

Fecundity and timing of oogenesis at high latitudes: reproductive traits in the brooding bivalve *Neolepton cobbi* (Cyamioidea: Neoleptonidae)

Cristián Ituarte¹ and María Laura Presta²

¹*División Invertebrados, Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina; and*

²*Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires & Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA), CONICET-UBA, Buenos Aires, Argentina*

Correspondence: C. Ituarte; e-mail: ituarte@macn.gov.ar

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ABSTRACT

Neolepton cobbi is a small bivalve (maximum length 3.4 mm) occurring on sandy or soft muddy bottoms in the southern Magellan Region. Its life span is 12–14 months and population turnover takes place in the late austral summer (December–January). It is a protandric hermaphrodite; sex change occurs at a non-defined size, although females smaller than 1.3 mm length were never found. During the functional male phase, clusters of oogonia were found in the posterodorsal region of the gonad. Among 152 specimens examined histologically, only one case of functional hermaphroditism was found, in which male and female tissues occupied differentiated regions of the gonad. *Neolepton cobbi* is a brooding species. Secretions from a glandular region of the mantle edge contribute to the agglutination of fertilized eggs, forming elongate, paired egg capsules that are attached to the anteroventral margin of the maternal individual. Up to three pairs of egg capsules were found on each female shell, with a total of about 150 eggs/embryos. Each pair of egg capsules corresponds to one of the three different spawning events during the sole breeding season (austral spring) in which each female participates. The hatchlings are released as small (260 µm length) crawling juveniles. The shell margin at the point of attachment of egg capsules is flattened due to a disruption of growth, resulting in a secondary sexual character. The timing of egg production and the possibility of increasing the number of egg capsules per female according to the time elapsed from the start of the breeding season are viewed as a solution to the limitations on fecundity and space available for brooding, which are imposed by small adult size.

INTRODUCTION

Neolepton Monterosato, 1875 is a shallow water genus that occurs worldwide. Seven species are known at the southwestern Magellan Region of southern South America and two species reach Antarctic waters in the South Georgia Islands (54°17'S, 36°30'W), the southern distributional limit for the genus in the southwestern Atlantic Ocean (Zelaya & Ituarte, 2002, 2004).

Brooding in marine invertebrates has been reported as particularly common in high latitudes and to be related to small adult size (Ramírez Llodra, 2002). It has also been recognized that there is a trade-off between egg production and the space available for brooding and, consequently, an evolutionary pressure to optimize reproductive effort by avoiding the production of a greater number of embryos than that which can be brooded (Strathmann, Strathmann & Emson, 1984; Kabat, 1985).

Parental care in bivalves comprises varied, both external and internal, modes of brooding (Malchus & Sartori, 2013). Most commonly, brooding takes place internally, either within the mantle cavity or within one or both demibranchs (Sastry, 1979). Such branchial incubation shows considerable structural diversity and complexity (reviewed by Sellmer, 1967; Sastry, 1979). In the

simplest case, reported for species of the marine genera *Lasaea* and *Cyamioocardium* (Beauchamp, 1986; Passos & Machado, 2014, respectively), some *Neolepton* species (Morton, 2015), the freshwater *Corbicula* and *Neocorbicula* (Ituarte, 1994) and others, fertilized eggs and embryos are retained within the nonmodified branchial space. In some cases, e.g. species of the sphaeriid genera *Musculium* and *Pisidium*, brooding takes place in brood sacs within the inner demibranchs (Burch, 1975). In contrast, external brooding has been reported in few cases; according to Collin & Giribet (2010), external egg masses (either attached to the shell or deposited on the substrate) have been reported in only five species of marine bivalves: *Nucula delphinodonta*, *Turtonia minuta*, *Parvicardium exiguum*, *Loripes lacteus* and *Abra tenuis*. Special cases of parental care such as nest building (*Modiolarca lateralis*), egg retention in ventilation tubes within the sediment (*Crenella* spp.) and deposition of egg strings (*Musculus discors* and *M. niger*) have been reported by Thorson (1935) and Ockelmann (1958). In other bivalves, care of their progeny includes the protection of eggs in gelatinous egg masses, e.g. *Phacoides pectinatus* (reported by Collin & Giribet, 2010) and *Carditamera floridana* (reported by Harvey, 1995). Not uncommonly,

the eggs can be provided with an individual demersal gelatinous coat (reviewed by Collin & Giribet, 2010), which is actually a persistent thick vitelline envelope; this is the case in several species of the families Astartidae and Tellinidae.

Brooding in representatives of the genus *Neolepton* has been reported for *N. sootryeni* (by Soot-Ryen, 1960, as *N. atlanticum*), *N. concentricum* and *N. cobbi* (by Zelaya & Ituarte, 2004; Presta, Cremonte & Ituarte, 2014) and *N. subtrigonum* and *N. salmoneum* (by Morton, 2015). Zelaya & Ituarte (2004) described for the first time the attachment of egg masses to the anteroventral shell surface in *Neolepton* species. Salas & Gofas (1998) suggested that a glandular region of the mantle participated in brooding by facilitating the agglutination of eggs in *N. sootryeni*. Nevertheless, details of the brooding process, the relationship with the process of oogenesis and the constraints imposed by the limited brooding space are still unknown.

Neolepton cobbi (Cooper & Preston, 1910) is a small bivalve occurring in the southern Magellan Region. Only a few details of its life history and ecology are known, concerning population dynamics and parasitism (Presta *et al.*, 2014). In the present study, the population dynamics of *N. cobbi* were followed at Puerto Deseado in the southwestern Atlantic Ocean. Its sexuality is described and the brooding process discussed in relation to the timing of oogenesis.

MATERIAL AND METHODS

Samples of *Neolepton cobbi* were obtained monthly or bimonthly over a 10-month period at Puerto Deseado (47°45'S, 65°55'W), Santa Cruz province, Argentina. The littoral at the study site is an extended rocky flat with shallow depressions of about 20 cm depth (herein referred to as tide pools) that retain water during low tides and have a thin layer of very fine sediments with high organic content covering the bottom. Bivalves were collected during the lowest tides from August 2009 to May 2010.

Samples were taken haphazardly from tide pools. A plastic sampling box (15 × 15 cm) was placed over the shallow pools and the very fine sediment was extracted with a vacuum pump, washed through a sieve (400 µm mesh size) and preserved in a 5% formalin solution. The shell length (SL) of the bivalves (maximum distance from anterior to posterior end in mm) was measured under a stereoscopic microscope using an ocular micrometre. Size–frequency distributions for each sampling event were calculated from the SL measurements and the polymodal frequency distributions analysed in order to follow the shifts in the mean SL of each cohort throughout the study period (for detailed methodology, see Presta *et al.*, 2014). Data on surface seawater temperature at Puerto Deseado were obtained from the national hydrographic service (http://www.hidro.gov.ar/oceanografia/tmareas/form_tmareas.asp).

Specimens for histology (about 20 specimens per sampling event) were fixed and decalcified in a 10% formalin solution with an added 5% of acetic acid or, in some cases, Bouin's fixative was used. Tissues (whole animals) dehydrated in ethanol series were embedded in Historesin (Leica), sectioned at 3.5 µm with a rotary motorized microtome (Leica RM 2255) and stained either with haematoxylin and eosin, toluidine blue or alcian blue (pH 2.5)/periodic acid–Schiff (AB/PAS), according to the information needed.

The expression of sexuality throughout the entire size range of the species was studied by determining the monthly/bimonthly size–frequency distributions discriminated according to sex of specimens (males, females or undifferentiated).

