New insights on the external features of egg capsules and embryo development in the squid *Loligo vulgaris*

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**ABSTRACT**

The embryonic development of the squid *Loligo vulgaris* was observed from 183 egg masses collected from special devices deployed throughout Cabrera National Park (Baleares Islands, western Mediterranean Sea). Sequence alignment analysis of the cytochrome oxidase I gene revealed that all embryos belonged to *L. vulgaris*. In total, 549 egg capsules were examined. Viable egg capsules (\(n = 420\)) were classified into one of five maturation stages according to the primary external features. The length of the viable egg capsules varied between 40 and 170 mm, and increased with embryonic development. The non-viable capsules (\(n = 129\)) were categorized into four groups: I (Ginger root), non-viable II and III, and empty egg capsule (IV). The percentage of non-viable capsules (i.e. grades I, II and III) was 92.25%. Empty capsules accounted for 7.75% of the total non-viable egg capsules. Embryonic development was classified into a second scale of eight stages. Egg capsule stage and embryonic stage were significantly related (\(n = 420; p < 0.001\)), facilitating the determination of the embryo developmental phase based on the outward appearance of the egg capsules. The embryo development stage based on the external features of the egg capsules might constitute an innovative tool for *in situ* embryological data collection. This new method is neither time consuming nor invasive, and could be helpful in fishing cruises, for scuba diving visual census in natural habitats and for laboratory culture. Slight variability in the developmental embryonic stages within egg capsules from the same egg mass was identified. The origin of this asynchrony is discussed. Chronological appearance of organs was similar to that of the six loliginid species previously examined. However, some developmental changes in the timing or rate of events (heterochronies) were observed: Hoyle’s organ was formed earlier in *L. vulgaris* and the appearance of ventral chromatophores was slightly delayed (2 days) compared with the other species considered.

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Introduction

The transition from egg to the exhaustion of the maternally derived yolk reserves and first feeding is a critical phase within the early life history of cephalopods (Robin et al. 2014). Almost all cephalopods have direct development, and the hatchling is a miniaturized form of the adult in many species. However, the term ‘paralarva’ is used as a general term for planktonic young cephalopods (only in the Teuthoidea and Octopoda) that meet certain ecological and, in some cases, morphological criteria (Young and Harman 1988). Although embryos develop under abiotic conditions within the perivitelline fluid and the capsules and chorions act as a barrier to the diffusion of dissolved gases and other substances, there are several environmental factors affecting the duration and success of this direct development. The effects of these factors (dissolved oxygen, accumulation of metabolic CO₂, seawater properties such as pH, salinity and temperature, essential and non-essential trace elements, etc.) on embryonic development have been recently reviewed (Robin et al. 2014). Steer et al. (2003) showed differences in the embryonic development rate within single egg strings of the loliginid Sepioteuthis australis, with eggs situated at the anchored or proximal end developing more slowly than those situated at the free or distal end. This asynchronous embryonic development and variation in the size of hatchlings within a single egg string have also been previously described by Chung (2003) for Sepioteuthis sepioidea and Sepioteuthis lessoniana. Strathmann and Strathmann (1995) suggested that the proximal embryos were not sufficiently oxygenated, and Chung (2003) observed that large egg clusters tended to obstruct the current, causing less circulation within the egg masses, thereby influencing oxygenation and survival. Therefore, the study of egg and embryos not only offers an interesting model system for understanding developmental biology (Boletzky 2003) but also has important implications for the prediction of embryo survival and the potential impacts of climate change (e.g. warming and acidification processes; André et al. 2010).

