Brooding and sperm storage by the deep-sea squid *Bathyteuthis berryi* (Cephalopoda: Decapodiformes)

STEPHANIE L. BUSH[1,2], HENDRIK J. T. HOVING[2], CHRISTINE L. HUFFARD[3], BRUCE H. ROBISON[2] AND LOUIS D. ZEIDBERG[4]

[1]University of Rhode Island, Department of Biological Sciences, 120 Flagg Road, Kingston, RI 02881, [2]Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, [3]Conservation International, 2011 Crystal Drive, Suite 500, Arlington, VA 22202, [4]California Department of Fish and Game, Marine Region, 20 Lower Ragsdale Drive, Suite 100, Monterey, CA 93940, USA

Squids of the family Bathyteuthidae have generally been infrequently encountered in their deep-sea habitat. Remotely operated vehicles were used to observe seven individuals in situ *in the Monterey Submarine Canyon, CA, USA. One of these was a female* Bathyteuthis berryi *holding a sheet with approximately 360 embedded embryos. Examination of this female after collection revealed the presence of a seminal receptacle on the buccal membrane. We present some potential costs and benefits of post-spawning egg care, a strategy that is now known for two families of deep-dwelling squids, but may turn out to be more common with the increasing exploration of the deep sea.*

INTRODUCTION

Bathyteuthids are small, solitary red squids that inhabit lower mesopelagic and upper bathypelagic depths worldwide (Roper, 1969). Three species are currently recognized: *Bathyteuthis abyssicola* Hoyle 1885 is nearly cosmopolitan, *B. bacidifera* Roper 1968 is known from the eastern equatorial Pacific, and *B. berryi* Roper 1968 occurs in the eastern North Pacific (Roper, 1968, 1969). *Bathyteuthis* sp. A, which is morphologically similar to *B. berryi* but may be a new species, inhabits the North Atlantic (Vecchione *et al.*, 2010). Despite their widespread distribution, individual *Bathyteuthis* have been relatively rare in trawl collections ever since the 'Challenger Expedition' collected the first specimens (Hoyle, 1885; Voss, 1956; Young, 1972a; Lu & Williams, 1994; Vecchione *et al.*, 2010). Direct observations from undersea vehicles have been even less frequent (Seibel *et al.*, 2004; Bush & Robison, 2007).

Reproduction and spawning in many deep-sea squids is poorly known. In general, squids appear to be semelparous with only a single gonad maturation period during the life cycle. However in some species, this period may be extended and eggs may be spawned multiple times (Rocha *et al.*, 2001). Some neritic species like *Doryteuthis opalescens* Berry 1911 leave their eggs in tough, protective cases attached to the sea floor, thus shielded from parasites, microbial infection and predation (Kaufman *et al.*, 1998). In many oceanic squids for which the details are known, spawning concludes when

a female releases a clutch of fertilized eggs embedded in a neutrally-buoyant, passively drifting gelatinous mass (Boyle & Rodhouse, 2005; Staaf *et al.*, 2008). For example, in *Thysanoteuthis rhombus* Troschel 1857 a double egg strand is wound around a cylindrical mass (Guerra *et al.*, 2002). In enoploteuthids, however, eggs are spawned singly or arranged within strings (Hayashi, 1989; Laptikhovsky, 1999), while egg balloons are produced in ommastrephids (Durward *et al.*, 1980; O'Shea *et al.*, 2004; Staaf *et al.*, 2008). No details are available for the Bathyteuthidae.

Evidence of an additional strategy, brooding, began to accumulate in 1995 (Okutani *et al.*, 1995; Arkhipkin & Bjorke, 1999; Seibel *et al.*, 2000) and was subsequently confirmed with direct observation (Seibel *et al.*, 2005). Female *Gonatus onyx* Young 1972 were observed in the Monterey Submarine Canyon (MSC) brooding their eggs deep in the water-column. The eggs were set in a single-layer, tube-shaped egg mass attached by the squid's arm hooks. Embryonic development is estimated to take up to 9 months. Because of the apparent advantages, Seibel *et al.* (2005) predicted that more deep-sea squids would be found brooding. We report here a second case of brooding by a deep-living squid—*Bathyteuthis berryi*—based on *in situ* observations, again made over the MSC off central California.

