2.69 $\pm$ 0.11]	52.78 ($<$ 0.01)	$<$ 0.01
105.0	651.10	1298	35	105.0	651.10	1298	35	4.28–4.88 [4.54 $\pm$ 0.17]	3.70–4.26 [4.14 $\pm$ 0.18]	3.20–3.35 [3.28 $\pm$ 0.07]	2.85–3.15 [3.02 $\pm$ 0.10]	58.83 ($<$ 0.01)	$<$ 0.01
114.0	500.00	1414	23	114.0	500.00	1414	23	3.83–4.43 [4.17 $\pm$ 0.19]	3.32–3.79 [3.55 $\pm$ 0.16]	3.00–3.25 [3.13 $\pm$ 0.08]	2.32–2.67 [2.47 $\pm$ 0.10]	53.29 ($<$ 0.01)	$<$ 0.01
115.0	745.00	12	2	115.0	745.00	12	2	–	–	–	8.71–9.00 [8.84 $\pm$ 0.13]	–	–
119.1	583.00	908	29	119.1	583.00	908	29	5.02–5.20 [5.09 $\pm$ 0.07]	4.51–5.28 [4.89 $\pm$ 0.25]	3.57–3.97 [3.74 $\pm$ 0.11]	2.91–3.61 [3.24 $\pm$ 0.29]	58.27 ($<$ 0.01)	$<$ 0.01
120.0	620.00	1478	64	120.0	620.00	1478	64	5.25–6.28 [5.85 $\pm$ 0.35]	3.76–4.87 [4.36 $\pm$ 0.39]	3.11–3.48 [3.37 $\pm$ 0.12]	2.38–2.58 [2.53 $\pm$ 0.11]	44.01 ($<$ 0.01)	$<$ 0.01
129.0	744.00	308	6	129.0	744.00	308	6	8.88–9.90 [9.37 $\pm$ 0.35]	8.18–8.75 [8.46 $\pm$ 0.19]	7.05–8.38 [7.97 $\pm$ 0.44]	6.02–7.33 [6.56 $\pm$ 0.48]	49.43 ($<$ 0.01)	$<$ 0.01
130.0	921.50	394	0	130.0	921.50	394	0	7.98–9.63 [8.66 $\pm$ 0.51]	6.92–7.60 [7.26 $\pm$ 0.23]	6.25–6.68 [6.48 $\pm$ 0.14]	4.80–5.90 [5.37 $\pm$ 0.43]	51.52 ($<$ 0.01)	$<$ 0.01
130.0	1018.20	685	20	130.0	1018.20	685	20	ND	ND	ND	ND	–	–
140.0	1000.00	2624	0	140.0	1000.00	2624	0	7.09–7.20 [7.14 $\pm$ 0.08]	5.62–6.52 [6.28 $\pm$ 0.25]	4.25–6.43 [5.44 $\pm$ 0.69]	2.51–3.98 [3.15 $\pm$ 0.61]	59.24 ($<$ 0.01)	$<$ 0.01
140.0	1114.30	450	6	140.0	1114.30	450	6	10.33–11.84 [10.70 $\pm$ 0.51]	8.49–9.38 [8.93 $\pm$ 0.23]	5.72–6.79 [6.33 $\pm$ 0.24]	4.62–5.71 [5.35 $\pm$ 0.31]	52.43 ($<$ 0.01)	$<$ 0.01
150.0	1126.00	267	23	150.0	1126.00	267	23	9.70–12.62 [10.92 $\pm$ 0.83]	7.43–7.80 [7.65 $\pm$ 0.10]	6.72–7.04 [6.91 $\pm$ 0.10]	4.96–5.20 [5.07 $\pm$ 0.08]	42.24 ($<$ 0.01)	$<$ 0.01
155.0	1440.10	845	15	155.0	1440.10	845	15	12.78–15.45 [14.57 $\pm$ 0.88]	9.72–13.35 [12.50 $\pm$ 0.86]	7.20–8.79 [8.15 $\pm$ 0.51]	4.18–6.47 [4.80 $\pm$ 0.70]	58.87 ($<$ 0.01)	$<$ 0.01
170.0	908.00	1259	0	170.0	908.00	1259	0	ND	ND	ND	ND	–	–

ML, mantle length; TW, total weight; ND, not determined.

Table 3

Histioteuthis bonnellii: regression equations and statistics of the correlation between mean spermatophores length (SpL) from different sections of Needham's sac and body size (ML and TW).