The relationships between the size of maternal individuals and the number of egg capsules and number of eggs carried on each valve were determined. Because the data were not normally distributed, a Kruskal–Wallis test was employed to study the relationship between the size of individuals and the number of egg capsules, and a Spearman's rank correlation analysis was used to

study that between the size of individuals and the number of eggs in the first pair of capsules.

RESULTS

Population dynamics

The population of *Neolepton cobbi* at Puerto Deseado showed a well-defined annual cycle (Figs 1, 2). The size–frequency distribution for most of the study period showed a bimodal pattern. From the late austral winter to late spring (August–November 2009), the intermediate and large-sized specimens (cohorts 1 and 2) dominated the population structure (Fig. 1). At the beginning of the

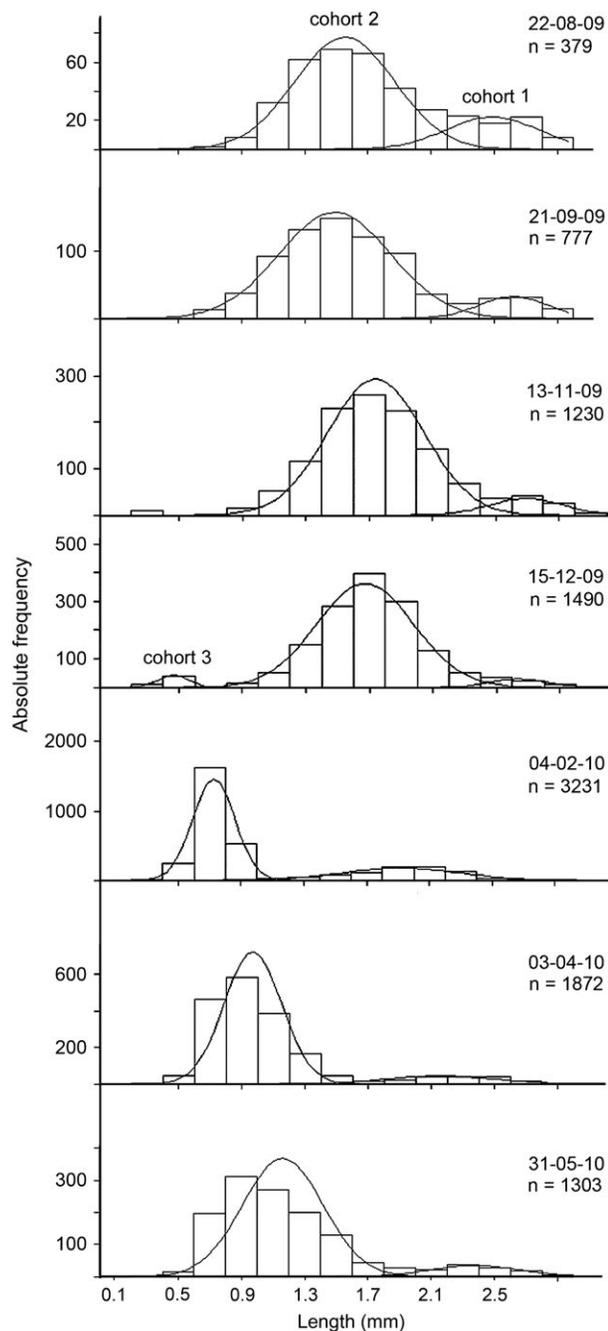


Figure 1. Size–frequency distributions of *Neolepton cobbi* at Puerto Deseado (reproduced from Presta *et al.*, 2014, with permission).

austral summer (December), a third cohort with mean (\pm SD) SL of 0.46 ± 0.09 mm appeared, whose first individuals likely recruited in mid- or late November. In February 2010 (austral midsummer), the size peak of cohort 3 shifted to 0.7 ± 0.1 mm SL and was the dominant size class in the population. From this time, cohorts 1 and 2 represented less than 25% of the population. This marked population turnover occurred in the austral summer, between December 2009 and January 2010. The maximum observed SL was 3.46 mm. Overall, the temporal changes in the population structure of *N. cobbi* followed the shifts in the mean monthly values of surface water temperature, which showed an increase from the late austral winter to late spring/early summer (August–December), with a peak temperature in midsummer (January–February), coinciding with the population turnover (Figs 1, 2).

Gonad and sexuality

The gonad of *N. cobbi* develops progressively from the posterodorsal end to the anterolateral portion of the visceral mass, either side of the digestive tract, where it invades the intervisceral connective

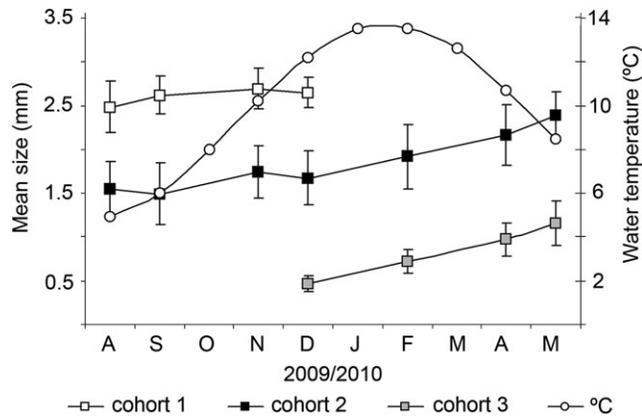


Figure 2. Population dynamics of *Neolepton cobbi* at Puerto Deseado. Shifts in the mean size monthly distributions for the three cohorts observed in the period August 2009–May 2010, related to the surface water temperature.

tissue (Fig. 3A, B). In specimens of about 1 mm SL, the size at which the gonad starts its differentiation, the germinal tissues are restricted to the posterodorsal portion of the visceral mass. *Neolepton cobbi* is a protandric hermaphrodite. An unusual finding was an individual of 2.3 mm SL (out of 152 specimens examined) showing male and female tissues equally developed in a case of a simultaneous hermaphroditism in which female tissues, from previtellogenic to late vitellogenic oocytes, occupied a dorsal position in the gonad and male tissues, represented by all stages of spermatogenesis and spermiogenesis, were ventrally located (Fig. 3C). Male individuals were found ranging from 0.8 to 3.1 mm SL (i.e. covering nearly the entire size range); female individuals were never found at sizes below 1.3 mm SL (Fig. 4).

Functional male phase

The male gonad comprised a few large acini, usually three or four per sagittal section (Figs 3A, 5A, B). Spermatogenesis and spermiogenesis were asynchronous processes within a single gonad, so that different acini or different sections within an acinus showed nonhomogeneous maturation stages. Irrespective of the size of the individuals and/or the season, 71.4% of the male individuals studied ($n = 90$) showed oogonia and/or primary oocytes, either isolated or in small clusters on the wall of the male acini, usually in low numbers and more frequently in the posterior portion of the gonad (Fig. 5C, D). From September 2009 to February 2010, the diameter of the oocytes present within male acini showed a tendency to increase (from $14.62 \pm 5.07 \mu\text{m}$ to $20.62 \pm 6.14 \mu\text{m}$). Vitellogenic oocytes were never found within a male acinus.

