

## Short Communication

# Female reproductive biology, and age of deep-sea squid *Histioteuthis miranda* from southern Africa

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Data on female reproductive strategy and male and female length–weight relationships and age are presented for the mesopelagic squid *Histioteuthis miranda*. Females exhibit synchronous ovulation, indicating that eggs are spawned at once or over a short period. The maximum potential fecundity seems to range from 89 000 to 148 000 oocytes. Assuming daily deposition of statolith increments, maturing females were 10–15 months old. Male *H. miranda* mature young (~6 months) and reach at least 15 months old.

**Keywords:** age, Cephalopoda, deep sea, *Histioteuthis miranda*, ovulation, reproductive strategy, squid, statoliths.

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### Introduction

The meso- and bathypelagic squid family Histioteuthidae consists of 19 species (Young and Vecchione, 2007), and despite their abundance and biomass virtually worldwide (Boyle and Rodhouse, 2005), age and growth rates are unknown for any of the species (Arkhipkin, 2004). Female reproductive patterns have only been studied in detail for six histioteuthids, although the sample size per species was small (Laptikhovskiy, 2001).

*Histioteuthis miranda* is the most common histioteuthid in southern African waters and the dominant squid taken in benthic trawls on the lower continental slope of the eastern South Atlantic between 700 and 900 m deep (Roeleveld *et al.*, 1992). It is known to range vertically between the subsurface and at least 1200 m. Juveniles tend to be found shallower than subadults and adults (Voss *et al.*, 1998). Sperm whales and other cetaceans consume large quantities of the species (Clarke, 1980), which itself preys on deepwater shrimps *Sergia* sp. (Lipiński, 1992). Clarke (1980) described mature males and females from the stomachs of sperm whales and provided general information on the species' reproductive system.

We investigated the female reproductive biology (potential fecundity, maturation, and ovulation) of *H. miranda*, and for both sexes provide length–weight relationships and estimated age by quantification of statolith increments. Statoliths are calcareous concretions in the statocysts of squid, and sectioned, they show microscopic concentric rings that can be counted to estimate age. For many species of neritic squid, validation of statolith increment numeration has revealed daily deposition of the increments (the first validation attempt was by Lipiński, 1986; for reviews, see Arkhipkin, 2004, and Jackson, 2004), but such deposition has not been validated yet for histioteuthids or other deepwater squid.

### Material and methods

Samples were collected by bottom trawl in 1988, 2005, and 2007 off southern Africa. Details of the squid and the data obtained from each are summarized in Table 1. Abbreviations used are: ML, mantle length; BW, body weight; NGL, nidamental gland length; and GSI, gonadosomatic index. Animals were assigned a maturity stage according to the maturity scale of Lipiński (1979) and Lipiński and Underhill (1995). Length was measured to the nearest 0.1 mm and weight to the nearest 0.1 g. During the 1988 cruise, reproductive organs were weighed immediately after capture, but in 2005, the organs were preserved in 10% formalin before weighing. The *H. miranda* sampled in 2007 were deep-frozen on board and measurements taken after defrosting. The digestive gland of those squid was large, and its contents were often lost during the defrosting process. Therefore, the weight of the digestive gland was weighed in 16 animals with intact digestive glands (12 females, BW 504.3–1249.8 g; 4 males, BW 450.5–804.3 g). For females, the digestive gland fluid contents ranged between 5.9 and 12.9% (mean 9%) of the total BW, and for males, between 6.9 and 9.8% (mean 8%). Consequently, to the recorded BW of the other 92 specimens, we added 9% weight for females and 8% weight for males to correct for the loss of the digestive gland fluid during defrosting.

The GSI was calculated for 87 females from the 1988 and 2007 collections using the formula  $GSI = RSW / (BW - SW) \times 100$ , where RSW is the total weight of the reproductive organs, and SW is the stomach weight.