The duration of embryonic development and the chronological appearance of different organs in the embryo have been studied in seven loliginid species of the former genus Loligo from different geographical areas: Doryteuthis pealeii from the northwestern Atlantic (Arnold 1965); Loligo forbesii from the eastern Atlantic (Segawa et al. 1988); Heterololigo bleekerii from Japan (Baeg et al. 1992); Loligo reynaudii from Southern African waters (Blackburn et al. 1998); Doryteuthis aff. gahi from Argentinian waters (Barón 2002); Doryteuthis sanpaulensis from Argentinian waters (Barón 2003) and Doryteuthis gahi from Chilean waters (Guerra et al. 2001) and Peruvian waters (Cardoso et al. 2005). Despite the similarity between the general organogenic patterns of these species, changes in the rhythm of the organogenic process (pattern heterochronies) and significant variations in the size of hatchlings were detected. The term, pattern heterochrony, describes an evolutionary shift of morphological characters in developmental timing (Wray and Raff 1990), which is important for generating morphological variations in adult forms (Richardson 1999). In coleoid cephalopods, the pattern heterochrony is known in many tissues and organs (e.g. Baeg et al. 1992; Watanabe et al. 1996; Boletzky 1997).

Although Loligo vulgaris is one of the most important species from a commercial point of view (Jereb et al. 2010) and the embryonic development of this species has been previously described (Naef 2000), until recently, there have been no comparative
studies on these aspects. To fill this gap, the first aim of the present study was to analyse the occurrence of heterochronies in *L. vulgaris* embryonic development.

Moreover, visual censuses undertaken in spawning zones of the Cabrera National Park (Mediterranean Sea) suggest that it is possible to identify the stage of the development of the embryos from the appearance of the egg capsules during field studies. Verifying this hypothesis would simplify sampling and provide a less time consuming and non-invasive method. Consequently, a second aim of the present study emerged: to validate whether a scale designed to classify egg capsule development based on the external appearance was correlated with the classification of the embryonic stages, and this objective was perhaps the most relevant subject addressed in the present study.

**Material and methods**

Thirty devices (cephalopod egg capsule aggregators; CECA; Figure 1) were randomly deployed between June 2012 and June 2013 throughout Cabrera National Park (Mallorca; northwest Mediterranean). The CECAs were recovered monthly, and the attached egg masses were collected (see Cabanellas-Reboredo et al. 2014 for sampling details). Three egg capsules from each egg mass were randomly collected. The egg capsules were preserved in 70% ethanol and transported to the laboratory for further analysis.

A genetic sequence similarity analysis (MEGA 5.2) of the cytochrome oxidase subunit I gene (COI) was performed using BLAST to determine the existence of one or more loliginid species in the samples studied. The analysis was performed using 10 embryos that showed clear morphological and textural differences. The molecular method used is described in Guerra et al. (2010). The sequences were considered as part of the same taxonomic unit when similarity was ≥ 98%, reflecting differences resulting from intra-specific variation and *Taq*-polymerase errors (Nei and Kumar 2000).

The first scale was established based upon external features to define the development stage of the egg capsules. The embryonic stages were determined according to a simplified second scale based upon the XX stage scale of Naef (2000). The first and second scales were contrasted in a contingency matrix and assessed using an independent chi-squared test (Zar 2010).

Embryonic development was classified based on morphological and anatomical features using both light and scanning electron microscopy. The embryos were fixed in 70% ethanol and examined using a compound microscope at magnifications ranging from 4× to 10×. For scanning electron microscopy, the embryos were fixed for 4 h in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3 at 4°C) and washed for 30 min in the same buffer. The sample was subsequently dehydrated in an ethanol series, critical-point dried in CO₂ using a Polaron E3000 and sputter-coated in a Polaron SC500 using 60% gold–palladium. The samples were subsequently examined using a Philips XC30 scanning electron microscope operating at 10–20 kV.

The order of appearance of the following 13 structures was recorded: eye vesicle closed (EC); eye vesicle invagination begins (EI); fully establish eyelid (PED); funnel formation begins (FF); Hoyle’s organ appears (HO); ink sack appears (IS); principal eyelid appears (PL); primordia of suckers appear (PS); primordia of shell gland appear (PSG); retinal pigmentation begins (RP); shell gland closed (SGC); statocyst first
presence (ST); and ventral mantle chromatophores appear (VCH). The order of formation of the studied structures was determined after examining embryos of different ages, and recording differences among them using an anatomical comparative approach according to Naef (2000) and Arnold (1965). The age of the embryos was estimated based on previously designated developmental stages.