Functional female phase

The female gonad was formed by a few large acini in which a relatively low number of oocytes developed (Fig. 3B). As the gonad approached maturity, two cohorts of oocytes were usually easily distinguished: one represented by oocytes in an advanced or late vitellogenic stage and ripe oocytes (the latter about $130 \mu\text{m}$ diameter, with a conspicuous vitelline membrane of $2.5 \mu\text{m}$ thick) and a second represented by previtellogenic or very early vitellogenic oocytes (Fig. 6A, B). Usually, in mature or nearly mature gonads that had not yet spawned, a few oogonia and growing oocytes

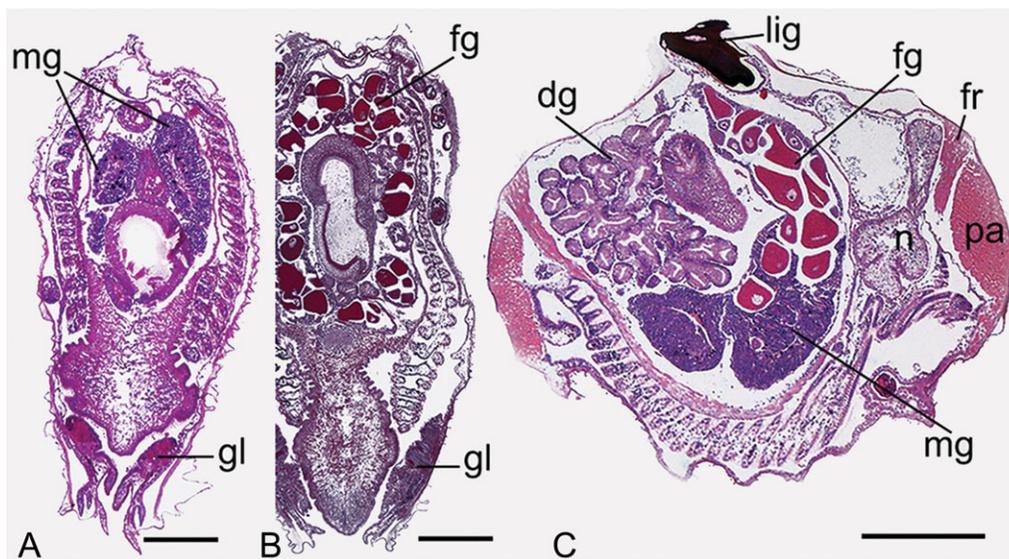


Figure 3. Expression of sexuality in *Neolepton cobbi*. **A.** Male functional phase. **B.** Female functional phase. **C.** A case of simultaneous hermaphroditism. Abbreviations: dg, digestive gland; fg, female gonad; fr, foot retractor muscle; gl, glandular portion of mantle; lig, ligament; mg, male gonad; n, nephridium; pa, posterior adductor muscle. Scale bars: **A, B** = 200 μm ; **C** = 500 μm .

were also present (Fig. 6C). After the first spawning event, indicated by the presence of egg masses adhering to the outer shell surface (see below), the second cohort of oocytes soon resumed their development and entered vitellogenesis (Fig. 6D).

Female gonads did not show remnants of the previous male phase, except in one case (a specimen of 1.47 mm SL) in which few spermatozooids were observed within a female acinus.

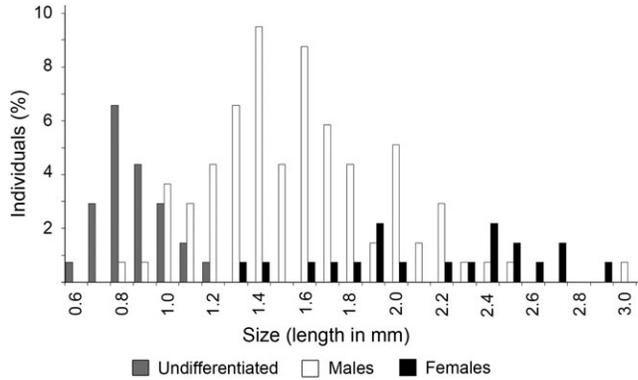


Figure 4. Proportion of sexes according to size classes in *Neolepton cobbi*.

Sex ratio through time

The proportion of sexes in the population generally showed a dominance of males (Fig. 7). From August to December 2009 (late austral winter–early summer), individuals in the male phase composed between 71.4% and 95.5%, with a peak in September. This percentage fell in February due to the massive incorporation in the population structure of the sexually undifferentiated individuals of the newly hatched cohort 3. Values over 50% were resumed by May 2010 as the percentage of sexually undifferentiated individuals dropped (mean SL of cohort 3 by this time was about 1 mm, the size at which differentiation starts). The percentage of female individuals oscillated between 4.54% and 28.57%, without definite seasonality.

Brooding

Neolepton cobbi is a brooding species. After spawning and egg fertilization (the precise place of fertilization is not known), females retained the ova in paired elongate egg masses protected within egg capsules that adhere to the outer shell surface on the anteroventral shell margin (Fig. 8A, B). As a result of the attachment of the egg capsules, the growth of the shell at the anteroventral margin of both valves is altered, leading to a flattening of the margin, which persists after the brooding process (Fig. 8C). This flattening of the anterior shell margin was never observed in males and