The potential fecundity was determined for 19 females (3 from the 1988 cruise, and 16 from 2007) ranging between 158 and 294 mm ML, corresponding to a BW of 483–1683 g. This was done by taking three samples from the ovary (each containing 132–632 oocytes) and weighing them on a calibrated microscale.

**Table 1.** Collections of *H. miranda* used for this study.

Variable	1988	2005	2007
Number of males	23	12	39
ML range (mm) of males	131–290	171–290	130–232
Number of females	21	14	69
ML range (mm) of females	180–294	173–290	125–239
Depth (m)	700–894	783–852	583–738
Position	32–36°S, 17–19°E	31–33°S, 16–17°E	34–36°S, 22–25°E
Vessel	FRS “Africana”	RV “Dr Fridtjof Nansen”	FV “Blue Bell”
Data collected	ML/BW Fecundity GSI – NGL	ML/BW – – Age material NGL	ML/BW Fecundity GSI – Age material NGL

For each sample, the total number of resorptive oocytes was recorded. For 10 females, the longest axis of 100–400 oocytes was measured to plot size frequency, and for all 19 females, the longest axis of the largest oocyte was recorded.

Statoliths were successfully prepared and read from 17 squid (9 females, 8 males) collected in 2005 and from 29 squid from 2007 (21 females, 8 males). Two of the statoliths were ground in the frontal plane *sensu* Lipiński and Durholtz (1994), the others in the transverse plane. A higher success rate was gained using Crystalbond as the mounting medium because the plane of grinding had to be adjusted frequently to prevent grinding away the nucleus. The statoliths were not polished because covering them with Canada balsam and a cover slide was deemed sufficient to remove the scratches caused by the grinding. Sometimes the Crystalbond was reheated to tilt the statolith slightly to obtain the most appropriate plane for further grinding. Sectioned statolith images were recorded under phase contrast by a Leica DFC 350 FX black-and-white camera mounted on an Olympus BH-2 Research microscope (×200 or ×400). Images were made with Leica Qwin Pro supplied by Leica Cambridge. In four statolith pairs, both statoliths were counted and differences between counts were <10%. Total increment number was taken as the mean of three replicate counts that differed by <10% of the mean.

The instantaneous relative growth rate (*G*) was calculated from  $G = (\ln W_2 - \ln W_1)/t$ , where  $W_1$  and  $W_2$  are the BW (g) at, respectively, the beginning and the end of time interval *t* (Forsythe and Van Heukelem, 1987).