Section	Regression equation	R^2	P
1	SpLS1 $= -11.7419 + 0.155168*$ML	0.7755	$<$ 0.01
	SpLS1 $= -0.458198 + 0.0100559*$TW	0.8695	$<$ 0.01
2	SpLS2 $= 7.34432 - 0.00896479*$ML	0.0030	$>$ 0.05
	SpLS2 $= 7.50893 - 0.00157732*$TW	0.0290	$>$ 0.05
3	SpLS3 $= 6.29259 - 0.00990047*$ML	0.0080	$>$ 0.05
	SpLS3 $= 5.93336 - 0.00108031*$TW	0.0260	$>$ 0.05
4	SpLS4 $= -0.233798 + 0.0364135*$ML	0.1296	$>$ 0.05
	SpLS4 $= 2.22838 + 0.00259533*$TW	0.1757	$>$ 0.05

ML, mantle length; TW, total weight.

the main fraction of the sampled population, similar to what observed in the western Mediterranean by Quetglas et al. (2010). Maximum sizes recorded for mature as well as spent animals in the present study are lower than the maximum size reported for the Catalonian population (330 mm ML; Morales, 1962); however they are comparable with sizes recorded more recently in the Balearic and adjacent seas (Quetglas et al., 2010).

The large size range of mature males (60.0–198.0 mm ML) indicates that the production of sexual products may start early in the individual life. According to an annual life cycle and to the age–size relationship proposed for this species in the Sardinian waters

(Mereu et al., 2011), the production of spermatophores would start at 6–7 months of life, to last for about half of the animal's life. A continuous production of sexual gametes is already known and has been investigated in females of deep-sea cephalopods, in which it is considered a reproductive adaptation to depth (Rocha et al., 2001). A similar strategy that increases the time interval in which reproduction can take place was proposed recently for *Histioteuthis miranda* (Berry, 1918) males from the South African waters by Hoving et al. (2010). Indeed, as evidenced by these authors, an early and continuous production of spermatophores allows a maximum time range for mating; this, in deep-sea environments where low density of individuals and scarcity of spawning aggregations may occur, would result in a winner reproductive strategy. The male reproductive apparatus of *H. bonnellii* differs from that of *Illex illecebrosus* described by Lipinski (1979) and from that of most Ommastrephids reviewed by Nigmatullin et al. (2003), but is similar to the apparatus of *Histioteuthis miranda* described by Hoving et al. (2010) in the peculiar structure of Needham's sac. As evidenced in *H. miranda*, also in *H. bonnellii* Needham's sac is partially curled up inside the genital sac. When dissected, the presence of a peculiar loop between the large bag-like structure where the spermatophores are stored and the tubular terminal organ is evidenced. In addition, sections of "discontinuity" are detectable in the large bag-like portion, similar to light shrinkages/bottlenecks, which "subdivide" the sac into three sections. As commented below, spermatophores size and composition differ according to the different sections they are located in.

This greater complexity of Needham's sac if compared to the structure generally observed and described for other squids (e.g., Nigmatullin et al., 2003) can be an adaptation to a continuous gametes production through an extended period of the life cycle, and subsequently need not only to store spermatophores properly but also to separate them according to the time of their production, as spermatophores' length and composition (in terms of proportion of spermatophores components mass), vary with time. The presence of the characteristic loop would be an additional structural help in this regard, possibly also reducing the chances of occasional losses of spermatophores during the long period of accumulation. As for the terminal organ, even if this structure does not extend beyond the mantle opening when looking at the internal organs in situ, after opening the mantle ventrally (Fig. 2a), it is nonetheless free in the mantle cavity for its entire length, not attached to other organs, thus in principle able to extend over the mantle opening in live specimens.
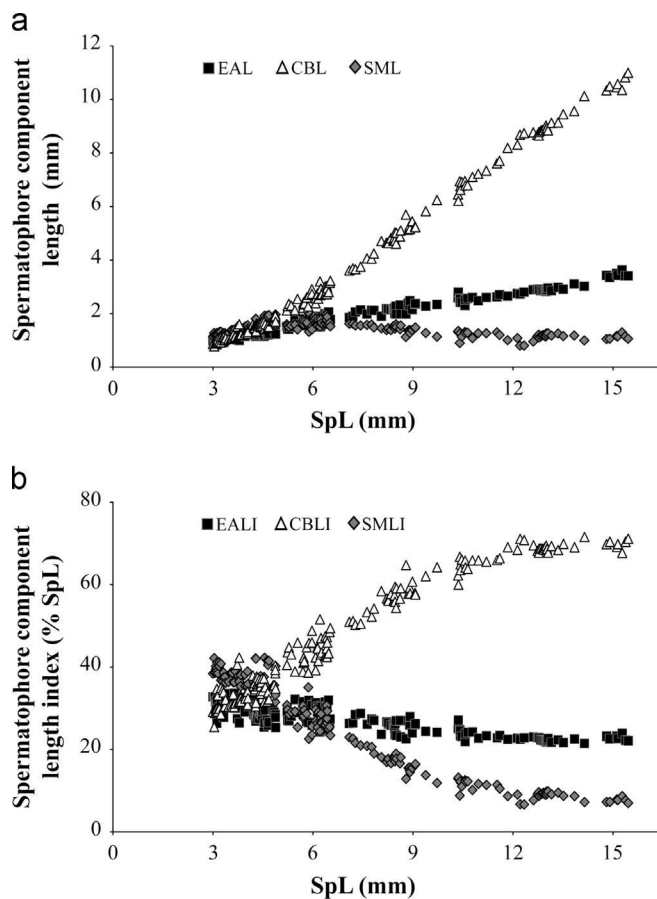