**Results**

A total of 183 egg masses were collected. The number of egg capsules per egg mass ranged from 7 to 274. A random sample of 549 egg capsules was examined. Viable egg capsules \( n = 420 \) were classified as one of five stages: I: incipient

![Figure 1. Cephalopod egg capsule aggregator (CECA) for Loligo vulgaris. Structure of the CECA using a rope (diameter 1.2 cm), a buoy to extend the rope and a weight to fix the structure in place. The first 2 m of the rope from the bottom contained five knots and 16 plastic flanges placed among these knots (to increase the attachment surface). The figure also shows details of the egg capsules attached to the rope and flanges.](image)
The length of the viable egg capsules varied between 40 and 170 mm, increasing with embryonic development (Figure 2). Non-viable capsules \((n = 129)\) were categorized into four groups: I (Ginger root), non-viable II and III, and egg capsule empty (IV) (Table 1). The percentage of non-viable capsules (i.e. I, II and III) was 92.25. Empty capsules accounted for 7.75% of the total non-viable egg capsules.

The sequence alignment analysis of the partial COI sequences revealed that all embryos belonged to \(L. vulgaris\) (MEGA 2.5, BLAST, \(\geq 98\%\) \(L. vulgaris\)).

Embryonic development was categorized as one of eight stages (Table 1 and Figure 3). These stages did not include gastrulation, but only organogenesis. Hence, the first stage (stage 1) encompassed the whole pre-organogenetic process, from the earliest cellular divisions (egg merely comprising the blastoderm and yolk). In stage 2, the first eye rudiments could be discerned. Stage 3 was characterized by the presence of arm rudiments and shell gland elongation. The embryo adopted a miniature adult shape between stages 4 and 5. Chromatophores and retinal pigmentation occurred in stage 6. Finally, the transitional phase between stages 7 and 8 was marked by the presence of the eyelid.

Ten full-grown egg capsules were randomly selected and the developmental embryo stages within the capsule were recorded. Total embryos examined (1130)
Figure 2. Aspect of the five (I–V) egg capsule stages preserved in 70% ethanol.
were in different stages of development: 0.97%, 5.75% and 93.28% in stages 6, 7 and 8, respectively. This slight asynchrony could represent between 4 and 8 days (Table 2).

Empty capsules and malformations in embryos were significantly lower ($p < 0.05$) in egg masses with many capsules (100–274) than in those with few capsules (7–99) capsules.

The correspondence between the embryonic development stages of Naef (2000) and Arnold (1965) and the stages proposed in the present study is shown in Table 2. The age of each stage was calculated according to Naef (2000, plates 1–7).

Table 3 shows the distribution of each embryonic stage (stages 1–8) in each viable capsule stage (stages I–V) in both absolute and percentage values. The chi-squared test showed that the scales were significantly related ($n = 420; p < 0.001$). This correlation facilitated the determination of the embryo developmental stage from the appearance of the egg capsules, confirming that the embryonic stages might be accurately predicted based on external egg capsule features.

From the observed sequence of the development of the major embryonic features, the developmental pattern of *L. vulgaris* was compared with that of *D. pealei*, *H. bleekeri*, *L. forbesii*, *D. aff. gahi*, *D. gahi* and *L. reynaudii* (Arnold 1965; Segawa

**Figure 3.** Embryonic development stages proposed in this study (Arabic numerals) compared with the stages of Naef (Roman numerals), 30× natural size for microphotographs 1–8. Scanning electron microphotography of an embryo at stage 8 (bottom right side). Details showing the closure of the eyelid fold with a small pore (top right side).
Table 2. Correspondence between embryonic development stages proposed by Naef (2000) and Arnold (1965) and the stages proposed in the present study.

| Naef (2000) | Arnold (1965) | Present study | Days old |
|-------------|---------------|---------------|----------|
| I–VI        | 1–15          | 1             | 1–5      |
| VII         | 16            | 2             | 6        |
| VIII        | 17            | 2             | 6⅓       |
| VIII        | 18            | 2             | 6⅓       |
| VIII–IX     | 19            | 3             |           |
| IX          | 20            | 3             | 7        |
| X           | 21            | 4             | 8        |
| XI          | 22            | 4             | 9        |
| XI–XII      | 23            | 4             | 9½       |
| XII         | 24            | 5             | 10       |
| XIII        | 25            | 5             | 11       |
| XIV–XV      | 26            | 5             | 12       |
| XVI–XVII    | 27            | 6             | 13       |
| XVIII       | 28            | 7             | 14       |
| XVIII–XIX   | 29            | 8             | 15–16    |
| XX          | 30            | 8             | 17–21    |

Age of each stage was calculated following Naef (2000, Plates 1–7).

