Reproductive biology of *Holothuria* (*Roweothuria*) *poli* (Holothuroidea: Echinodermata) from Oran Bay, Algeria

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Abstract

Our study is a first contribution of the reproductive biology of the aspidochirotid sea cucumber *Holothuria (Roweothuria) poli* at Kristel Bay at Ain Franine in Oran Province, Algeria. Sampling was conducted on 305 individuals (129 males, 131 females and 45 of indeterminate sex) from October 2016 to September 2017. Five macroscopic and microscopic sexual maturity stages have been identified in the gonadal tubules: recovery (I), growing (II), early mature (III), mature (IV) and spent (V). Also, the size at first sexual maturity within the entire population is 135 mm. Our results show that the maturation of the gonads (stages III and IV) occurs from March until May. From May to July, the entire sampled population is at full sexual maturity, and it is only in July that spawning begins, which extends up to September. The period of non-reproductive activity is between October and November.

Key words: *Holothuria poli*, reproduction, sexual maturity, southwest Mediterranean Sea

Introduction

Holothuria poli Delle Chiaje, 1824 is a frequent and abundant sea cucumber along the Algerian coast (Mezali 2008). It plays an important role in the recycling of organic matter in marine bottom sediment and it is now considered a target species for the Mediterranean fisheries (Purcell et al. 2013). The main countries that exploit *H. poli* are Turkey, Spain, Greece, Italy and Portugal. The Turkish sea cucumber fishery started in 1996 and reached about 600 tonnes (t) in 2011; *Holothuria poli* represented 80% of the catches (González-Wangüemert et al. 2014). Sea cucumbers are mainly exported to Asian countries in the form of frozen, dried and salted products (Aydin 2008; Aydin et al. 2011).

In Algeria, detailed studies have been carried out on the population dynamics of *H. poli* (densities and biomasses) (Mezali et al. 2006). Biological aspects such as biometrics, growth and feeding behaviour have also been detailed (Mezali and Semroud 1998; Mezali et al. 2003, 2006; Mezali and Soualili 2013). Although some aspects of reproduction have been studied for the two main sea cucumber species *Holothuria sanctori* and *H. tubulosa* (Mezali et al. 2014; Mezali and Soualili 2015), no studies exist on the reproductive cycle and the maturation of the gonads of *H. poli*.

Material and methods

Study area

The Ain Franine station is located on the western end of Algeria's coast, and is in the Bay of Oran, 8 km from Kristel (35° 46'52.40"N and 0° 30'50.12" W) (Fig. 1). This area is considered as being little impacted by anthropogenic activity (Hebbar 2013).

Identification and characteristic of the studied species

Identification of *H. poli* is based on morphological, anatomical, and endo-skeletal criteria (Koehler 1921; Tortonese 1965). This species does not present a defence organ (Cuvierian tubules), but does present white podias that are scattered throughout the ventral part of the body (Mezali 2008). Microscopic observation of the ossicles showed the presence of different forms of regular and smooth loops and pseudo-buttons and small tables (Mezali 2008). The gonad of *H. poli* is located in the left of the dorsal mesentery and has a single genital duct at its base to the outer part of gonopore, and consists of a single clump of tubules.

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Figure 1. Sampling site location: A) Oran Bay, B) Ain Franine station. Source: adapted from Google Earth, 2018

Sampling

In total, 305 individuals of *H. poli* were harvested over 12 months (from October 2016 to September 2017). The sampling was carried out randomly by hand using snorkelling and scuba equipment at an average depth of 8 m. During sampling, to avoid the loss of internal organs (gonads) due to the process of autotomy practiced by these marine invertebrates, each individual was isolated in a plastic bag. In the laboratory each individual was dissected, and the gonads carefully removed, drained and weighed (Wg ± 0.01 g). Each gonad was stored in a pill box and fixed in formalin (10%).