Fig. 4. *Histioteuthis bonnellii*: trends of spermatophores functional components with increasing spermatophore size; (a) spermatophores components measurements; (b) spermatophores components indices; EAL: ejaculatory apparatus length; EALI: ejaculatory apparatus length index; CBL: cement body length; CBLI: cement body length index; SML: sperm mass length; SMLI: sperm mass length index.

Though low, the GSI is comparable to values observed in other squid species, in which the reproductive investment is not comparatively high (e.g., *Dosidicus gigas*; Markaida et al., 2004) but the HI is definitely high in *H. bonnelli* (up to 0.94) even in comparison with species with much higher GSI (e.g., *Illex argentinus*; Perez et al., 2009). This confirms that the main reproductive effort in male *H. bonnellii* is focused on spermatophores production and storage.

4.2. Spermatophores and spermatangia

Spermatophore size range of the Sardinian sample was broad (2.32–15.45 mm) and the maximum size recorded was conspicuously larger than the maximum size reported for spermatophores of specimens from the Atlantic (i.e., 4.6 mm; southwest Atlantic) by Voss et al. (1998), the only information available to date for *H. bonnellii* spermatophores. According to the great difference in size observed in spermatophores found in the same specimen, also the spermatophoric index was broad (2.63–9.97) and the maximum SpLI value was larger than what was previously recorded (Voss et al., 1998).

Indeed, in the Sardinian sample spermatophore size gradually decreased from the proximal to the distal sections of Needham's sac, so that spermatophores found in the proximal part were always larger than those found in subsequent sections and in the terminal organ and the difference in length was conspicuous in large animals (i.e., up to over 300% in a male of 155 mm ML).

In addition, a positive correlation existed between body size and size of the spermatophores from the proximal tract of Needham's sac while no correlation was found between body size and spermatophores from the other sections. Both factors combined indicate that spermatophores from the proximal tract are those most recently produced and those from the terminal organ are the oldest.

The high variation in spermatophores number (12–3097), along with the broad range of spermatophoric complex weights, neither of which correlated with body size, indicates that males may mate and release their sexual products at different times while continuing to grow.

No disruptive, tentative spermatophores were found in mature males and all spermatophores were equipped with the complete set of functional components (i.e., sperm mass, cement body and ejaculatory apparatus). However, a different investment in spermatophore production and storage clearly characterized animals'

Table 5
Histioteuthis bonnellii: regression equations and statistics of the correlation between total spermatophore length (SpL) and ejaculatory apparatus index (EALI), cement body index (CBLI) and sperm mass index (SMLI), and between spermatangia length (SgL) and oral sperm mass (OSM) and trailing end (TE).

Regression equation	R^2	P
EALI = 32.8199−0.795821∗SpL	0.7021	< 0.01
CBLI = 19.521 + 3.92755∗SpL	0.9300	< 0.01
SMLI = 47.6602−3.13181∗SpL	0.9106	< 0.01
OSM = 0.34683 + 0.232588∗SgL	0.7270	< 0.01
TE = −0.34683 + 0.767412∗SgL	0.9666	< 0.01

Table 4
Histioteuthis bonnellii: actual length and corresponding indices of spermatophores (SpL, SpLI), ejaculatory apparatus (EAL, EALI), cement body (CBL, CBLI) and sperm mass (SML, SMLI).