Table 3. Contingency table of the stages of egg capsules (SEC) versus embryonic development stages (ED).

| SEC | ED I | II | III | IV | V | Total |
|-----|-----|----|-----|----|---|-------|
| 1   | 131 | 0  | 0   | 0  | 0 | 131   |
|     | 31.09| 0.00| 0.00| 0.00| 0.00|31.09 |
| 2   | 0   | 29 | 0   | 0  | 0 | 29    |
|     | 0.00| 6.90| 0.00| 0.00| 0.00|6.90  |
| 3   | 0   | 51 | 0   | 0  | 0 | 51    |
|     | 0.00|12.14|0.00|0.00|0.00|12.14 |
| 4   | 0   | 0  | 16  | 0  | 0 | 16    |
|     | 0.00|0.00|3.81 |0.00|0.00|3.81  |
| 5   | 0   | 0  | 46  | 0  | 0 | 46    |
|     | 0.00|0.00|10.95|0.00|0.00|10.95 |
| 6   | 0   | 0  | 0   | 10 | 0 | 10    |
|     | 0.00|0.00|0.00 |2.38|0.00|2.38  |
| 7   | 0   | 0  | 0   | 32 | 0 | 32    |
|     | 0.00|0.00|0.00 |7.62|0.00|7.62  |
| 8   | 0   | 0  | 0   | 86 | 19| 105   |
|     | 0.00|0.00|0.00 |20.48|4.52|25.00 |
| Total|131|80|62|128|19|420|
|     |31.19|19.05|14.76|30.48|4.52|100.00|

The numbers within each cell represent the number of individuals in absolute and relative terms.

et al. 1988; Baeg et al. 1992; Blackburn et al. 1998; Guerra et al. 2001; Barón 2002) (Figure 4). The pattern of chronological appearance was similar for the seven examined species. However, some heterochronies were identified. Hence, the Hoyle’s organ formed earlier in *L. vulgaris* and the appearance of ventral chromatophores was slightly delayed (2 days). The primary eyelid (PEL) or corneal fold was introduced here as a determinant of the last stage of *L. vulgaris* embryogenesis; this character was not considered in the other species.
Discussion

Protective egg capsules had increased turgidity with increasing maturity when the eggs were preserved in 70% ethanol (Figure 2). However, the visual observations in the field revealed that the capsules with the most developed eggs were more gelatinous and flaccid, potentially simplifying hatching, as in other loliginid squids (Arkhipkin et al. 2000). Consistently, the correlation between the egg capsules and embryo development must be determined using capsules preserved in 70% ethanol.

The range of lengths of L. vulgaris egg capsules was slightly shorter (40 mm) and slightly longer (170 mm) than that according to the Worms review of 1893 (60–160 mm). Mangold-Wirz (1963) previously described fecundity, in terms of number of eggs produced, as the process in which a female deposits 30–60 egg capsules each containing 50 to 130 eggs. However, we observed that 13.66% of the egg masses collected in the CECA contained more than 60 egg capsules. Hence, a different hypothesis has emerged: (1) CECAs could operate as effective spawning attractors, as most loliginid species frequently attach their eggs onto ropes, nets, traps and other fishing gear when these artificial structures are available; (2) the large eggs masses could be spawned by several females, although sampling was designed to individually

Figure 4. Comparison of the chronological appearance of selected organs in seven loliginid species from previous studies (Arnold 1965; Segawa et al. 1988; Baeg et al. 1992; Guerra et al. 2001; Barón 2002) and the present study. The embryonic stages of Arnold were used because these stages were employed in previous comparisons (Guerra et al. 2001). Abbreviations: EC, eye vesicle closed; EI, eye vesicle invagination begins; FF, funnel formation begins; HO, Hoyle’s organ appears; IS, ink sack appears; PED, fully establish eyelid; PL, primary lip; PS, primordia of suckers appear; SGC, shell gland closed; ST, statocyst first presence; and VCH, ventral mantle chromatophores appear.
select the egg clutches of each female squid; or (3) the fecundity of female squid could be much larger than expected. In any case, further studies must be conducted to determine the true hypothesis.