Gonad maturity stages

The macroscopic characteristics of each gonad were observed (colour, number, length, diameter and branching of the tubules). Histological analysis was performed on a monthly subsample of 10 gonads (males and females) selected from an average sample of 25 individuals collected each month (120 individuals of the 305 individuals collected in total). One piece (0.5 mm) of each part of the tubules was removed, dehydrated, embedded in paraffin, sectioned at 7 µm, stained with hematoxylin-eosin, and observed under an optical microscope at x100 and x400 magnification. Sex was determined primarily on the basis of gonad colour. The tubules of the female gonads are usually yellow or orange, and in the male, they are whitish. We noted that determining sex in some cases was not easy macroscopically, and necessitated microscopic observations. The identification of the various stages of sexual maturity was based on the gonad maturity scale established by Conand (1981) and modified by Ramofafia and colleagues (2000): I) recovery, II) growth, III) early mature, IV) mature and V) spent.

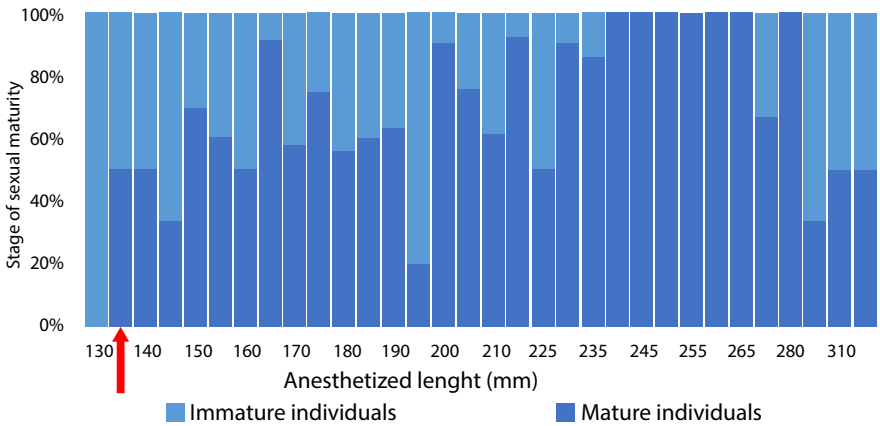

Figure 2. Distribution of immature individuals (% of individuals at stages I and II) and mature (% of individuals at stages III, IV and V), depending on the size of *Holothuria poli* at the Ain Franine station. The red arrow indicates the size at first sexual maturity.

Results

Size at first sexual maturity

The analysis of the variation of sexual maturity stages (depending on the size of the holothuroid) showed that 50% of individuals are mature when they reach 135 mm (Fig. 2).

Macroscopic and microscopic observations of female and male gonads

During stage I (recovery), determining the sex of the gonads is impossible. The ovarian tubules are thin, white and transparent, and very slightly branched and short (Figs. 3A and 3a). In females, the lumens of the tubules are almost empty. The pre-vitellogenic oocytes are attached to the germinal epithelium of the tubule with the presence of some more rounded early vitellogenic oocytes (Fig. 3B). In males, the wall of the gonad is at its maximum thickness. The presence of early stages of sperm maturation is noted on the borders of the tubules with the presence of empty zones (Fig. 3b).

In stage II (growth) in both sexes, the number of gonad tubules increases and they can become divided. They have a whitish colour (Figs. 3C and 3c). In females, the tubules are very pale white to orange colour. The number of oocytes increases and the lumen of the tubules is not completely occupied by oocytes of different sizes (Figs. 3D and 3E). In males, the germinal epithelium extends towards the lumen of the tubule, giving rise to the development of spermatocytes colonies (Fig. 3d).

At stage III (near maturation) in both sexes, the gonad tubules are longer, more numerous, dilated and branched (Figs. 3F and 3e). In females, their colour is light pink to orange. The presence of different stages of development is observed (e.g. mature oocytes in the centre of the tubules and small oocytes near the tubular wall, see Fig. 3G). In males, the tubules are cream colour, long, numerous, dilated and branched (Fig. 3f).

At stage IV (maturity) in females, the number of tubules and their ramifications reach their maximum. During this stage, ovary weight is generally higher than that of the testicle; the largest ovary is 17.94 g; female gonads are orange in colour and are completely dilated (Fig. 3H). There is a predominance of mature oocytes with a single nucleolus (Fig. 3J) and few immature oocytes are also observed (Fig. 3I). In males at this stage, the gonad weight reaches 12.64 g, and the tubules are milky in colour, dilated to a maximum diameter (Fig. 3g). The walls of the tubules are thin, and the spermatozoa are numerous and mature (Figs. 3h, 3i).