Spermatophores		Ejaculatory apparatus		Cement body		Sperm mass	
SpL (mm)	SpLI	EAL (mm)	EALI	CBL (mm)	CBLI	SML (mm)	SMLI
2.32–15.45	2.63–9.97	0.82–3.63	21.36–33.52	0.77–10.99	25.33–71.57	0.80–2.05	6.49–42.2
(7.11 ± 3.49)	(5.08 ± 2.04)	(1.83 ± 0.70)	(27.17 ± 3.31)	(3.84 ± 2.93)	(47.43 ± 14.20)	(1.43 ± 0.24)	(25.41–11.44)

growth; the percentage of sperm mass contents decreased with increasing spermatophores size, while the percentage of cement body contents increased, so that the largest/ more recently produced spermatophores contained comparatively lower quantities of sperm and larger cement bodies.

Hoving et al. (2010), analyzing the same trend in *H. miranda*, commented that spermatophores from the proximal section of the Needham's sac may be those less likely to be used because of their location and because mating opportunities presumably decrease as the animals grow older (after reaching an optimum). However, these authors also mentioned that spermatophores with large cement bodies are considered better equipped to implant into female tissues (see also Hoving and Lipinski, 2009), thus, better suited to maximize males effort to transfer sperm to females. Indeed, the large size of spermatangia observed in the present study (up to over 8 mm long) support the hypothesis that even the largest spermatophores are capable of a normal functional inversion. In addition, the small-sized spermatophores found even in large specimens, confirm that mating events may be rare occasions in deep-sea environments.

The lack of hectocotyli in the Sardinian specimens, in which no arms modification was observed either (as potential replacement of a functional hectocotylus; Voss et al., 1998), leaves open the question of spermatophores transfer to females and leads to speculate on the possibility that the terminal organ may be used for this purpose. As already commented, the terminal organ observed in *H. bonnellii* lays free for nearly its full length in the mantle cavity, as it occurs in unhectocotylized species which use it for spermatophores transfer. It makes up for about half of Needham's sac organ, thus being comparatively longer than the homologous structures observed in species provided with hectocotyli, definitely longer than the terminal organ observed in the congener *miranda*, in which spermatophores transfer is attributed to the modified basal portions of dorsal arms (arms I; Hoving et al., 2010; Voss et al., 1998).

Even though the terminal organ observed in *H. bonnellii* did not exceed the mantle opening and is smaller than the analogous structures used to transfer spermatophores in other species (i.e., it reaches only 34.4% of ML in the Sardinian specimens) it is not possible to exclude that it may be able to extend in alive specimens. Recent observation of penis elongation in *Onykia ingens* (Smith, 1881) (Arkhipkin and Laptikhovsky, 2010) help support this hypothesis.

Mated histioteuthid females *Histioteuthis celetaria celetaria* (Voss, 1960) were found with spermatophores embedded in their tissues, while *Histioteuthis hoylei* (Goodrich, 1896), *Histioteuthis celetaria pacifica* (Voss, 1962) and *Histioteuthis atlantica* (Hoyle, 1885) carried spermatophores inside the mantle cavity (Laptikhovsky, 2001; Voss et al., 1998).

The presence of spermatangia in males' terminal organs recorded in the present study is similar to what is observed in another deep-sea species, *Neorossia caroli* (Joubin, 1902), in which spermatangia implantation in female tissues has been confirmed (Cuccu et al., 2011b). Therefore, a potential similar strategy is not to exclude in *H. bonnellii*, although traces of copulation have never been observed in females examined by the authors, neither in those where at least some oocytes in the oviducts were found (Mereu et al., 2011).

Overall, maturity condition evolution, male reproductive anatomy and spermatophores production and storage indicate a male strategy focused to start producing spermatophores early in the life cycle to extend the time window in which mating may occur, as observed for the congener, deep-sea species *H. miranda* (Hoving et al., 2010). An effort to maximize sperm investment and storage (not a trivial cost in the animals' energy-costs balance; Wells and Clarke, 1996) and enhance spermatophore attachment to females

also is apparent by the production of spermatophores equipped differently as the animals grow older: from smaller spermatophores with comparatively larger quantity of sperm, to larger spermatophores with comparatively smaller quantity of sperm but actual and comparatively larger cement bodies to better insure potential spermatophores implantations. To summarize, *H. bonnellii* males are likely to extend the time window in which mating may occur and maximize reproductive inputs, with the production of spermatophores better equipped in cement bodies (thus potentially more efficient in sperm transferring) as the animals grow older. All winning elements for a deep-sea squid successful adaptation to the environment.

Acknowledgments

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