The egg capsule increased in size during egg development. The sharp increase in egg size was observed in stages XVI–XIX of embryonic development, when the embryo experiences a strong size increase, particularly in the longitudinal axis, whereas the outer yolk is rapidly reduced, partially through the utilization of the nutritive material and the active transfer of the yolk mass to the inner yolk organ. Nevertheless, no statistically significant increase in capsule dimensions during D. gahi egg development was observed in the cold waters of East Falkland. This observation might partially reflect differences in capsule gel properties and the partial destruction of capsule envelopes during long embryonic development at low temperatures (6.5–9°C) and lower capsule turgidity (Arkhipkin et al. 2000). Moreover, the colour of the egg capsules changed during capsule development, turning a brownish hue. This colour change primarily reflects the appearance of chromatophores in embryos, which occurs during the later embryonic phases (Naef 2000). We observed a subsequent graduation in the brownish colour of the egg capsules: the greater was the chromatophores’ expansion the deeper was the brown colour of the capsule.

Variability in the stages of embryonic development within an egg capsule from the same egg mass was observed. This asynchronic embryo development within the same egg capsule suggests that the eggs do not progress simultaneously, as previously suggested for other loliginid species. Nevertheless, such variability has been observed in other loliginid species, such as S. australis and S. lessoniana. Different rhythms of embryonic development in a single egg capsule could be provoked through external environmental factors (Chaffee and Strathmann 1984; Hanlon et al. 1989; Arkhipkin et al. 2000; Cronin and Seymour 2000; Oosthuizen et al. 2002; Gutowska and Melzner 2009; Rosa et al. 2012). However, not only the external environment influences the embryonic development rate, but also embryo mortality and the environment within the egg capsule. Hence, the number and position of egg capsules within the egg mass and the position of embryos within an egg capsule might lead to differences in hatching traits, such as the developmental rate, survival and growth resulting from insufficient oxygenation. We observed that there were more empty capsules and malformations in the embryos in egg masses with many capsules (n > 100) than in those with few capsules (n < 99). The need to ensure the adequate oxygenation of all the egg capsules in an egg mass, and, therefore, adequate rates of survival, might explain the preferred spawning habitats of L. vulgaris in Cabrera National Park being located in zones where currents prevail. The asynchrony in the embryo development within a capsule was a few days (4–8 days) and affected three embryo stages (Table 2). This observation has some implications for the accuracy of the classification of the mass based on the external appearance of the egg capsule and should be considered. However, we considered that this factor is negligible in the present case because 93.28% of the embryos were close to hatching (stages XIX and XX).

The non-viable egg capsules included cases of empty capsules and types of Ginger Root and non-viable embryos II–III. Aside from environmental effects, such phenomena might also represent capsules from exhausted females, which frequently lay egg capsules without embryos.
As indicated above, the pattern of the chronological appearance of organs is similar in the loliginid species examined so far. However, several heterochronies are evident among these species. A slight delay in the appearance of the first ventral chromatophores was observed between *L. vulgaris* and the other six species. However, the formation of Hoyle’s organ on the examined individuals of *L. vulgaris* occurred at least two stages earlier than in the other loliginid species. The appearance of this hatching gland is closely related to hatching time (Boletzky 1987). The length of embryonic development was assumed in the present study to be 30 days, which is an appropriate value considering the water temperature (18–20°C) in Cabrera at the time and depth of egg mass collection. Nevertheless, the length of the embryonic development of this species is strictly dependent on temperature (when oxygen supply is optimal) and can take 70 days at 10°C (Boletzky 1987).