MATERIALS AND METHODS

In situ observations

We used two of the Monterey Bay Aquarium Research Institute (MBARI)'s remotely operated vehicles (ROVs),

Corresponding author:
S.L. Bush
Email: stephanie_bush@mail.uri.edu

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ROV 'Tiburon' and ROV 'Doc Ricketts', for *in situ* observation and sampling of bathyteuthids. Each vehicle was equipped with a high-resolution video system that served as a visual connection to the deep water-column. The primary video camera was a broadcast-quality, Panasonic WV-E550 (800 TVL resolution) fitted with a Fujinon S8.5 × 4.2BERM-16B zoom lens. This system allowed us to make detailed observations with the ROV at a distance of 5–10 m from a subject. This standoff ability, combined with the vehicle's variable ballast system that allows the ROV to be trimmed to neutral buoyancy, optimizes these platforms for mid-water observations. Each vehicle had a complement of collection devices for retrieving specimens with minimal disturbance, allowing for further observations and experimentation in the laboratory at sea and ashore. Both vehicles carried instrumentation for the precise measurement of depth, temperature, salinity and oxygen concentration (Robison *et al.*, 2010). These methods have been used successfully for observing deep-pelagic cephalopods for many years (Hunt *et al.*, 2000; Robison *et al.*, 2003; Zeidberg, 2004; Seibel *et al.*, 2005; Bush & Robison, 2007; Bush *et al.*, 2009).

Video sequences of escape jetting by four bathyteuthids were analysed to compare the swimming speeds of brooding (N = 1) and non-brooding (N = 3) individuals. The sequences ranged in duration from 0.83–2.57 seconds. Non-compressed video frames were captured with IrfanView, shareware that converted the individual video frames to bitmap images. ImageJ shareware was then used to quantify the swimming speeds of the individual squids (Zeidberg, 2004). Mantle length (ML) was measured in pixels by drawing a line over this body part in 4–16 frames. This allows the size of the animal to be recalculated frame-by-frame as the animal escapes. The ImageJ marking tool was used to give an x and y coordinate to both the mantle tip of the squid and an inanimate particle judged to be at the same depth of field as the focal animal. Distance moved was then calculated from the difference of movement between the particle and the squid via the instantaneous equation. Squid escape jet velocity, in ML s^{-1}, was calculated by dividing the distance by ML, and then this quotient by the time (1/29.97 seconds) for each video frame. Estimates of velocity along the x- and y-coordinates ignore depth of field; therefore any movement along the z-axis would cause an underestimation in our calculations. Thus measurements of velocities and swimming capabilities are probably conservative.

Laboratory examinations

A single brooding female, and much of the egg sheet she was carrying, were collected and brought to the surface for further study. Measurements of the female specimen's dimensions

were made and then used to estimate embryo sizes from video frame grabs. Oocytes from the ovary were measured using a stereomicroscope. An organ was found on the female's buccal membrane, which we suspected was a seminal receptacle for the storage of sperm. Histological sections of this organ were therefore obtained by dehydrating the tissue in a graded ethanol series, clearing it with toluene, and embedding it in paraffin wax. Cross-sections of 3 μm thick were mounted on slides and stained with haemotoxylin and eosin.

RESULTS

In situ observations

Seven specimens of *Bathyteuthis* spp. have been observed by MBARI ROVs during hundreds of dives through their known depth-range (Table 1). Individuals are easily identified as members of the genus by their small, round fins and anteriorly directed eyes as compared to other squids (Young & Vecchione, 2008). However, identification to species is not possible from *in situ* video alone because many of the identifying characteristics (e.g. tentacle sucker counts) cannot be determined without specimen collection. Collected specimens were identified as either *B. berryi* or *B. bacidifera* (Table 1). The shallowest sighting occurred at 942 m and the deepest at 1424 m. Temperature ranged from 2.97 to 4.25°C, salinity from 34.24 to 34.48 psu, and oxygen concentration from 0.24 to 0.79 ml/l (Table 1).