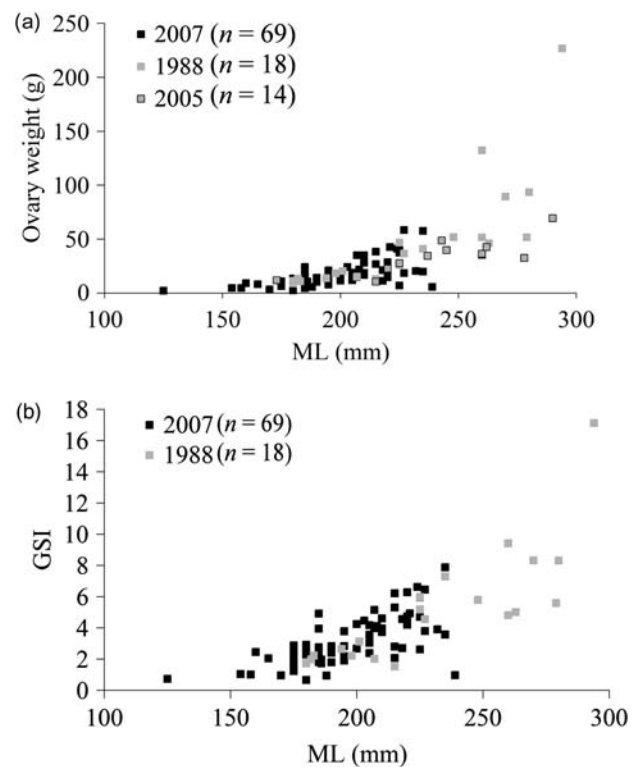
**Results**

**Sexual size dimorphism**

All males examined were mature (stage V; ML 130–290 mm; BW 267–1770 g; *n* = 74). Maturing females (stages II and III) were of similar size (ML 125–294 mm; BW 353–1950 g; *n* = 104). The similar size of mature males and immature females in our sample shows female-biased sexual size dimorphism. The ML–BW relationship was  $BW = 0.0069 ML^{2.20}$  (*n* = 104,  $r^2 = 0.76$ ) for females and  $BW = 0.0379 ML^{1.86}$  (*n* = 74,  $r^2 = 0.78$ ) for males.

**Female reproductive strategy**

The relationship between NGL and ML was significant:  $NGL = 0.6 ML - 52.7$  ( $r^2 = 0.71$ , *n* = 87,  $p < 0.001$ ). The weight of the ovary increases gradually up to a body size of ~1000 g and 225 mm ML, after which it increases more steeply (Figure 1). The largest ovary had a relative weight of 13.5% BW (294 mm ML, 1683 g BW). The GSI ranged from 0.7 to 17.1 (*n* = 87; Figure 1).

