Observations on age and reproduction of the oceanic squid Ancistrocheirus lesueurii (d’Orbigny, 1842) (Cephalopoda: Ancistrocheiridae)

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Despite the importance of Ancistrocheirus lesueurii in the diet of a wide variety of oceanic predators, many aspects of its biology are unknown. We report new observations on the reproductive system of the species and provide age estimates of one normal and two intersexual males based on the number of increments in the statoliths. The age of the examined mature males was estimated to be more than 2 years, increasing the maximum age known for males of the species. Female A. lesueurii have specific modified areas for spermatangia reception in the nuchal region. The morphology of the right hectocotylized ventral arm and the relatively large spermatophore are also described.

**Keywords:** Cephalopoda; Ancistrocheirus lesueurii; spermatophore; mating; hectocotylus; deep sea; statoliths; age

**Introduction**

The oceanic squid Ancistrocheirus lesueurii (d’Orbigny, 1842) is important in the deep sea foodweb, being heavily preyed upon by sharks and cetaceans (Clarke 1980, 1996; Smale and Cliff 1998). Females attain larger sizes and mature later than males, and growth rates in both sexes are among the lowest known for squid (Arkhipkin 1997). Asynchronous ovulation suggests that Ancistrocheirus lesueurii spawns its eggs intermittently in separate batches (Laptikhovsky 1999). Males carry up to 77 spermatophores (maximum length 112 mm) in the Needham’s sac, and the right fourth ventral arm is hectocotylized (Hoving et al. 2006). In a recent study on A. lesueurii from southern Africa, almost half of the males examined were found to have nidamental glands (reported exclusively in females in all other cephalopod species to date) and a larger body size than normal males. This phenomenon is known as intersexuality, but neither the function nor cause is known (Hoving et al. 2006). One question that was raised in that study was whether intersexuality was a stage that normal males reach after some time of being normal males or if intersexual males are different large forms that are ontogenetically faster growing and are then of similar age to normal males at sexual maturity. One way to answer this question is by estimating the age of individual squid. We estimated the age of intersexual individuals by quantifying the number of increments in the squids’
statoliths. Statoliths are aragonite concretions in the organs of balance in which increments are laid down periodically (Arkhipkin 2004). In coastal squid species these increments are laid down daily (e.g. Lipinski 1986). These structures are also widely used for age and growth studies in oegopsid squid (Arkhipkin 2004) and are here used to estimate the age of male _A. lesueurii_. In addition to the first age estimates of intersexual male _A. lesueurii_, we report aspects of the male and female reproductive systems including specific areas for spermatangia deposition in females.

**Material and methods**

For the current study we examined a large mature female _A. lesueurii_ (ML 540 mm, BM 5.36 kg) (Figure 1A) that was caught on 1 October 2005 by bottom trawl on the R/V _Dr Fridtjof Nansen_ at 33°46' S, 17°29' E, at 400 m depth. Additional material of _A. lesueurii_ that we examined included the specimens described in Hoving et al. (2006). Abbreviations used here follow Roper and Voss (1983) and are as follows: ML = mantle length; BM = body mass; SAM = South African Museum; SpL = spermatophore length; SpLI = spermatophore length index (SpL/ML); CBL = cement body length; CBLI = cement body length index (CBL/SpL); EAL = ejaculatory apparatus length; EALI = ejaculatory apparatus length index (EAL/SpL).

Three male statoliths were prepared according to methods used by Arkhipkin (1997) after the total statolith length was measured using a calibrated stereomicroscope. The sections were viewed using 100 × and 400 × magnification. A series of high-resolution pictures were taken from the nucleus to the rostrum. This created a complete counting axis, where individual increments were quantified using ImageTool software (University of Texas Health Science Center, San Antonio, TX, USA). The increments in each statolith were counted twice.

**Results**

The examination of the large mature female (Figure 1A) revealed that the species has one specialized area on each side of the nuchal cartilage for the reception of spermatangia (inverted spermatophores). These areas are located on each side of the nuchal cartilage in the dorsal neck region, under the outer neck collar (Figure 1B and C). Sperm was also found loose on the outer neck collar (Figure 1B). The areas under the outer neck collar are approximately 20 mm long and consist of a simple depression lined with rugose tissue. Spermatangia were found inside the depressions, and also implanted into the tissue close to the depressions (Figure 1). An estimated number of five to ten spermatangia were deposited in or near each receptacle. However, because the areas are located underneath the neck collar, several spermatangia were sometimes squeezed into a single mass. Another female (# 11 in Hoving et al. 2006) was re-examined, and similar specialized areas for spermatangia deposition with spermatangia were found; the same was true in a female from a sperm whale stomach (SAM-S3533, see Hoving et al. 2006). Because of the association between these rugose areas and spermatangia in females and because males lacked such areas on both sides of the nuchal cartilage, the areas were considered to be specialized areas for spermatangia deposition.
The two trawl-caught females had a wide range of oocytes in their ovaries (0.12–2.4 mm) and filled oviducts, with 14,666 and 13,761 eggs (ML 540 mm) and 14,545 and 29,159 eggs (ML 435 mm), in the left and right oviducts, respectively. The egg size was 2–2.5 mm and each egg weighed 4–5 mg. The preserved total mass of the reproductive system was 16.6% BM (ML 540 mm) and 20.2% BM (ML 435 mm).