In all encounters, the squid was solitary, and initially positioned vertically in the water column with the head and arms down. All individuals remained dark red on the dorsal side, and while sometimes the same colour ventrally, the head and anterior–ventral mantle were commonly white. Specimens were either motionless at first sighting or were gently undulating the margins of their fins, apparently relying on neutral buoyancy to maintain their vertical position (Seibel *et al.*, 2004). As the ROV approached, fin movement typically expanded to involve the entire fin. Continued approach by the vehicle always led to fast escape attempts, and included rapid and unpredictable changes in speed and direction. Inking was a common component of escape behaviour (Bush & Robison, 2007).

A single brooding female *Bathyteuthis* specimen was encountered at a depth of 1351 m during the ascent phase of a dive at 36.33°N 122.90°W in September 2005 (Table 1). This individual was situated among numerous ink releases, indicating that the squid had moved around a relatively large area before stopping to maintain position. The squid

Table 1. Encounters with *Bathyteuthis* spp. by Monterey Bay Aquarium Research Institute remotely operated vehicles (ROVs). Collected individuals, which could be identified to species, are indicated, brooding female *Bathyteuthis berryi*[1], male *Bathyteuthis bacidifera*[2].

| Date	ROV dive	Depth (m)	Temperature (°C)	O₂ (ml/l)	Salinity (psu)
17 November 2000	T240	942	4.25	0.24	34.32
20 February 2002	T395	1342	3.09	0.70	34.28
20 March 2002	T409	1424	2.97	0.71	34.24
21 September 2005[1]	T895	1351	3.07	0.67	34.48
8 August 2007	T1114	1140	3.59	0.53	34.28
3 November 2007	T1149	1307	3.17	0.79	34.35
7 May 2009[2]	DR20	1324	3.08	0.72	34.48

was holding an elongate, single-layer egg sheet with its arms (Figure 1A). The sheet was translucent white, tapered at both ends, and was hanging beneath the vertically-oriented adult. An irregular, lengthwise split in the sheet ran from the distal end up about a third of its length. The egg sheet was slightly wider than the female's mantle width (MW = 26.8 mm) and nearly three times the mantle length (ML = 72 mm); therefore the egg sheet was approximately 30 mm wide by 200 mm long. In the bright white lights of the ROV, the sheet had an iridescent sheen (Figure 1B).

The egg sheet appeared to be principally attached to suckers at the base of arms I (the dorsal pair; cephalopod arms are annotated as left (L) or right (R), I (dorsal)–IV (ventral)). The distal portion of arm LI was curved over the top of the tapered end of the sheet, while distal RI was curved underneath. Arms II provided lateral support and arms III and IV supported ventrally without appearing to be attached. Only a portion of one tentacle was visible, tucked inside the crown of arms.

Each embryo was contained within its own capsule in the sheet and these capsules were mostly arranged in rows; central capsules had a roughly hexagonal outline whereas capsules on the edge of the sheet were pentagonal (Figure 1C). *In situ* digital images revealed approximately 360 individual embryos encapsulated within the sheet. Each embryo rotated freely within its capsule to maintain a head-down position when the orientation of the sheet changed (Figure 1A). All embryos were at the same stage (22–24 in Watanabe *et al.*, 1996), and were estimated to be 5 mm total length (TL).

When we first encountered the brooding squid, it appeared to be neutrally buoyant as evidenced by: (1) the fins were not moving; (2) ink was slowly trickling out of the siphon, indicating that the squid was not jetting rapidly; and (3) the specimen's position relative to the surrounding pseudomorphs did not change. As the neutrally buoyant ROV approached slowly, the animal's fin margins began to undulate. After a sequence of evasive ink-jet manoeuvres instigated by the ROV bow wave reaching the animal, the squid stopped in a vertical posture and resumed sculling its fins asynchronously by moving the leading and trailing edges of each fin in opposing directions. These fin movements rotated the individual around its vertical axis so that the ventral mantle faced the vehicle and shielded the squid's eyes from the ROV's lights. As the ROV manoeuvred, the squid again jetted downward trailing the egg sheet, changing direction frequently. During these abrupt movements eggs were not dislodged, nor did the sheet shred when dragged across ROV hardware during capture.