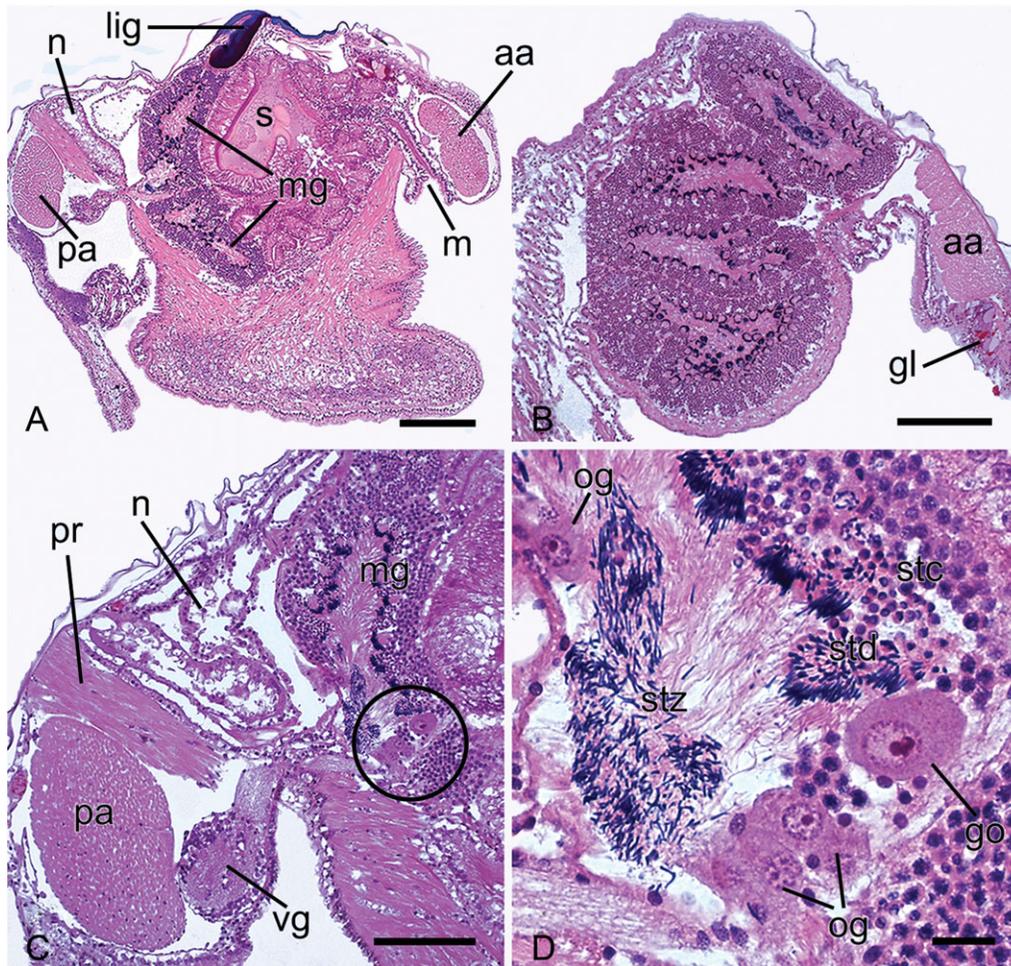


Figure 5. *Neolepton cobbi*: functional male phase. **A.** Mid-sagittal section of specimen showing structure of male gonad. **B.** Detail of gonad structure; only four large male acini are viewed in a slightly laterally shifted sagittal section. **C.** A cluster of female germinal cells (circle) within a male acinus. **D.** Detail of oogonia and growing oocyte in a mature male acinus. Abbreviations: aa, anterior adductor muscle; gl, glandular portion of mantle; go, growing oocyte; lig, ligament; m, mouth; mg, male gonad; n, nephridium; og, oogonia; pa, posterior adductor muscle; stc, spermatocytes; std, spermatids; stz, spermatozooids; vg, visceral ganglion. Scale bars: **A, B** = 200 µm; **C** = 100 µm; **D** = 20 µm.

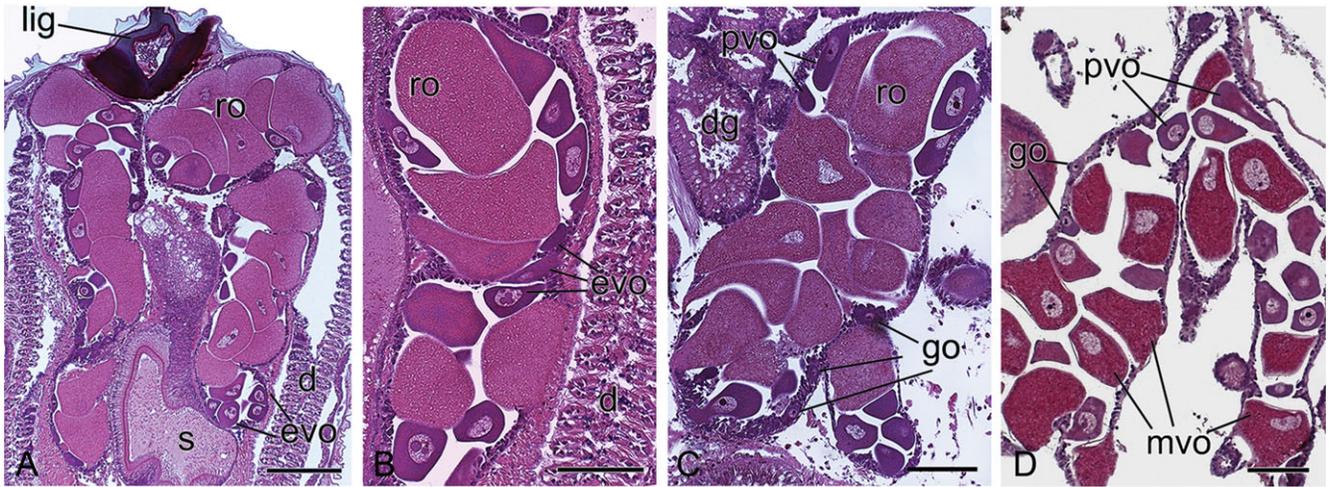


Figure 6. *Neolepton cobbi*: functional female phase. **A.** Transverse section showing gonad structure; four large female acini showing two well differentiated cohorts of oocytes. **B.** Detail of the two cohorts of oocytes. **C.** A gonad with three cohorts of female germinal cells. **D.** Fast recovery of a recently spawned gonad (at the moment of processing for histology the specimen carried a pair of egg capsules with developing embryos) showing a cohort of midvitellogenic oocytes. Abbreviations: d, demibranch; dg, digestive gland; evo, early vitellogenic oocytes; go, growing oocytes; lig, ligament; mvo, midvitellogenic oocytes; pvo, previtellogenic oocytes; ro, ripe oocytes; s, stomach. Scale bars: **A** = 200 μm ; **D** = 100 μm .

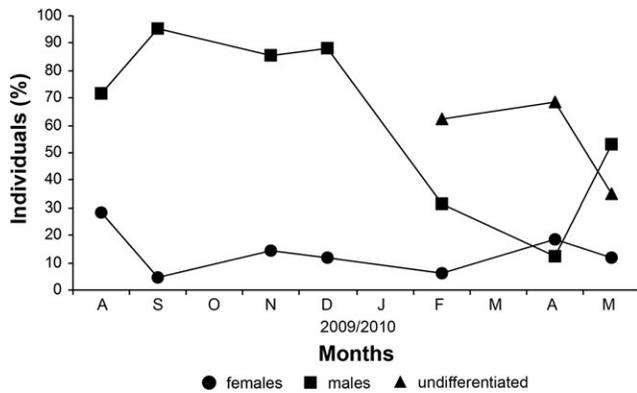


Figure 7. Variation of proportion of sexes over time in *Neolepton cobbi*.

develops only as a secondary sexual character (Fig. 8D). Embryos are retained until they attain about 260 μm SL (Fig. 9A–C).

A single female can bear up to three paired egg masses on each valve, each pair corresponding to one spawning event within a single reproductive period, the oldest occupying the upper position (Fig. 9B). The total number of eggs/embryos carried by a female with three pairs of egg capsules was about 150.

Pallial glands and egg-capsule formation

The free mantle border of *N. cobbi* shows a peculiar morphology. Viewed in transverse section, the outer mantle fold splits into two parts; the outermost is placed between the inner shell surface and the inner portion of the outer fold (Fig. 10A, B). The middle mantle fold is also divided into two parts—the outer, which is slender and closely applied to the periostracum and the inner, which is wider and elongate. The inner mantle fold is large, quite distant from the middle fold, showing differences in morphology according to the section considered, but usually comprising two or three indistinct lobes above which a well-differentiated glandular portion (see below) is developed (Fig. 10B, C). A series of well-developed radial muscles in the middle and inner mantle folds (Fig. 10A) imparts motility to the free mantle border. The free mantle border contributes to egg-capsule formation by actively

coating the egg mass with the glandular secretions of the mantle and attaching the egg capsule to the outer shell surface by moulding and positioning the agglutinated egg mass (Fig. 10D, E).

Along the anterior two-thirds of its length, the portion of the mantle epithelium above the inner fold is differentiated into a large, 100 μm thick, glandular region (Fig. 11A–F). This glandular region is present throughout the life of *N. cobbi*, from the early male phase to the female stage (Fig. 11A, B). The glandular tissue develops between the outer and inner mantle epithelia, immediately above the inner mantle fold (Fig. 11C, D). Two different types of subepithelial secretory cells are present, one with highly acidophilic secretory granules and the other with slightly basophilic content; with toluidine blue and AB/PAS staining they showed orthochromatic (light blue) staining and shades of purple (the latter a metachromatic reaction compatible with acid mucopolysaccharides) (Fig. 11C, E). It is possible that these putatively different secretory substances could actually represent different stages of maturity of the same secretion. In addition, deep in the glandular region, a third type of gland cell showed a finely granulated metachromatic secretory product (Fig. 11E).

