An investigation of the macroscopic and microscopic characteristics of gonadal tubules in the sea cucumber *Holothuria leucospilota* (Persian Gulf, Iran)

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Abstract

In this study, we present data obtained from monthly samplings of *Holothuria leucospilota* done between July 2007 and June 2008 (except December and January) along Iran's Bustaneh Coast in the Persian Gulf. In total, 124 females and 108 males were collected. Macroscopic and microscopic features of the gonads were used as the main criteria for determining maturity stages. Observed macroscopic features included gonad colour, weight, length, diameter and number of tubules, while microscopic examinations consisted of histological studies of stained slides with haemotoxylin and eosin. Based on these studies, five stages of maturity were determined for both sexes: early growth (I), growth (II), advanced growth (III), mature (IV) and post-spawning (V). The tubules were found to be much longer and narrower in males than in females. Gonadal tubules developed from stage I to stage IV, declining in stage V. The possible relationship between tubule numbers and gonad weight was evaluated, and was found to be 0.73 for males and 0.80 for females.

Introduction

Holothuroidea (sea cucumbers) is a diverse class of worm-like and usually soft-bodied echinoderms. They are found in nearly every marine environment, but are particularly diverse in tropical shallow-water coral reef ecosystems. They range from the intertidal, where they may be exposed briefly at low tide, to the floor of the deepest oceanic trenches. Sea cucumbers are a group of marine invertebrates that are harvested worldwide, mostly for human consumption in Asian countries. Over the past several decades, a significant increase in the demand for sea cucumbers has led to an explosion in exploitation, which often results in population declines in many producing nations. Because of their critical importance as a source of livelihood for many artisanal fishers from developing countries, and as a globally traded product, there is considerable interest in the biology, ecology and fisheries management of sea cucumbers (Conand and Muthiga 2007). There have been few ecological studies conducted on sea cucumbers in the Persian Gulf. The importance of detailed studies on holothuroids is necessary due to their key role in the conservation of the marine environment. They are essential economically and used as food, are important members of benthic communities, and are responsible for causing significant changes in the composition of the sea floor. They also have medical significance such as treating weakness, impotence, senility, constipation due to intestinal dryness, and frequent urination. The worldwide decline of wild stocks of sea cucumbers has generated a great deal of interest in their reproduction and biology. Such information could lead to the development of better management programmes as well as aquaculture of this valuable resource.

The goals of the present study are to describe the macroscopic and microscopic characteristics of gonadal tubules and determine the gonadal development stages in *H. leucospilota* on the Bustaneh coast of Iran's Hormozgan Province in the Persian Gulf.

Materials and methods

Sea cucumbers were collected from the intertidal area at Bustaneh in the Persian Gulf of Iran (26°31'N and 54°39'E) (Fig. 1). From July 2007 to June 2008, 25–30 animals were collected each month. Sex was determined by dissecting the animals. Male tubules were always creamy white in colour. Female tubules were more transparent, with the interior having a granulated appearance. Female tubules became reddish-orange with the development of fecund ovaries. The gonads were preserved in buffered formalin (10% dilution). In a holothurian population

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with simultaneous gonadal development, spent tubules are absorbed after the reproductive season; therefore, gonads may not be visible for a certain period (Purwati and Luong-van 2003).

Identification of maturity stages in this study was done using several methods. Macroscopic features were assessed and then a microscopic observation of a fragment of gonad was made (Conand 1981). We then confirmed our results with histological examinations. The macroscopic and microscopic features were used to assess maturity stages of gonads, as described by previous studies (Drumm and Lonergan 2005; Ong-Che 1990; Purwati and Luong-van 2003). Gonadal macroscopic features, including gonad colour, total number of tubules in a gonad, length of tubules,[3] diameter of tubules,[4] and presence of gametes in squash preparations were studied. The amounts of all these features depend on the stage of maturity. The relationship between gonad weight and the number of tubules was calculated for both sexes. The microscopic study consisted of histological examinations. A subsample of 10 animals (representing the available size range) was taken from each monthly sample for histology. Small sections of gonad tubules were dehydrated, embedded in paraffin, sectioned (5-μm thick) and stained with haemotoxylin and eosin (H/E). The histological stages of gametogenesis were based on those used in previous studies of sea cucumber gonads (Drumm and Lonergan 2005; Purwati and Luong-van 2003; Rasolofonirina et al 2005; Shiell and Uthicke 2006). Based on these criteria, a five-stage maturity scale was determined: early growth (Stage I), growth (Stage II); advanced growth (Stage III); mature (Stage: IV); and post-spawning (Stage: V). Each gonad was examined and assigned in order to determine its sexual stage. These five maturity stages were verified by histological examination and macroscopic features.

Results

Sexes were separate in *Holothuria leucospilota* but there was no sexual dimorphism. Therefore, it was not possible to differentiate males from females externally. The gonads of females and males are different colours so could be sexed by dissection. The gonad was a single tuft in *H. leucospilota* and consisted of tubules hanging freely in the coelom from a transparent, saddle-like gonadal base located at the side of the anterior part of the intestine. Each tubule formed two or more branches. When mature (Stage IV), female tubules were pink to reddish-orange, and the oocytes were visible as small as white spots in the fecund ovary. After spawning (Stage V), the tubules deteriorated and turned brown; unspawned gametes were likely reabsorbed. Male gonadal tubules in advanced stages of maturity were long and creamy-white to beige in colour. The length, diameter, colour and number of tubules in both sexes were related to the stages of development.