Non-brooding squid escape speeds averaged 6.6 ± 3.7 ML s^{-1} (N = 3 squids, four jet escapes total, Table 1, supplemental data). The brooding female's one escape run averaged 2.3 ± 1.4 ML s^{-1}. In a typical sequence, the squid used its fins to rotate from a vertical orientation, with the dorsal mantle facing away from the ROV, to a dorsal-side-up position. It then executed its escape in four steps: (1) hyperinflation of the mantle; (2) after a vigorous flap the fins are streamlined against the mantle; (3) arms snap like the C-start of fish; and (4) the squid jets out of the video frame. Variations in the pattern included ink release at the start of
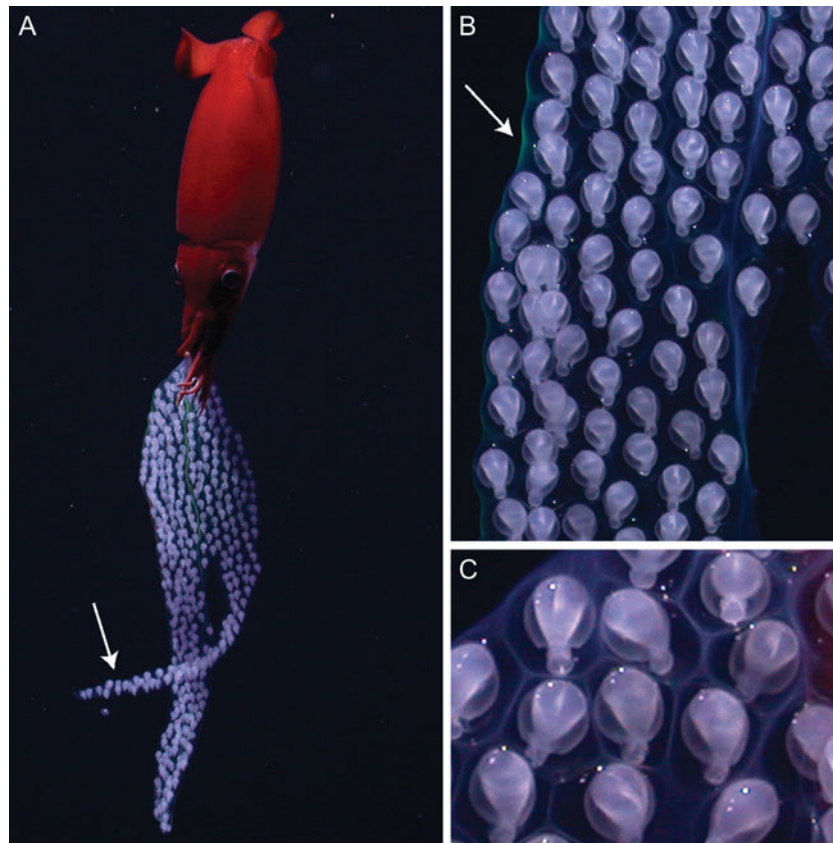
Fig. 1. *In situ* images of brooding *Bathyteuthis berryi*. (A) Female *B. berryi* holding single layer egg sheet. Note that the embryos maintain a head-down orientation (arrow) no matter the orientation of the sheet; (B) egg sheet showing iridescence along the edge (arrow); (C) embryos within individual capsules.

a jet pulse, pulse frequency, changes in escape direction and angle, and curvature of the path. The jet escape of the brooding female was performed with the propulsion from one mantle contraction and the peak speed was achieved before completely exiting the frame of view. For the non-brooding squids, the average speed of the escape jet was achieved or exceeded within 2–6 frames or < 0.23 seconds. The brooding female took ten frames or 0.33 seconds to reach her average escape jet speed. While the speed attained by the non-brooding individuals was greater than that of the brooder, low sample sizes precluded statistical analysis.

Laboratory examinations

The brooding female was identified as an individual of *B. berryi* because each of the arms in pairs I–III had 250–300 suckers, and the protective arm membranes were well-developed, but lacked free trabeculae (Roper, 1968). There was a high concentration of very small suckers at the bases of arms I and II. Arms III and IV had fewer suckers at their base. Fragments of the egg sheet base were still attached to suckers on the female's arms I, with at least one strand attached to arm LII. The specimen measured 72 mm dorsal ML, 60.4 mm ventral ML, 26.8 mm MW, 27.6 mm head width, and 7.2 mm eye diameter (Table 2, supplemental data). The left and right tentacles were both intact and measured 118.8 and 111.9 mm, respectively. Internally, the brooding female had a well-developed digestive gland that was suffused with orange-red oil, some of which had seeped into the mantle cavity.