The gland cells, when full of secretory product, are ovoid or pear shaped (40 and 25 μm maximum and minimum diameter) with a long neck that traverses the thickened mantle border and carries the secretion to the surface of the inner mantle epithelium, which is formed by cuboidal or low columnar cells that at this point are densely ciliated (Figs 10C, 11C, F). After secretion, the gland cells collapse (Fig. 11D).

The secretory products of the glandular portion of the mantle are released in the form of globules and smaller droplets into the pallial space (Fig. 10C), where the recently spawned and fertilized eggs are progressively enrobed with the secretions to form the egg capsules before being attached to the outer shell surface (Fig. 10D, E). The ventral edge or tip of the foot, together with the inner and middle mantle folds (see above), contributes to the process of arranging and enrobing a defined number of ova to form an egg capsule, which progressively leaves the pallial cavity to be attached to the outer shell surface (Fig. 10D, E).

Size of brooding females and brood size

Brooding females ranged between 1.6 and 3.4 mm SL, although most were between 2.4 and 2.8 mm. The number of egg capsules

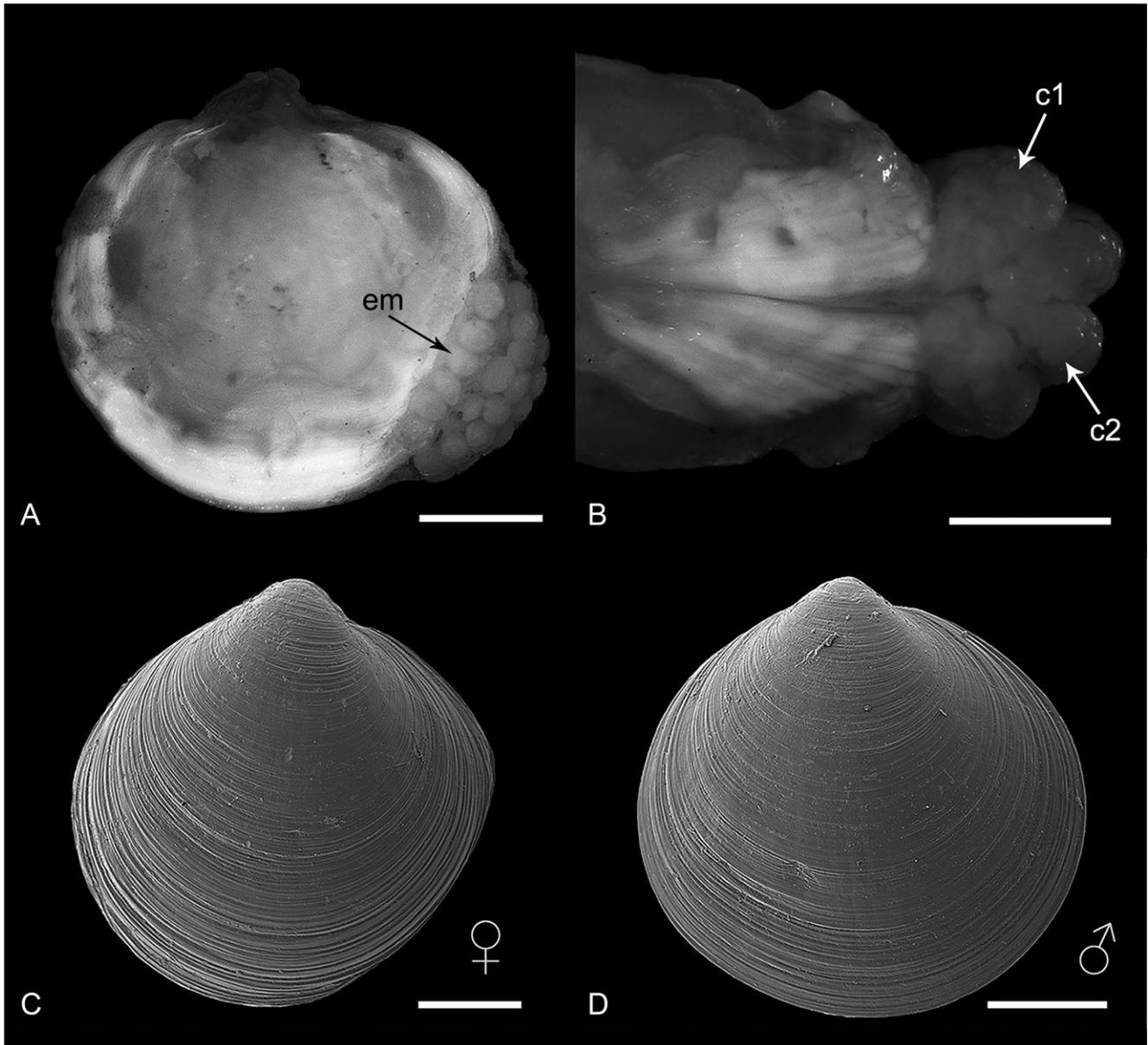


Figure 8. *Neolepton cobbi*: brooding. **A.** Lateral view of partially decalcified specimen bearing two egg capsules. **B.** Dorsal view of partially decalcified specimen with two pairs of egg capsules attached to each valve. **C.** Lateral view of right valve of female specimen showing flattened anteroventral shell margin. **D.** Right valve of male specimen showing normal shell outline. Abbreviations: c1, outer (older) egg capsule; c2, inner (newer) egg capsule; em, embryos. Scale bars = 500 μ m.

per female (one, two or three pairs) was not correlated with female size ($H = 5.43$, $P = 0.065$), but with the time elapsed from the start of the reproductive period (Tables 1, 2). By August 2009 (mid- to late austral winter) females between 2.2 and 3 mm SL showed a single pair of egg capsules. From September (austral spring), a low percentage of females within this same size interval showed two pairs of egg capsules, while females between 1.8 and 3 mm length carrying a single pair were also observed. By November–December (austral late spring–summer), females from 1.8 to 3.2 mm SL with up to three pairs of egg capsules were found, although most had only one or two pairs, and by February only one female larger than 2.6 mm length showed three pairs of egg capsules (Tables 1, 2). The number of eggs or developing embryos was positively correlated with the size of the brooding females ($R = 0.85$, $P < 0.001$) (Fig. 12).

Each egg capsule of a pair contained approximately the same number of eggs or embryos, all of them showing the same developmental stage (Fig. 9B).

Seasonality of reproduction

Overall seasonality was observed in the reproductive process. A peak in the percentage of brooding females was observed in October–November 2009 (austral early mid-spring), reaching 10.1%, coinciding with the period of fast increase in the surface water temperature (Fig. 13). In the periods May–July 2009 (austral late autumn–early winter) and February–May 2010 (late summer–autumn), the percentage of ovigerous females (those bearing egg capsules) was less than 2%.