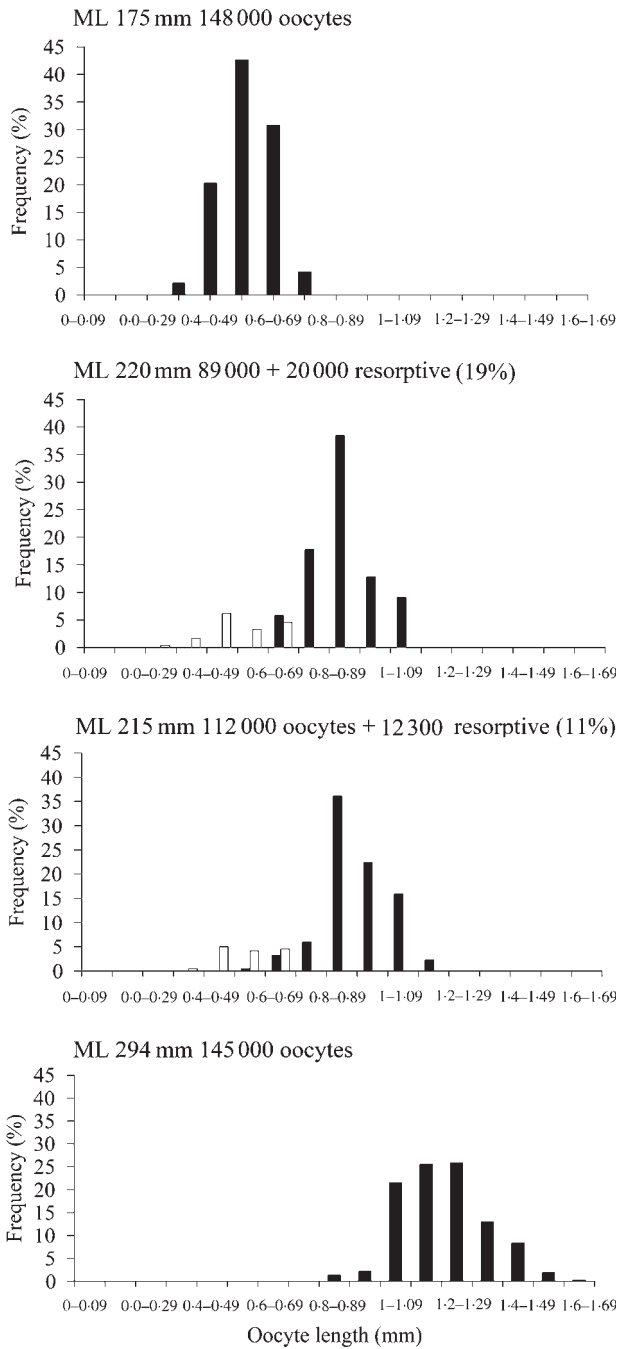


**Figure 1.** Female reproductive system of *H. miranda*: (a) ovary weight and ML; (b) GSI and ML.

The ovulation pattern is synchronous, with one size group of oocytes developing (Figure 2). The maximum oocyte length in the ovary increases linearly with ML (maximum oocyte length =  $0.0068 ML - 0.38$ ;  $r^2 = 0.77$ , *n* = 19,  $p < 0.001$ ), indicating that maturation coincides with somatic growth. The highest levels of resorption (20% of all oocytes inside the ovary) were found in a female of 220 mm ML and 358 g BW. The potential maximum fecundity for 19 females (i.e. excluding resorptive oocytes) ranged from 89 000 (ML 220 mm, BW 684 g) to 148 000 (ML 175 mm, BW 603 g). The correlations between potential fecundity and body size (ML,  $r^2 = 0.20$ ,  $p > 0.05$ ; BW,  $r^2 = 0.19$ ,  $p > 0.05$ ) were not significant.

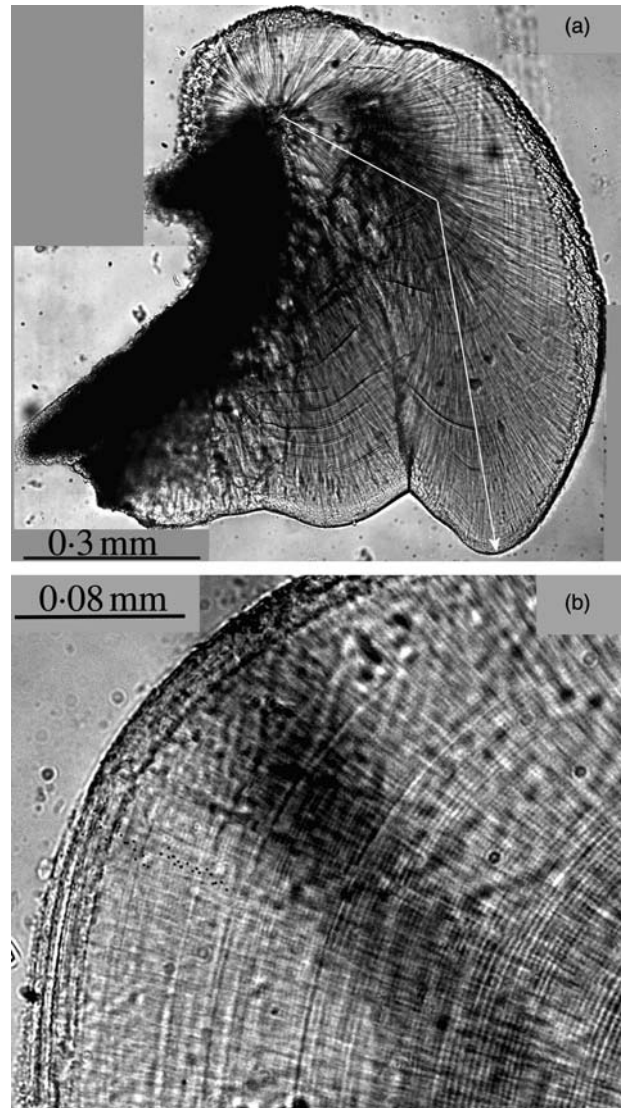
**Age estimates**

Statolith increments were well resolved (Figure 3). The smallest and youngest (mature) male (ML 150 mm, BW 266 g) had 276



**Figure 2.** Length frequency distribution of oocytes in the ovaries of four female *H. miranda*. White bars are the resorptive oocytes.