The egg sheet, while appearing strong and flexible before capture, had disintegrated in the collection container by the time the ROV returned to the surface two hours later. Most of the 205 (of the estimated 360) embryos were still alive when they reached the shipboard laboratory, however, observations under the microscope revealed that the embryos were sufficiently damaged, perhaps because of premature hatching or the change in pressure between the collection depth (1351 m) and the surface, as to preclude measurements. All embryos died within the next several hours.

The female's oviducts were empty, but the ovary contained about 300 post-ovulatory follicles. Post-ovulatory follicles remain in the ovary after ovulation and are therefore used as indicators of previous spawning activity (Hunter & Macewicz, 1985; Melo & Sauer, 1998). As these follicles are the remains of recently ovulated eggs, the number of post-ovulatory follicles may be used to estimate batch fecundity, i.e. the number of eggs spawned in the last batch of eggs. The correspondence between the number of post-ovulatory follicles and the spawned eggs (~360) indicates that this female probably had not spawned more eggs than those carried in the egg sheet reported here. *Bathyteuthis berryi* therefore probably has a single spawning event.

Inside the ovary, 367 atresian or resorptive oocytes (0.1–1.0 mm) were also present. Atresia is the process of oocyte and follicle resorption, altering the oocyte structure as an indicator for the destruction and resorption of oocytes (Bagenal, 1978; Melo & Sauer, 1998). The potential fecundity (spawned eggs + resorptive oocytes) of the brooding female *B. berryi* was 730–750 oocytes, of which about half were being resorbed. It is not uncommon to find unspawned eggs in female cephalopods that have died following spawning (e.g. Nesis, 1995; Nesis *et al.*, 1998; Laptikhovsky *et al.*, 2007).

On the ventral side of the buccal membrane a small (~1 mm long by ~0.5 mm wide) droplet-shaped organ was present, with an opening directed slightly to the left (Figure 2). Histological sections showed the presence of sperm, confirming that this organ functions as a seminal receptacle, where sperm from spermatangia are stored (Figure 3). An exact reconstruction of the internal structure of the seminal receptacle was not possible, but it appears to be divided into at least 7 convoluted spaces consisting of cuboidal, ciliated epithelial cells with oval nuclei (Figure 3).