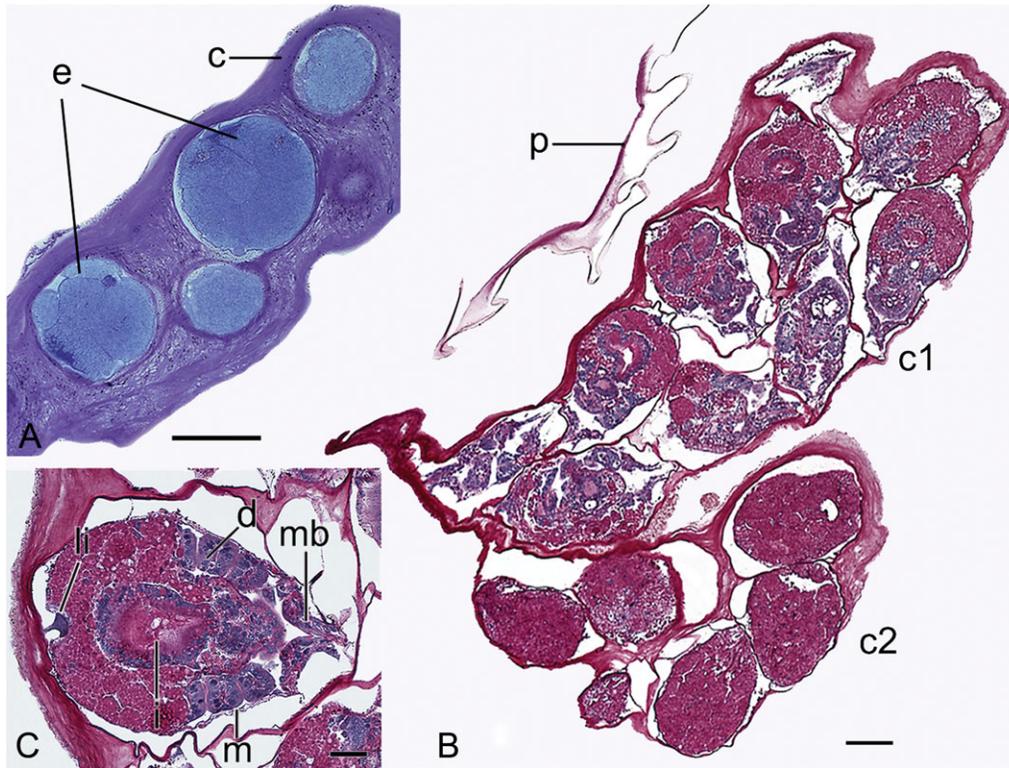


Figure 9. *Neolepton cobbi*: histology of egg capsules. **A.** Recently laid egg capsule containing embryos in an early developmental stage (toluidine blue). **B.** Transverse section of two egg capsules corresponding to different cohorts of embryos showing different developmental stages. **C.** Transverse section with detail of an embryo in advanced stage of development. Abbreviations: c, wall of the egg capsule; c1 and c2, egg capsules of two different cohorts; d, demibranch; e, embryos; I, intestine; li, ligament; m, mantle; mb, mantle border; p, periostracum. Scale bars: **A** = 100 µm; **B** = 50 µm; **C** = 20 µm.

DISCUSSION

Sexuality

Bivalves show a wide range in the expression of sexuality. Most species (at least among those in which sexuality is known) are gonochoristic. Hermaphroditism is also known, either simultaneous or consecutive, showing a variety of alternatives according to species (reviewed by Sastry, 1979). Information obtained from our nine-month study of a population at Puerto Deseado revealed that *Neolepton cobbi* is a protandric consecutive hermaphrodite, with a non-definite size at which sex change takes place. Hermaphroditism, either simultaneous or consecutive, is common among other small-sized bivalves, particularly at high latitudes. Lützen & Nielsen (2005) reported 18 hermaphroditic galeommatids belonging to the genera *Ephippodonta*, *Galeomma*, *Pseudogaleomma*, *Scintilla* and *Scintillona*; other small Galeommatidae described as hermaphrodites are species of the genus *Waldo* (Zelaya & Ituarte, 2002, 2013; Valentich-Scott, Ó Foighil & Li, 2013). Fox, Jaspersen & Lützen (2007) reported the cyamioid *Tellimya ferruginosa* as a protandric hermaphrodite and the same condition was also reported in *Tellimya tehuelcha* by Zelaya & Ituarte (2012). The consecutive sexuality of *N. cobbi* differs from other described cases in that the sex change from male to female can take place over a wide size interval. The minimum size at which the change takes place is slightly below 1.3 mm, but male individuals up to 3.1 mm length were found. This suggests that some specimens may never experience sex reversal, remaining as males throughout their entire life. Regarding the expression of sexuality of other *Neolepton* species, Salas & Gofas (1998), based on anatomical data, reported that in *N. sulcatulum*, *N. antipodum* and *N. sootryeni*, the sexes are separate, and Morton (2015) also reported *N. salmoneum* and *N. subtrigonum* as dioecious (but it should be noted that these studies were based on instantaneous observations, not on time series).

Egg capsules

The place of fertilization in *N. cobbi*, either in the suprabranchial chamber or, more likely, the pallial cavity, could not be determined.

Brooding of embryos in the suprabranchial chamber of inner or both demibranchs is a common feature in small bivalves, e.g. the Galeommatoida (Lützen & Nielsen, 2005) and the Cyamioidea (Shabica, 1974; Ituarte, 2009; Zelaya & Ituarte, 2009; Passos & Machado, 2014; Morton, 2015). However, brooding outside the branchial space, particularly in external structures, has been reported in only a few genera other than *Neolepton*. Recently, Collin & Giribet (2010) reported cohesive gelatinous egg masses in the lucinid *Phacoides pectinatus*, which are laid on the surface of the sediment. A similar behaviour, although retaining the egg mass attached to the posterior part of shell, was described in *Carditamera floridana* by Harvey (1995). Coan (1999: 138) reported individuals of *Basterotia panamica* as brooding “their young along its ventral mantle margin”. However, Goto, Hamamura & Kato (2011: 233) reported that other *Basterotia* species “harboured hundreds of veligers in the ctenidium”, suggesting that the description by Coan (1999) may be the result of the reattachment of larvae released from the ctenidium. The same could be true for the finding by Ponder (1971) of ‘shelled larvae’ attached by a byssal thread to the overlapping periostracum of the sportellid *Anisodonta alata*.

The various brooding processes reported in the tiny species of the genus *Neolepton* (less than 5 mm SL) seem to be rather diverse. Soot-Ryen (1960) reported that eggs of *N. sootryeni* (as *N. atlanticum*) were attached to the glandular portion of the anterior inner surface of the mantle. A conspicuous glandular differentiation, similar to that described here for *N. cobbi*, was also reported by Salas & Gofas (1998) in *N. sulcatulum* and *N. antipodum*. Based on both