increments, and the largest (mature) and oldest male (ML 290 mm, BW 1550 g) had 467 increments. The smallest maturing female (ML 180 mm, BW 457 g) had 351 increments, and the youngest female (ML 215 mm BW 950 g) had 293 increments. The largest maturing female (ML 290 mm, BW 1950 g) had 406 increments, and the oldest maturing female (ML 210 mm, BW 926 g) had 464 increments. The correlation between age and body size was weak but significant for females: age and BW,  $r^2 = 0.154$ ,  $p = 0.03$ ,  $n = 30$ ; age and ML,  $r^2 = 0.207$ ,  $p = 0.011$ ,  $n = 30$ . The correlation between age and body size was better for males: age and BW,  $r^2 = 0.458$ ,  $p = 0.004$ ,  $n = 16$ ; age and ML,

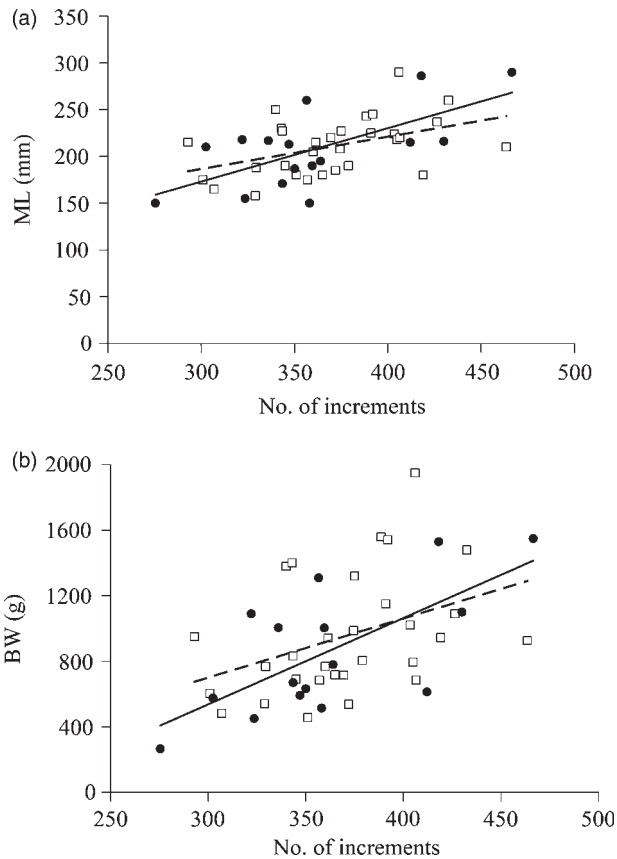


**Figure 3.** (a) Transverse section of the statolith of *H. miranda* (female, ML 278 mm, BW 1910 g, 406 increments). (b) Detail of the periphery of a transversely sectioned statolith of *H. miranda* (female ML 210 mm, BW 926 g, 464 increments), with the narrow increments (<1  $\mu\text{m}$ ) indicated by black dots.

$r^2 = 0.433$ ,  $p = 0.0055$ ,  $n = 16$ . The instantaneous growth rate  $G$  was obtained for BW. For females, it was  $0.4\% \text{ BW d}^{-1}$ , and for males  $0.66\% \text{ BW d}^{-1}$ . Fitting linear curves resulted in an absolute growth rate of  $0.35 \text{ mm d}^{-1}$  and  $3.6 \text{ g d}^{-1}$  for females, and  $0.57 \text{ mm d}^{-1}$  and  $5.3 \text{ g d}^{-1}$  for males (Figure 4).

**Discussion**

*Histioteuthis miranda* most likely exhibits female-biased sexual size dimorphism, because the largest females, which were still maturing, were of similar size to the largest males, which were fully mature. The largest female (ML 294 mm) in our sample had an ovary weight of 226 g and nidamental glands 126 mm long, but was still maturing. These data do not match the observations of Clarke (1980), who reported the ovary of a “ripe” female (ML = 160 mm) from Albany weighing 23.9 g and a nidamental gland of another “ripe” female (180 mm ML) as being 80 mm long.