DISCUSSION

As predicted by Seibel *et al.* (2005), another deep-living squid species has been found that provides post-spawning egg care. The Gonatidae and Bathyteuthidae are not closely related families (Lindgren & Daly, 2007), suggesting that brooding has evolved independently at least twice. Brooding in these squids differs in a number of important details (Table 3, supplemental data). First, *B. berryi* broods its egg sheet within the normal adult depth-range. In contrast, *G. onyx* broods its eggs substantially deeper (1500–2500 m) than the depth-range where adults are typically found (0–1000 m), indicating that females migrate vertically from mesopelagic, relatively predator-rich waters to the bathypelagic to brood (Hunt &
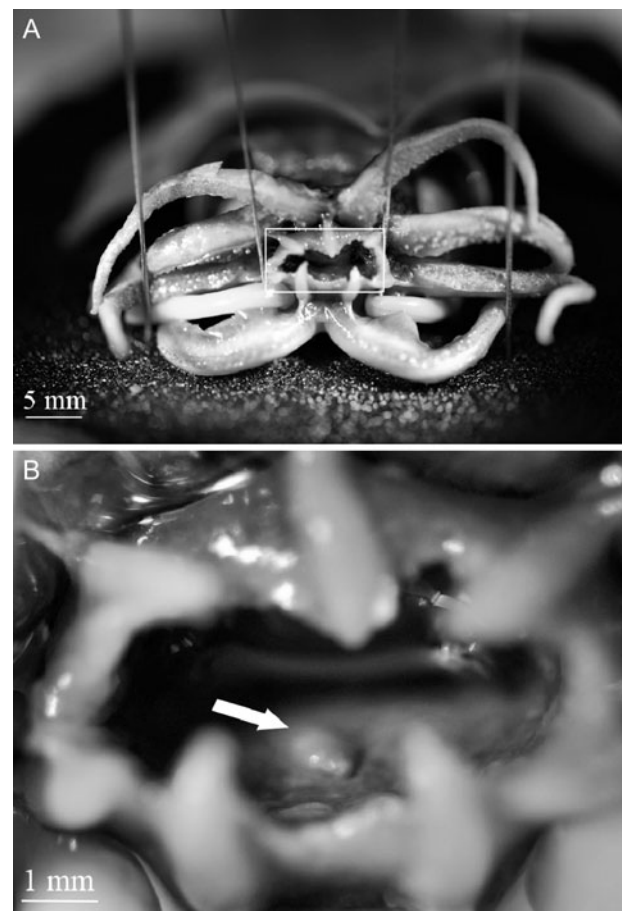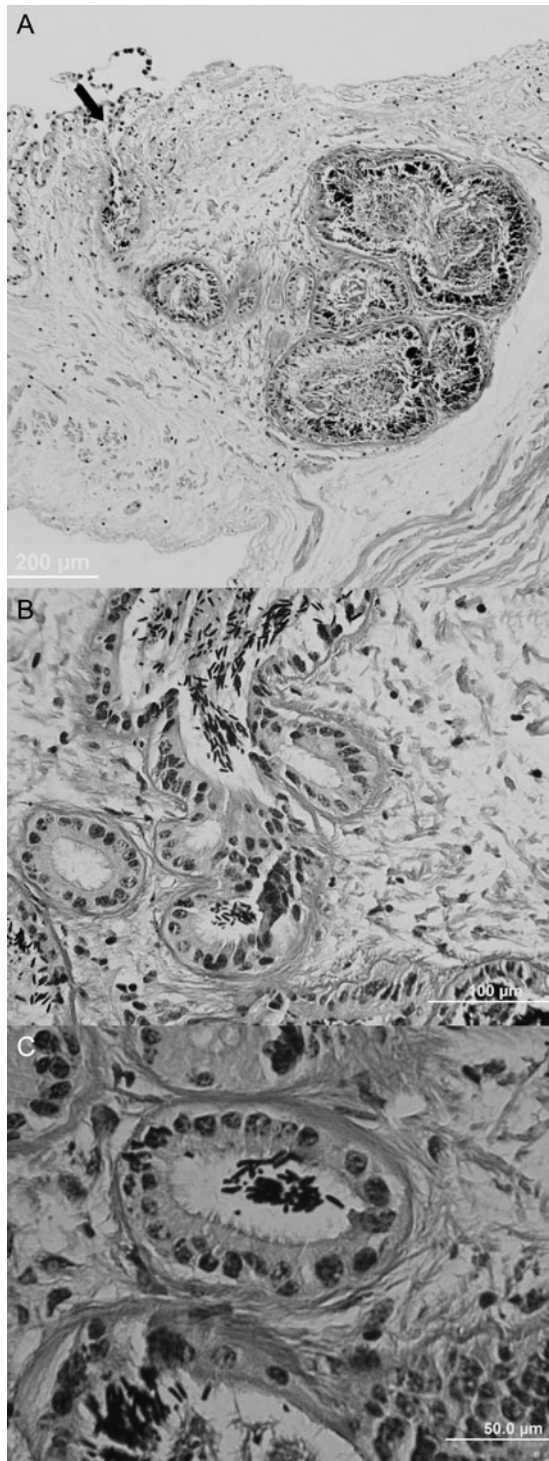

Fig. 2. Oral view of the arm crown of *Bathyteuthis berryi* showing (A) the buccal membrane; (B) a close-up of the buccal membrane of *B. berryi* with the seminal receptacle (arrow).

Fig. 3. Histological sections of the seminal receptacle of *Bathyteuthis berryi*. (A) A longitudinal section through the seminal receptacle showing what presumably is the opening of the seminal receptacle to the exterior (arrow); (B) a close-up of part of the seminal receptacle showing the convoluted spaces of the receptacle; (C) a section through one of the spaces of the seminal receptacle showing the walls of the space consisting of cuboidal, ciliated epithelial cells with oval nuclei. In the centre the black elongated nuclei of the spermatozoa are visible.