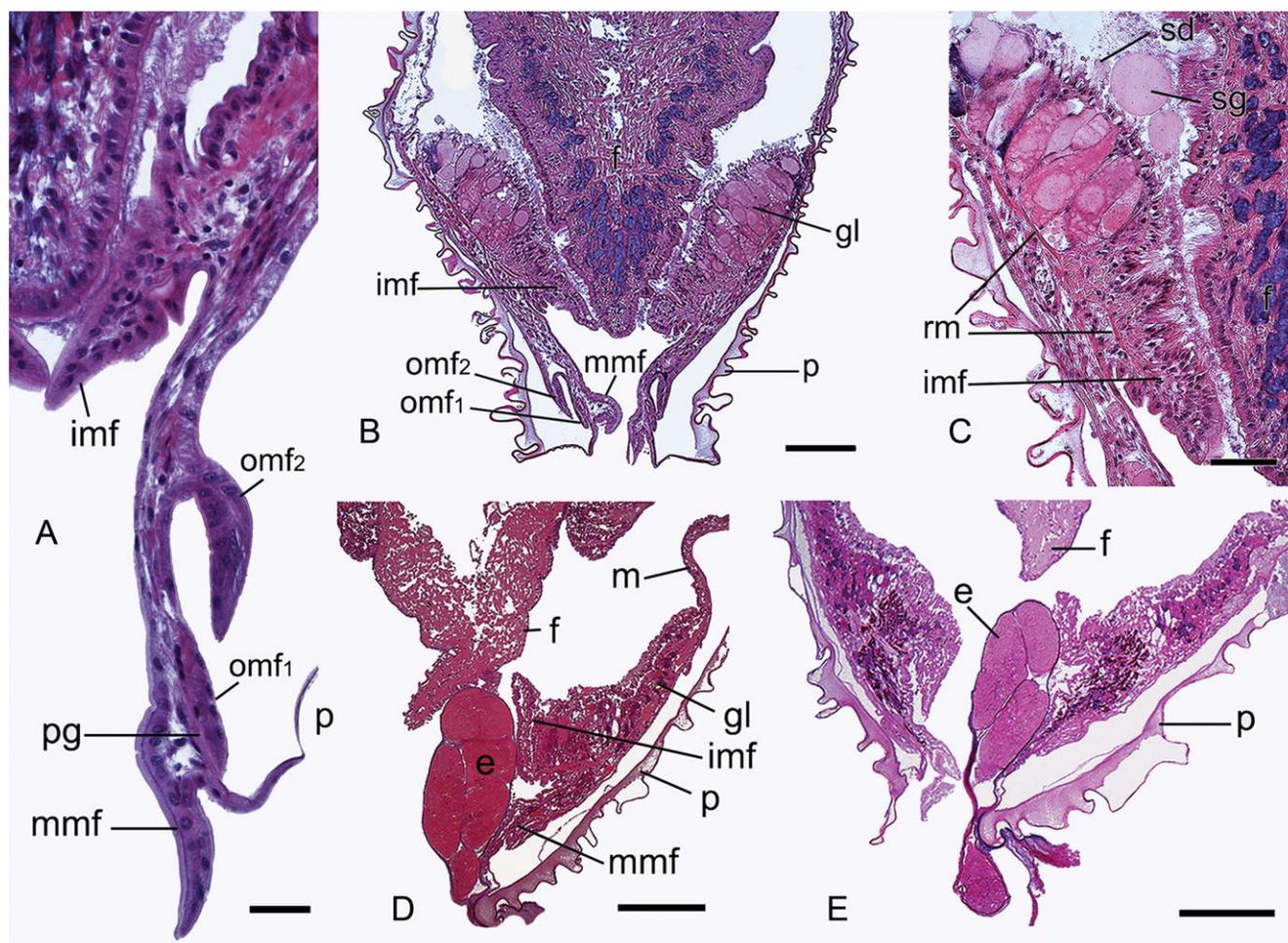


Figure 10. *Neolepton cobbi*: mantle border and egg-capsule formation. **A.** Transverse section of mantle folds. **B.** Mantle folds and mantle glands. **C.** Detail of mantle glands showing secretory globules released into the mantle cavity. **D.** Early stage of egg-capsule formation, showing embryos enclosed in gland secretions moulded by middle mantle fold and foot. **E.** Egg capsule being moved to outer shell surface. Abbreviations: e, embryos; f, foot; gl, glandular portion of mantle; imf, inner mantle fold; mmf, middle mantle fold; omf 1 and omf 2, components 1 and 2 of outer mantle fold; p, periostracum; rm, radial mantle muscles; sd, secretory droplet; sg, secretory globule. Scale bars: **A** = 20 μm ; **B** = 100 μm ; **C** = 50 μm ; **D**, **E** = 200 μm .

observations, the latter authors suggested the involvement of the glandular portion of the mantle in the brooding process. The above-noted observation by Soot-Ryen (1960) may actually correspond to an initial step in the brooding process, i.e. the agglutination of the egg mass near the glandular mantle border prior to being transferred outside the shell. Salas & Gofas (1998) also reported the distorted [anterior] margin of the larger examined specimens of *N. cobbi*, although they did not relate this growth alteration to the brooding process. The results of the present study confirm the involvement of the glandular mantle in the formation of external egg capsules in *N. cobbi*. Other neoleptonids as *N. salmonum* and *N. subtrigonum* have been reported to brood their embryos within inner demibranchs (Morton, 2015).

Small adult size, reduced fecundity and limited space for brooding: consequences for a semelparous reproductive strategy

The positive relationship between female size and fecundity is recognized as a fundamental reproductive characteristic in many invertebrate taxa, being the result of morphological and physiological constraints in energy allocation to reproduction (Ramírez Llodra, 2002). The trade-off between fecundity and egg size is also significant and forms a basic component of all life history models (Ramírez Llodra, 2002), because energy resources available for

reproduction are limited, particularly in small-sized species. Furthermore, the space available for brooding constitutes an additional restriction in small-sized brooding species. Different ways to overcome this limitation have been described, mainly involving either reduction in fecundity or development of some kind of flexibility in the brooding capacity.

In the case of bivalves, if development is confined to the branchial space or within egg capsules (the latter a rather uncommon trait) until a fully grown juvenile stage, the maternal individual must provide eggs with enough nutrients to complete their embryonic development. This is achieved by the production of large yolky eggs (about 130 μm diameter in the case of *N. cobbi*), which constitute a significant problem if the space available for brooding is limited. In this regard, the restricted oocyte production in *N. cobbi* is viewed as a response to both small adult size and to the limited space available for brooding—because the limited volume of a single egg capsule limits the number of eggs it can contain. In other words, the restriction in volume of the brooding structures is a limiting factor for egg production.

Considering the population dynamics over the study period and the reproductive characteristics observed, *N. cobbi* appears as a strict semelparous species with a definite annual life cycle.

Neolepton cobbi exemplifies well the association of small adult size (maximum SL 3.4 mm), brooding and hermaphroditism, three