At stage V (spent), in females, the tubules regress and become flaccid, wrinkled and more or less empty. At this stage, tubules or the tubular region are seen and they always occupy un-expelled gametes in atresia (Fig. 3k). In males, after the release of gametes, the tubules regress and become transparent. They are flaccid and more or less empty, with the presence of residual gametes (Fig. 3j).

Monthly variability of sexual maturity stages

The monthly variability in sexual maturity stages of mature individuals (all confused sexes) is followed in order to specify the spawning period. Figure 4 shows that 70% of individuals are at full sexual maturity (stage IV) in July 2017, while the percentage of individuals observed at stage V is greater than 90% in October and November. Stage I was observed from November 2016 to January2017 and is marked by the presence of a maximum number of individuals at this stage (in November 2017). The growth of the tubules was noted during stages II and III from February to June 2017, with a presence of less than 10% of the gonads in stage III during July and August.

Discussion

Holothuria poli is a common species in the Mediterranean Sea, but data on its reproduction are lacking. Sampled individuals of *H. poli* are gonochoric and show no sexual dimorphism, which is observed in many aspidochirotid holothurians (Despalatovic et al. 2004; Asha and Muthiah 2007; Navarro et al. 2012; Mezali et al. 2014). Sea cucumbers often show an annual reproductive cycle (Tuwo and Conand 1992; Conand 1993a, b; Chao et al. 1995). Although half yearly or even a continuous reproductive cycle throughout the year is also common, especially in tropical regions (Harriott 1985; Conand 1993b). In total, 305 individuals of *H. poli* (129 males, 131 females and 45 of indeterminate sex) were considered in the present study. During our survey, the absence of hermaphroditic individuals was noted. This result is similar to that obtained by Bardanis and Batjakas (2018) for the same species at Lesvos Island in Greece.

Holothuria poli is a typical temperate sea cucumber, which presents a single annual reproductive cycle (Tuwo and Conand 1992; Hamel and Mecier 1996). Maximum reproductive activity was observed during the warm months of the year (July and August 2017) while minimum activity (rest) was observed during the coldest months of the year (November and December 2016). The macroscopic and microscopic observations suggest that the development of male and female gonads of *H. poli* adapts an asynchronous 'tubular recruitment model', showing the tubules in different stages of development