According to Shigeno et al. (2001), the overall organogenesis pattern is highly conserved in loliginid embryonic development. However, heterochronic and morphological variations are easily recognized. Hence, for example, eye pigmentation is not initiated at the same stage as in other loliginids, and it is unlikely that the heterochronic variation in eye pigmentation is correlated with embryonic size. The results of Shigeno et al. (2001) might be used to interpret these differences as an adaptation for certain post-embryonic modes of life, suggesting that an environmental variable such as temperature, which strongly influences the duration of embryonic development, has little relevance.

Although the embryonic character primary lip was not examined in the other species, we examined this parameter in *L. vulgaris* because it is particularly important for the Myopsida lineage. Embryos at stage 6 (corresponding to Naef’s stage XVI) had a fully established primary eyelid. However, the formation and subsequent closure of the eyelid fold with a small pore (myopsid eye) was definitively adopted at the end of embryonic development (stages 7–8, Table 2), consistent with Naef (2000) and Arnold (1965).

Taken together, these results showed that the *L. vulgaris* embryonic stage could be predicted based on the external aspect of the egg capsules. The embryo development stage designed from egg capsules might constitute an innovative tool for *in situ* embryological data collection. This new method is less time consuming and non-invasive. Consequently, this new tool could be useful for fishing cruises in which loliginid egg masses are accidentally caught. This method could also be helpful in obtaining the scuba diving visual census of natural habitats and in laboratory culture.

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References

André J, Haddon M, Pecl GT. 2010. Modelling climate-change-induced nonlinear thresholds in cephalopod population dynamics. Glob Change Biol. 16:2866–2875.

Arkhipkin AI, Laptikhovsky VV, Middleton DAJ. 2000. Adaptations for the cold water spawning in squid of the family Loliginidae: Loligo gahi around the Falkland Islands. J Mollusc Stud. 66:551–564.

Arnold JM. 1965. Normal embryonic stages of the squid, Loligo pealei (Lesueur). Biol Bull. 128:23–32.

Baeg GH, Sakurai Y, Shimazaki K. 1992. Embryonic stages of Loligo bleekeri Keferstein (Mollusca: Cephalopoda). Veliger. 35:234–241.

Barón PJ. 2002. Embryonic development of Loligo gahi and modeling of hatching frequency distributions in Patagonia. Bull Mar Sci. 71:165–173.

Barón PJ. 2003. Embryonic development of the South American long-fin squid Loligo sanpaulensis Brakoniecki. 1984. J Mollus Stud. 69:221–227.

Blackburn S, Sauer W, Lipinski MR. 1998. The embryonic development of the chokka squid, Loligo vulgaris reynaudii. Veliger. 41:249–258.

Boletzky S. 1987. Cephalopod life cycles, Vol II. Embryonic phase. London: Academic Press; p. 5–31.

Boletzky S. 1997. Developmental constraints and heterochrony: a new look at offspring size in cephalopod molluscs. Geobios m. sp. 30:267–275.

Boletzky S. 2003. Biology of early life stages of cephalopods. Adv Mar Biol. 44:147–159.

Cabanellas-Reboredo M, Calvo-Manazza M, Morales-Nin B, Palmer M, Hernández-Urcera J, Garci ME, González ÁAF, Guerra ÁA, Morales-Nín B. 2014. Using artificial devices for identifying spawning preferences of the European squid: usefulness and limitations. Fish Res. 157:70–77.

Cardoso F, Baltazar P, Bautista J. 2005. The early development of the Patagonian squid Loligo gahi d’Orbigny, 1835 in Peruvian Waters (Cephalopoda: Loliginidae). Rev Peru Biol. 12:369–376.

Chaffee C, Strathmann R. 1984. Constraints on egg masses. I. Retarded development within thick egg masses. J Exp Mar Biol Ecol. 84:73–83.

Chung WS. 2003. Effects of temperature, salinity and photoperiod on the deposition of growth increments in statoliths of the oval squid Sepioteuthis lessoniana Lesson, 1830 (Cephalopoda: Loliginidae) during early stages [MSc thesis]. National Sun Yat-sen University.

Cronin ER, Seymour RS. 2000. Respiration of the eggs of the giant cuttlefish Sepia apama. Mar Biol. 136:863–870.

Guerra A, Rocha F, González AF. 2001. Embryonic stages of the Patagonian Squid Loligo gahi (Mollusca: Cephalopoda). Veliger. 44:109–115.