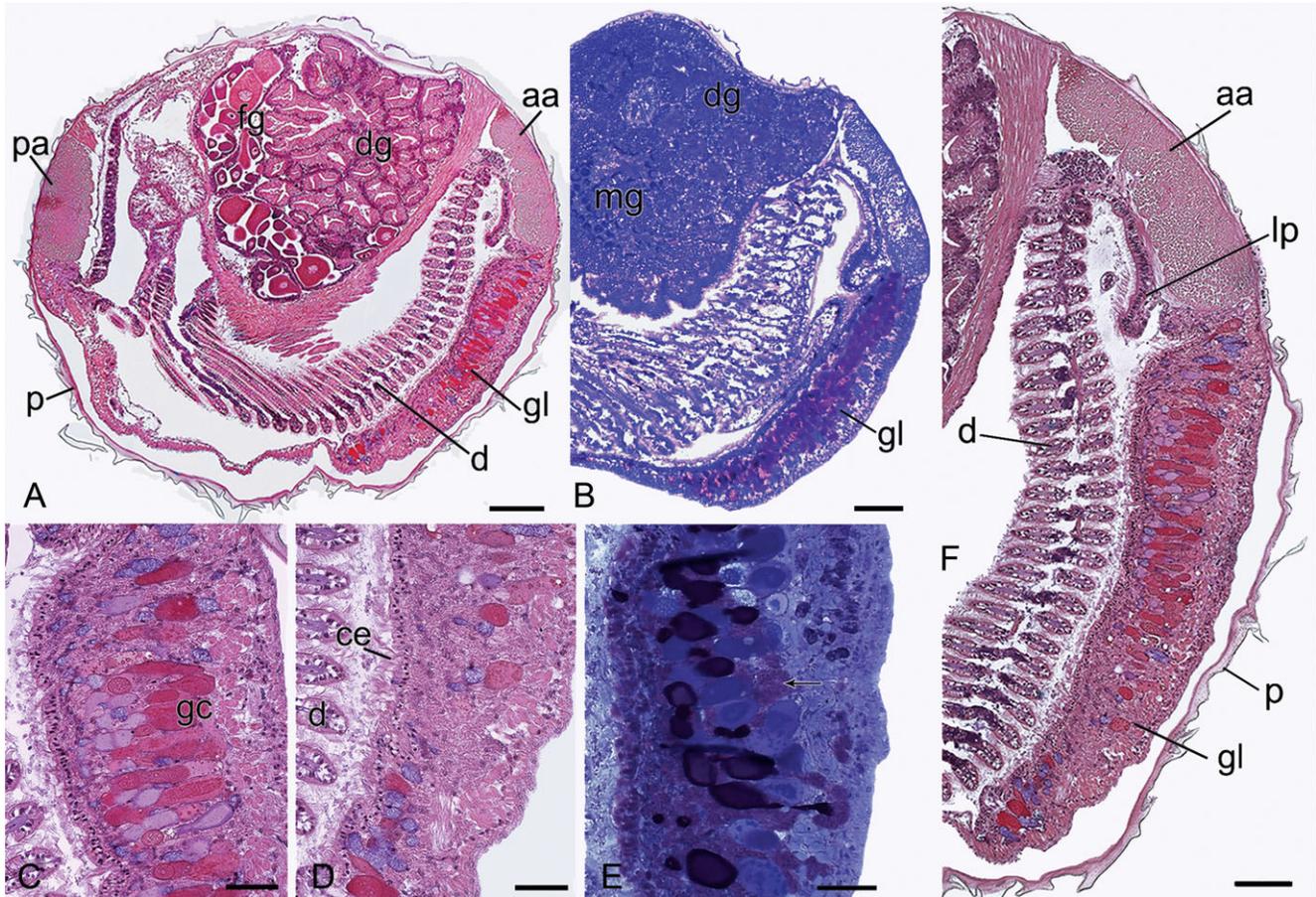


Figure 11. *Neolepton cobbi*: mantle gland differentiation. **A.** Sagittal section of specimen in functional female phase showing extent of glandular differentiation of anteroventral portion of mantle. **B.** Sagittal section of specimen in functional male phase with mantle gland differentiation (Toluidine blue). **C.** Detail of histology of fully developed mantle glands. **D.** Mantle glands after secretion of their products. **E.** Section of mantle glands showing the three types of secretory products (arrow indicates finely granular acidophilic secretion) (toluidine blue). **F.** Detail of mantle gland. Abbreviations: aa, anterior adductor muscle; ce, ciliated epithelium; d, demibranch; dg, digestive gland; fg, female gonad; gc, gland cell; gl, gland differentiation of mantle; lp, labial palp; mg, male gonad; p, periostracum; pa, posterior adductor muscle. Scale bars: **A** = 200 μm ; **B, F** = 100 μm ; **C–E** = 50 μm .

Table 1. Size range (mm) of females of *Neolepton cobbi* bearing one, two or three pairs of egg capsules.

Pairs of egg capsules	August 2009	September 2009	November 2009	December 2009	February 2010
1	2.2–3 (100)	1.8–3 (72.7)	1.6–3 (38.2)	1.6–3 (37.2)	1.8–2.6 (47.6)
2	–	2.2–3 (27.5)	1.6–3 (47.1)	1.6–3 (55.1)	1.6–2.8 (47.6)
3	–	–	1.8–3.2 (14.7)	1.8–2.8 (7.7)	2.6 (4.8)

The percentage of females of each size class per month is shown in parentheses.

Table 2. Results of Kruskal–Wallis test for comparing sizes of *Neolepton cobbi* females carrying one, two or three pairs of egg capsules.

Pairs of egg capsules	<i>n</i>	Mean size (mm)	SD	Median size (mm)	<i>df</i>	<i>H</i>	<i>P</i>
1	128	2.30	0.36	2.33	2	5.43	0.0657
2	115	2.34	0.35	2.33			
3	22	2.50	0.32	2.57			

reproductive traits that are recognized as associated variables in invertebrate life histories (Strathmann *et al.*, 1984; Ramírez Llodra, 2002). Small body size of adult females is a limiting factor on the number of eggs produced, leading to the commonly pattern in which small organisms produce a few large eggs that are

brooded to advanced developmental stages (Ramírez Llodra, 2002). In this context, and particularly in species with an annual cycle such as *N. cobbi*, the number of eggs produced in a single spawning event might not suffice to maintain the population and consequently, semelparity would not be a viable reproductive

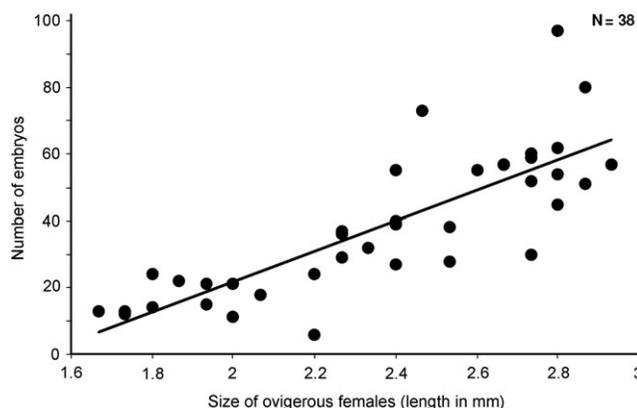


Figure 12. Relationship between number of brooded embryos and SL of brooding females in *Neolepton cobbi*. SL, shell length.

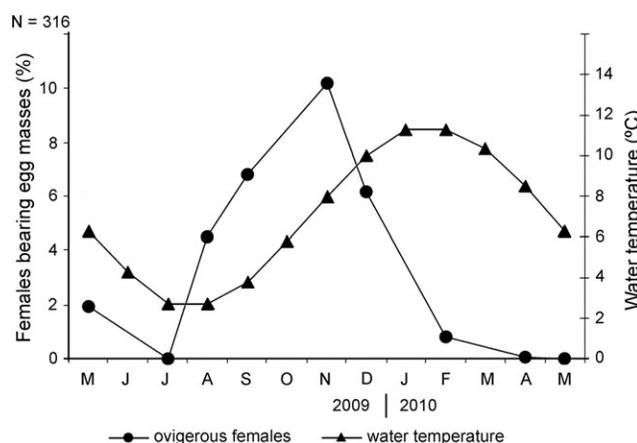


Figure 13. Variation in the number of brooding females of *Neolepton cobbi* over time and relationship with water surface temperature.

strategy. Staged oogenesis (two or three cohorts of developing oocytes) and production of successive pairs of egg capsules to accommodate the successive cohorts of ova are two traits that allow an increase in the effective fecundity and in the capacity for brooding. In other words, the mechanism ensures that egg production does not exceed the brooding capacity of each female.

A similar strategy to overcome constraints imposed by low fecundity and restrictions in space for brooding has been described in other bivalves. The cyamioid *Gaimardia bahamondei* is a semelparous species in which females are able to brood up to three cohorts of embryos, producing these cohorts of oocytes within the sole breeding season in which each female participates (Chaparro *et al.*, 2011). The small hermaphroditic brooding mysellid *Mysella moelleri* also shows a similar oogenesis dynamic with two oocyte generations, brooded in both inner and outer demibranchs (Petersen & Lützen, 2008). In this way, the undesirable consequences of a strict semelparous reproductive strategy in a species with reduced brood size are avoided.

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