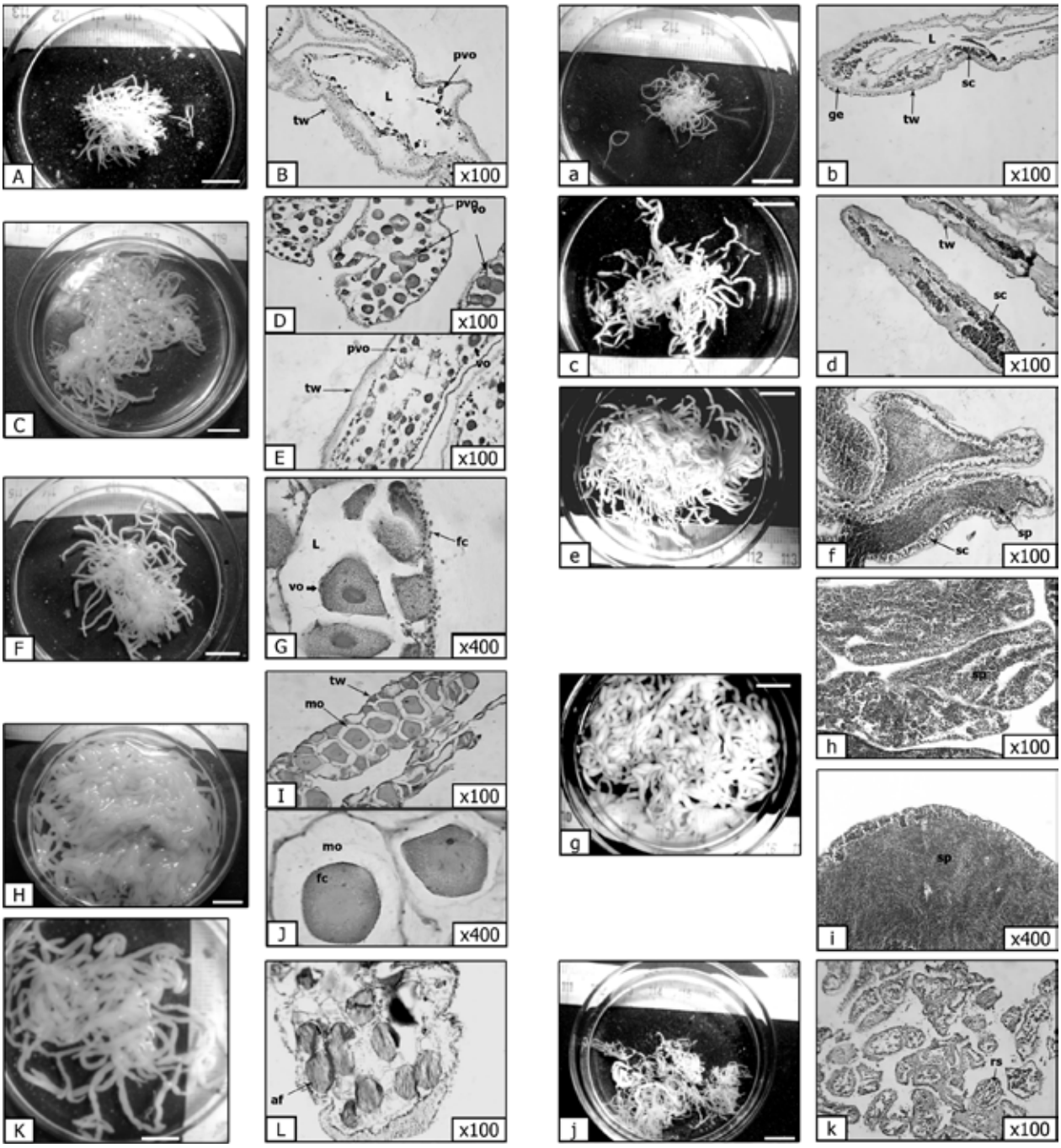

Figure 3. Macroscopic and microscopic description of female (left) and male (right) gonads characteristics of *Holothuria poli* at different maturity stages, scale bar = 10mm. **A, a.** Gonad at recovery stage. **B, b.** Empty tubule [Light (L), thin tubule walls (tw); with pre-vitellogenic oocytes (pvo), spermatocytes (sc) along the germinal epithelium (ge)]. **C, c**. Gonad at growth stage. **D, E.** details of tubules with various development stages [Early vitellogenic oocytes (vo) detached from the thin tubule wall (tw); pre-vitellogenic oocytes (pvo)]. **d.** Thin tubule wall (tw) with spermatocytes (sc); **e, F.** Gonad at early mature stage**. f.** Male tubule lumens are occupied by several stages of spermatogenesis, spermatocytes (sc) and spermatozoa (sp); **G.** Early mature tubules [with vitellogenic oocytes (vo) and follicular cells (fc)]. **H, g.** Gonad at mature stage. **I.** Tubule [with densely packed fully mature oocytes (mo) and thin tubule walls (tw)]. **J.** Mature oocyte (om) separated from its follicle (fc). **K.** Gonad at partially spawned stage. **L.** Residual oocytes with atretic follicle (af). **H, i.** Tubules engorged with mature spermatozoa. **j.** Gonad at spent stage (tubules turn thin and translucent). **k.** Extremely wrinkled wall almost empty tubules, only relict spermatozoa (rs) patches.