The examined males (# 1, 2 and 10; SAM-S2174 and SAM-S2175 from Hoving et al. 2006) have a Needham’s sac and short terminal organ (combined length 44–73% ML, maxW 5–12% ML) that open at the base of the funnel and do not extend beyond the mantle edge. The opening is simple and spermatoophores are probably expelled individually. The distal 20–27% of the right ventral arm is hectocotylized (Figure 2), the modification consisting of a flap that extends approximately for the first half of the hectocotylus, and a terminal portion.
that narrows distally (Figure 2). A photophore (~ 2 mm long) is present at the distal part of each ventral arm (Figure 2A), similar to *A. lesueurii* described from Hawaii (Vecchione and Young 2008). On the hectocotylus the photophore is situated in the middle of the modified arm tip.

Measurements of the spermatophores from three males (#1, #10 and SAM-S2175 from Hoving et al. 2006) show that the spermatophores are relatively large (absolute lengths are 62–72 mm (n = 19), 97–102 mm (n = 19) and ~ 65 mm (n = 20) respectively; relative lengths are between 22 and 46% of ML). They contain a large sperm mass (SpLI = 50–70% SpL), a cement body (CBLI = 7–14% SpL; Figure 2B), and a slightly convoluted ejaculatory apparatus (EALI = 21–38% SpL). The maxW is 1–2 mm at approximately the middle of the sperm mass.

The statoliths are large (~ 3 mm) and the increments were very well resolved and could be counted continuously from the nucleus to the tip of the rostrum (Figure 3). The statoliths of the normal male with ML 270 mm had 658 increments, the intersexual males of ML 236 mm and ML 275 mm had 540 and 761 increments, respectively.

**Discussion**

The female of ML 540 mm analysed herein is the largest individual reported so far for this species.
The presence of modified tissue in the nuchal region for sperm reception in *A. lesueurii* is typical for the squid families Enoploteuthidae, Pyroteuthidae and Lycoteuthidae (Burgess 1998; Young and Harman 1998; Hoving et al. 2007). These structures have been described as “seminal receptacles” (Burgess 1998; Hoving et al. 2007) or “spermatophore receptacles” (Young and Harman 1998). Receptacles for spermatangia occur singly (as a median receptacle posterior to the collar) in Lycoteuthidae (Hoving et al. 2007) and Pyroteuthidae (Young and Harman 1998), while in Enoploteuthidae they may be paired (each side of the nuchal cartilage beneath the collar), single (“median pocket” posterior to the collar), or located at the posterior junction of retractor muscles of head and funnel (Burgess 1998; Young

Figure 3. *Ancistrocheirus lesueurii*. (A) Statolith section of a mature intersexual male (ML 275 mm) in which 761 increments could be counted. The areas in boxes are enlarged in B, C and D, where B is the nucleus, C shows regular increments past the mid part of the rostrum and D shows the increments in the periphery of the rostrum.
and Harman 1998). Both the single median receptacle and the paired receptacles under the collar may be present in some species. Additionally, in the cases where a single tissue modification is present, spermatangia may be found on both sides of the location. The location of the specialized area for spermatangia reception of *A. lesueurii* resembles the paired receptacles beneath the collar of some Enoploteuthidae (Burgess 1998), although some enoploteuthids have wrinkled outer collar tissue, which was not found in *A. lesueurii*. In general the “seminal receptacles” (here called specialized areas for spermatangia reception) of the enoploteuthid group of families (Ancistrocheiridae, Enoploteuthidae, Lycoteuthidae and Pyroteuthidae) differ structurally from the seminal receptacles of loliginids, ommastrephids and bathyteuthids (Nesis 1995; Bush et al. 2012). In these latter families, sperm from spermatangia that are attached in the buccal area are removed or migrate into a closed storage organ present on the buccal membrane. This organ may be properly called a seminal receptacle, since sperm are stored in here in an inactive state until fertilization of the eggs.

The asynchronous ovulation pattern described for *A. lesueurii* by Laptikhovsky (1999) was confirmed here, because a wide range of oocytes were present in the ovary of the two females, and the oviducts were full of eggs, while ripe ova were also present in the ovary. This indicates an intermittent spawning strategy (Rocha et al. 2001) where several batches of eggs are spawned successively. Based upon the oviduct fullness these batches may comprise 15,000–30,000 eggs in total.

The males of *A. lesueurii* have a short terminal organ and therefore they presumably use their hectocotylized right ventral arm to transfer spermatophores (Nesis 1995), probably one spermatophore at a time because the diameter of the opening of the terminal organ is as wide as the diameter of the spermatophore. The spermatophores have a large sperm mass and the highest total number of spermatophores present in one male was 77, a relatively low number compared with other oceanic squid (e.g. 100–1800 in 18 ommastrephid species; 250 in *Lycoteuthis lorigera*; up to 1000 in *Octopoteuthis sicula*) (Nigmatullin et al. 2003; Hoving et al. 2007, 2008).

In an earlier study on *A. lesueurii* nine mature females were reported with “sperm reservoirs” (here called spermatangia) on the inner mantle wall (Arkhipkin 1997). Given the present description of the specialized areas for spermatangia deposition and the previous observations by Arkhipkin (1997), *A. lesueurii* may have three sites for the reception of spermatangia.

The age estimations from the statoliths revealed that the largest intersexual male was more than 2 years old (assuming daily deposition of the increments). The slightly smaller, but normal male was estimated to be less than 22 months old. This is similar to the age reported for mature females (Arkhipkin 1997). Although our study includes a very limited dataset, it may suggest that intersexual males of the same size may grow older than normal males. Also our data indicate that the lifespan of males is longer than previously estimated (c.1 year; Arkhipkin 1997).

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