Seibel, 2000; Seibel *et al.*, 2005). Additionally, *B. berryi* retains both feeding tentacles for at least part of the brooding period, while *G. onyx* does not. The smaller squid, *B. berryi*, has fewer, relatively larger eggs than *G. onyx*, which has an order of magnitude more, but smaller eggs (Seibel *et al.*, 2005). *Gonatus onyx* regularly pushed water through its voluminous, tubular egg sheet, presumably to keep it ventilated inside as well as out. The *B. berryi* female was not observed pulsing water over its eggs, perhaps because the single-layer flat sheet kept them well exposed to ambient O_2 levels during locomotion.

Most deep-living squids achieve neutral buoyancy by storing ammonia in the mantle musculature or coelom (Seibel *et al.*, 2004). This results in poor swimming ability in those ammoniacal species, which are usually found only at bathypelagic depths, where the selective pressure to have quick-escape abilities is reduced (Childress, 1995). By contrast, *B. berryi* is unusual among bathypelagic squids in that it is a rather muscular, strong swimmer and partially achieves neutral buoyancy through high concentrations of a yet unidentified cation rather than using ammonia (Seibel *et al.*, 2004). *Bathyteuthis berryi*, like *G. onyx*, also appears to have high lipid stores that aid neutral buoyancy (Seibel *et al.*, 2000, 2004). Gill diffusion capacity of bathyteuthids is among the highest measured, suggesting further adaptations for rapid jetting in low oxygen environments (Madan & Wells, 1996).

Female care of developing embryos is common among benthic octopuses (Hanlon & Messenger, 1996). Four deep-living pelagic octopuses, *Balitaena pygmaea* Verrill 1884, *Haliphron atlanticus* Steenstrup 1861, *Japetella diaphana* Hoyle 1885, and *Vitreledonella richardi* Joubin 1918, also brood their eggs at depth by holding egg masses in their arms, attached by suckers (Young, 1972b, 1995, 2008a, b; Joubin, 1937 in Young *et al.*, 2010; MBARI, unpublished data). However, feeding during brooding is uncommon in cephalopods (Hanlon & Messenger, 1996). *Gonatus onyx* and *B. berryi* probably stop feeding, even though tentacles were still present in the latter. It appears that suckers on *B. berryi*'s buccal crown may help to hold the proximal end of the egg sheet, which would impede feeding. Both species apparently rely on lipids stored in the digestive gland for nutrition while brooding, and to provide buoyancy (Seibel *et al.*, 2000). The resorption of oocytes provides an additional nutrient source for *B. berryi* during brooding, which may occur when parental energy reserves are inadequate to proceed with oocyte maturation (Melo & Sauer, 1998).

To date there have been no reports on how and where bathyteuthids store sperm. According to Nesis (1995), male bathyteuthids lack a hectocotylus and use their terminal organ to transfer spermatophores to the female during mating. Spermatangia are expelled from the spermatophores, and attach to the female. In *B. berryi*, spermatangia attachment is probably to the buccal membrane as this is the location of the seminal receptacle. How sperm reach the seminal receptacle from the spermatangia in *B. berryi* is not known. The female may pick up the spermatangium by extending the buccal mass and transferring sperm to the receptacle. Sperm may also actively swim from the spermatangium into the seminal receptacle as recent evidence on pygmy squid suggests (Sato *et al.*, 2010). It is unknown how sperm from the seminal receptacle get to the eggs. Perhaps during spawning eggs are moved across the seminal receptacle, which will activate sperm and bring them in contact with eggs. For *Illex illecebrosus* it was suggested that the nidamental gland secretion activates sperm (Duward *et al.*, 1980). Of the

28 families of oceanic decapodiform cephalopods, a specialized storage organ for sperm was previously known only in the Ommastrephidae and Spirulidae (Nesis, 1995); to these we can now add the Bathyteuthidae. In all other known species, sperm remain in spermatangia that are stored on or within the female's body (Nesis, 1995; Hoving et al., 2004, 2008, 2010).