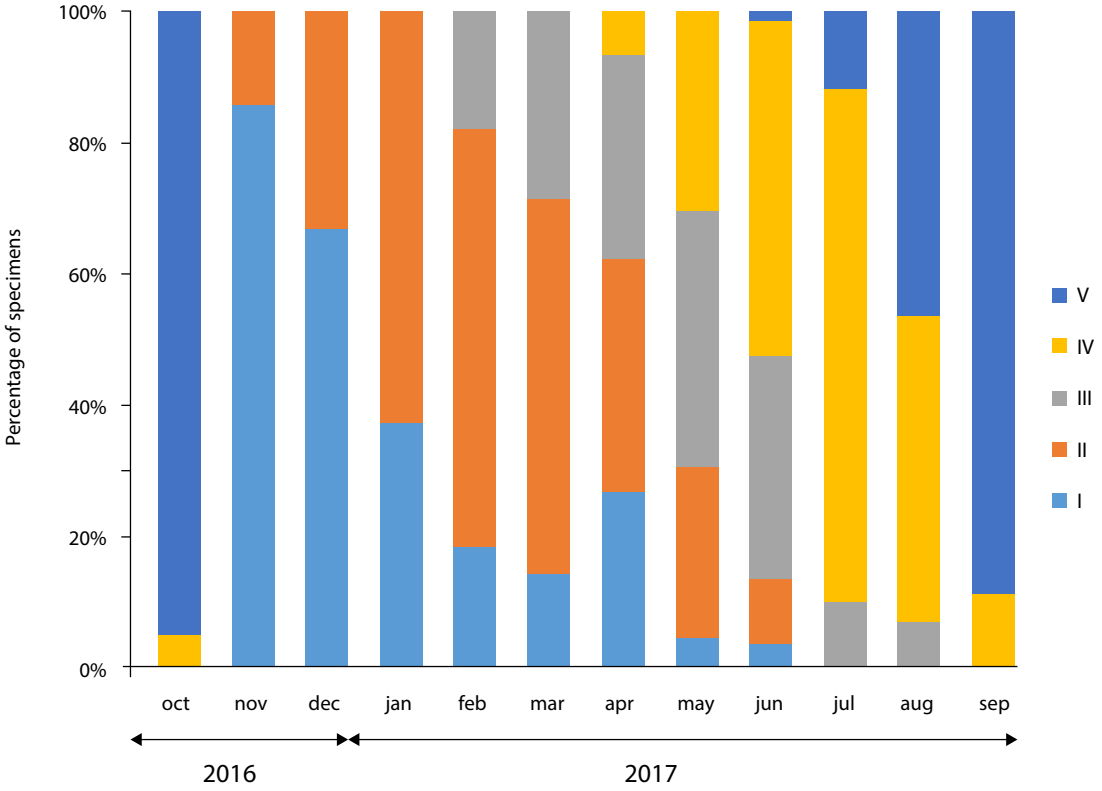

Figure 4. Monthly evolution of sexual maturity stages in mature individuals of *Holothuria poli* at Ain Franine station.

together. This case is not unusual, because there are many other species of sea cucumbers that do not follow the model of recruitment of the tubules, such as *H. leucospilota* (Ong Che 1990), *H. atra* (Chao et al. 1994), *H. fuscogilva* and *H. mauritiana* (Ramofafia and Byrne 2001).

In *H. poli*, the percentage of sexual maturity stages of the sampled individuals varies from March to June 2017, where stage III is predominant in both males and females. From the end of June 2017 and into early July 2017, individuals reach full sexual maturity, which is marked by the presence of a high percentage of individuals in stage IV. At the last stage of maturation (stage V), a regression of tubule size in length and diameter in males and females is observed, and this is most probably due to post-emission gonad regression, In their studies on *H. leucospilota,* Purwati and Luong-Van (2003) explain that this species has the ability to 'reintegrate' its gonad once its gametes are expelled. This regression of male and female tubules was also observed by Ong Che (1990). In work conducted in New Caledonia, Conand (1981) explains the presence of individuals without gonads during the resting phase, showing that the gonad grows throughout its maturation, until fertility, then retracts after spawning. This was observed for *H. poli*, which presents a large number of non-gonadal individuals in November.

Conclusion

Our study shows that size at first sexual maturity is around 135 mm (both sexes combined). As for maturation, *H. poli* sampled from Ain Franine station present a general reproduction pattern marked by an increase in the percentage of mature individuals (stages III and IV) from March to June, with spawning beginning in July and extending until September, while the period of rest is observed from October to November. The reproduction parameters obtained in the present work will provide a database for the establishment of regulatory measures for the exploitation of *H. poli* on the western end of Algeria's coast, such as defining a harvest season, and establishing a ban on catching this species during the breeding period in order to manage sea cucumber stocks on Algeria's coast.

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