Guerra A, Roura A, González AF, Pascual S, Cherel Y, Pérez-Losada M. 2010. Morphological and genetic evidence that Octopus vulgaris Cuvier, 1797 inhabitst Amsterdam and Saint Paul Islands (southern Indian Ocean). ICES J Mar Sci. 67:1401–1407.

Gutowska M, Melzner F. 2009. Abiotic conditions in cephalopod (Sepia officinalis) eggs: embryonic development at low pH and high pCO2. Mar Biol. 156:515–519.

Hanlon RT, Bidwell JP, Tai R. 1989. Strontium is required for statolith development and thus normal swimming behaviour of hatchling cephalopods. J Exp Biol. 141:187–195.

Jereb P, Roper CFE, Vecchione M. 2010. Family Loliginidae. In: Jereb P, Roper CFE, editors. Cephalopods of the world. An annotated and illustrated catalogue of species known to date. Volume 2. Myopsid and Oegopsid Squids. FAO species catalogue for fisheries purposes. No 4, Vol. 2. Rome: FAO; p. 38–117.
Mangold-Wirz K. 1963. Biologie des céphalopodes bentiques et nectoniques de la Mer Catalane. Vie et Milieu. 13:285.

Naef A. 2000. The Cephalopoda-Embryology. Smithsonian Institution Libraries. English translation by Boletzky S.v. of: Die Cephalopoden (Embryologie). Fauna e Flora del Golfo di Napoli: monograph No 35. Editori: Bardi G (Roma) and Friedländer & Sohn (Berlin), 1928.

Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. New York: Oxford University Press.

Oosthuizen A, Roberts MJ, Sauer WHH. 2002. Temperature effects on the embryonic development and hatching success of the squid Loligo vulgaris reynaudii. Bull Mar Sci. 71:619–632.

Richardson MK. 1999. Vertebrate evolution: the developmental origins of adult variation. BioEssays. 21:604–613.

Robin JP, Roberts M, Zeidber L, Bloor I, Rodriguez A, Briceño F, Downey N, Mascaró M, Navarro M, Guerra A, et al. 2014. Transitions during cephalopod life history: the role of habitat, environment, functional morphology and behaviour. Adv Mar Biol. 67:361–404.

Rosa R, Pimente MS, Boavida-Portugal J, Teixeira T, Trübenbach K, Diniz M. 2012. Ocean warming enhances malformations, premature hatching, metabolic suppression and oxidative stress in the early life stages of a keystone squid. Plos One. 7:e38282.

Segawa S, Yang WT, Marthy H-J, Hanlon RT. 1988. Illustrated embryonic stages of the eastern Atlantic Squid Loligo forbesi. Veliger. 30:230–243.

Shigeno S, Tsuchiya K, Segawa S. 2001. Conserved topological patterns and heterochronies in loliginid cephalopods: comparative developmental morphology of the oval squid Sepioteuthis lessoniana. Invertebr Reprod Dev. 39:161–174.

Steer MA, Moltschaniwskyj N, Jordan AR. 2003. Embryonic development of southern calamary (Sepioteuthis australis) within the constraints of an aggregated egg mass. Mar Freshw Res. 54:217–226.

Strathmann RR, Strathmann MF. 1995. Oxygen supply and limits on aggregation of embryos. J Mar Biol Assoc UK 75:413–428.

Watanabe K, Sakurai Y, Segawa S, Okutani T. 1996. Development of the ommastrephid squid Todarodes pacificus, from fertilized egg to rhynchoteuthion paralarva. Am Malacol Bull. 13:73–88.

Worms J. 1983. Loligo vulgaris. Cephalopod life cycles, Vol. I. Species account. London: Academic Press; p. 143–157.

Wray GA, Raff RA. 1990. Pattern and process heterochronies in the early development of sea urchins. Semin Dev Biol. 1:245–251.

Young RE, Harman RF. 1988. “Larva” “paralarva” and “subadult” in cephalopod terminology. Malacologia. 29:201–207.

Zar JH. 2010. Biostatistical analysis. 5th ed. Upper Saddle River (NJ): Prenticet-Hall/Pearson.