All living coleoid cephalopods are semelparous in their egg production, but their spawning strategies can vary widely (Rocha et al., 2001; Laptikhovsky et al., 2007). In B. berryi the oocytes appear to mature in unison and the ripe ova are spawned in a single event, i.e. synchronous ovulation followed by synchronous spawning. Synchronous ovulation is found in other oceanic squid, including Gonatus antarcticus Lönnberg 1898, Onykia ingens Smith 1881 and Histioteuthis miranda Berry 1918 (Laptikhovsky et al., 2007; Hoving & Lipinski, 2009). This may be a common strategy in deep-sea species. An alternative strategy, asynchronous spawning, allows higher relative fecundities because the total number of eggs that can be spawned is not limited by space in the coelomic cavity (Boletzky, 2003; Laptikhovsky et al., 2007). The brooding of eggs, however, is presumably at the cost of multiple spawning events. Though the duration of senescence is unknown for most deep-sea squids, death is assumed to follow quickly after the final spawning event, as in D. opalescens and O. ingens (Hixon, 1983; Jackson, 1997).

Bathyteuthis berryi incorporates low fecundity into its reproductive strategy but it balances this with large egg size and parental care. Brooding provides protection from predators and parasites, and also allows the parent to determine the depth and thus the hydrographic conditions (T°, S and O_2) under which the eggs will develop, as well as the depth at which they will hatch. Bathyteuthis berryi lives in the upper bathypelagic depth zone, at the deeper end of the distribution gradient of active pelagic predators, and where the pace of life appears to be much slower than in the kilometre above (Robison et al., 2010). The single brooding specimen we observed was found well within the normal depth-range recorded for this species (942–1424 m). Following the equation for cephalopod embryonic development at 5–7°C (Boletzky, 1994; Seibel et al., 2000), and assuming a Q_{10} of 2, we calculate that it may take 9–12 months for B. berryi embryos to reach 5 mm TL at 2–4°C. In B. abyssicola smaller individuals are generally found shallower than adults, so it is assumed that in B. berryi either the female swims into shallow water to initiate hatching or, more likely, paralarvae must migrate to shallow water (Roper, 1969).

For B. berryi, some potential costs and benefits of brooding are outlined below.

Potential costs:

1. Pre-brooding investment in nutrient storage instead of somatic growth, but note that this same investment may also contribute to neutral buoyancy and associated savings in locomotory energetics.
2. Metabolic cost of maintenance; e.g. cleaning and ventilating eggs, if any.
3. Metabolic cost of protection; e.g. predator avoidance and egg sheet production to protect the eggs from parasites.
4. Metabolic cost of maintaining depth in the preferred habitat, which may be minimal for neutrally buoyant species.

5. The extension of life beyond egg laying and the cessation of feeding.
6. Potential cost of reduced fecundity through the inability to extend spawning into multiple or prolonged sessions, and allocation of energy stores to survive brooding rather than egg production.
7. A reduced escape response compared to non-brooding individuals of the same species.

Potential benefits:

1. Large size at hatching leading to increased survival rates of paralarvae.
2. Brooding allows the female to determine the abiotic conditions under which the eggs will develop and the depth where they will hatch.
3. If the egg sheet is neutrally buoyant, it may assist the female in maintaining neutral buoyancy, reducing the energy spent on swimming.

Calow (1987) points out that the benefits derived from brooding can favour semelparity over multiple spawning periods. Additionally, in deep-sea squids the energetic costs of brooding may be high enough to preclude any selective advantage of extended spawning in this already semelparous lineage. Bathyteuthis berryi is a small species, which is thereby limited to low fecundity, and has evolved a strategy that may increase the survivability of embryos by producing relatively large eggs that are protected until hatched. Alternatively, the fact that members of two deep-sea squid families broad eggs in mid-water may indicate that these species are constrained to do so by having a common ancestor that attached eggs to the ocean floor. In either case, as more directed submersible observations are performed, we expect that deep-brooding by meso- and bathypelagic squids will be a more widespread strategy than currently known (Nesis, 1995).

Supplementary materials and methods

The supplementary material refered to in this paper can be found online at journals.cambridge.org/mbi.

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Correspondence should be addressed to:
S.L. Bush
University of Rhode Island
Department of Biological Sciences
120 Flagg Road, Kingston, RI 02881
email: stephanie_bush@mail.uri.edu