**Figure 4.** Estimated total number of increments in relation to body size of *H. miranda*. (a) Male ML = 0.57 Age + 1.89 ( $r^2 = 0.43$ ), female ML = 0.35 Age + 82.37 ( $r^2 = 0.21$ ). (b) Male BW = 5.27 Age - 1042.5 ( $r^2 = 0.46$ ), female BW = 3.64 Age - 391.45 ( $r^2 = 0.15$ ). Fitted linear growth curves are shown for males (line and dots) and females (dashed line and open squares).

The absence of mature females in our sample may indicate that spawning is in water deeper than 900 m or that spawning is season-related and takes place outside the sampling period of February and March.

The ovulation pattern of *H. miranda* is synchronous, with significant resorption of oocytes. Oocyte resorption is a known phenomenon in cephalopods and may function to remove surplus oocytes for which energy reserves are inadequate to proceed with oocyte maturation (Melo and Sauer, 1998). Because of resorption, one would expect the total fecundity to decrease with increasing body size, as found for the deepwater squid *Onykia ingens* and *Gonatus antarcticus* by Laptikhovsky *et al.* (2007). The absence of a correlation between fecundity and body size in *H. miranda* is likely because of the small sample size and the great individual variation in fecundity. Additional resorption of oocytes with advancement towards full maturity may further reduce the fecundity.

Although fully mature females were absent from our samples, it seems that *H. miranda* has “avalanche”-like maturation, with a sudden steep increase in GSI and ovary weight. Such a maturation pattern is similar to that of other deepwater squid with synchronous ovulation (Arkhipkin and Björke, 1999), but different from squid with asynchronous ovulation, for which GSI increases more gradually with increasing ML (Laptikhovsky *et al.*, 2007,

and references therein). The synchronous ovulation pattern observed for *H. miranda* is also found in other histioteuthids (Laptikhovsky, 2001) and in *O. ingens* and *G. antarcticus* (Laptikhovsky *et al.*, 2007), indicating that all eggs are spawned at once or over a short period. Compared with asynchronous spawning, synchronous ovulation and spawning is detrimental to the overall fecundity because the female will only spawn as many eggs as the coelom can hold. In semelparous organisms, relatively low rates of adult survival and high rates of juvenile survival are expected to select for synchronous ovulation and simultaneous terminal spawning (Crespi and Teo, 2002).

Assuming that a single statolith increment represents one day, male *H. miranda* attain full maturity in 6 months, but may live as long as 15 months or more. The oldest maturing female was estimated to be some 15 months old. The absence of final ontogenetic stages of *H. miranda* in our sample restricts us from estimating longevity, but it seems that females grow older than males. The instantaneous growth rates of mature males ( $G = 0.66\% \text{ BW d}^{-1}$ ) and maturing females ( $G = 0.4\% \text{ BW d}^{-1}$ ) of *H. miranda* were lower than that of females of the southern African mesopelagic squid *Lycoteuthis lorigera* ( $G = 1.4\% \text{ BW d}^{-1}$ ; Hoving *et al.*, 2007), and female *Ancistrocheirus lesueurii* ( $G = 1-3\% \text{ BW d}^{-1}$ ), but similar to mature male *A. lesueurii* ( $G = \sim 0.5\% \text{ BW d}^{-1}$ ; Arkhipkin, 1997). The absolute growth rates of *H. miranda* (0.35 and 0.57 mm  $\text{d}^{-1}$  for females and males, respectively) were also lower than most oceanic squid studied to date (range 0.8–2.7 mm  $\text{d}^{-1}$ ; see Table 8 of Jackson and Choat, 1992; Jackson, 1997). The absence of all ontogenetic stages in our sample prevented reconstruction of a complete growth curve for *H. miranda*.

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