Figure 1. Study site: Bustaneh, Persian Gulf, Iran

3 This was done by measuring the lengths of 20% of the total number of tubules for each gonad, to the nearest 0.02 mm measured from tip to end by means of colis in order to determine the average length of gonad).

4 This was done by measuring the diameter of 20% of the total number of tubules for each gonad, by means of an ocular micrometer, in order to determine the average diameter of gonad).

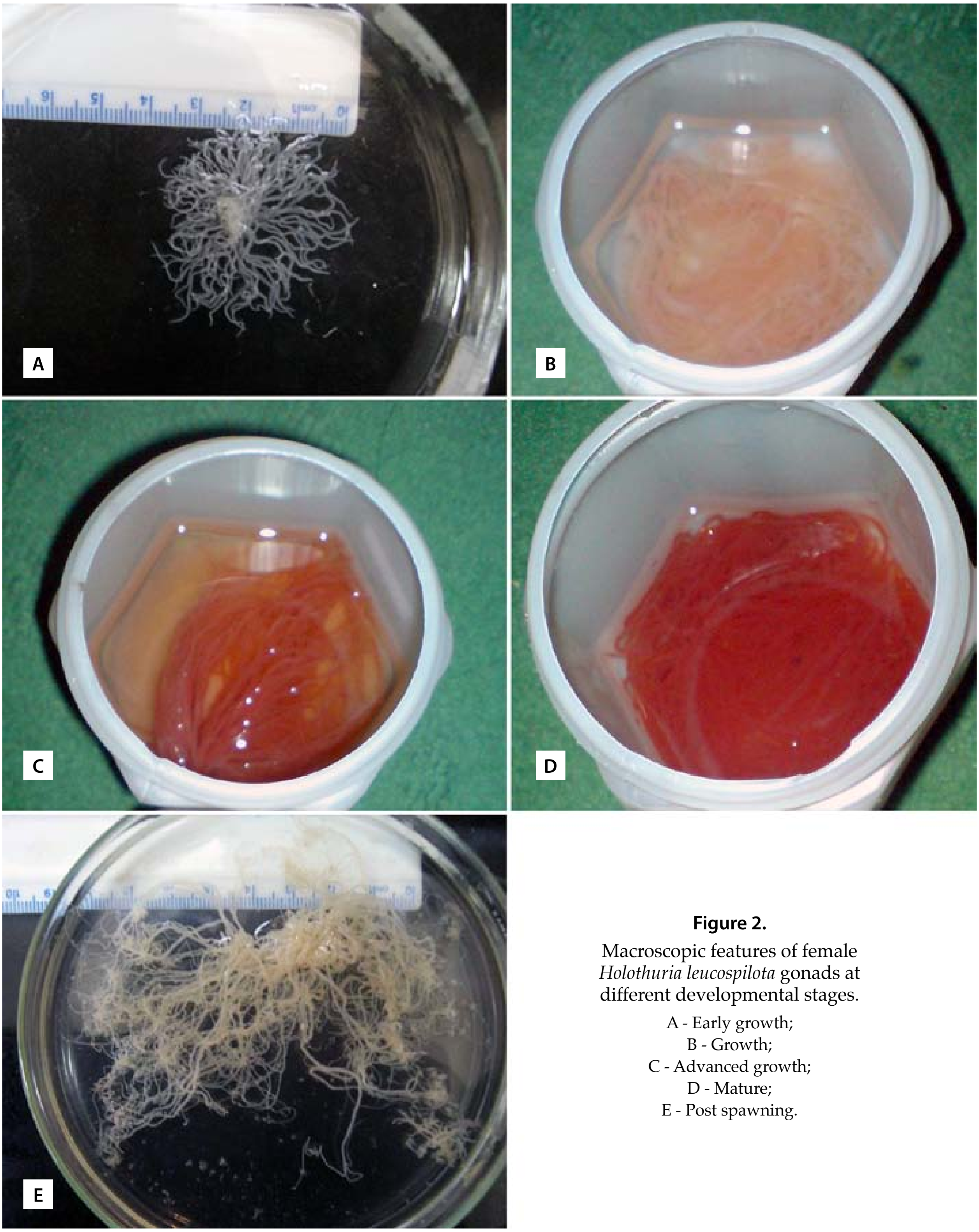

Figure 2.
Macroscopic features of female *Holothuria leucospilota* gonads at different developmental stages.
A - Early growth;
B - Growth;
C - Advanced growth;
D - Mature;
E - Post spawning.

Macroscopic features of gonadal maturity stages

Stage I – Early growth

Gonads at this stage could not be sexed by microscopic examination. And sex could only be identified by histological examination. The tubules in both sexes were thin, white to transparent, slightly branched, and were small and numerous (Fig. 2A, 3A).

Stage II – Growth

The tubules' dimensions, numbers and branches increased in both sexes. Female gonads were white to very light-pink. Sex could be determined microscopically by the presence of developing oocytes (Fig. 2B) and male gonads were pale-white (Fig. 3B).

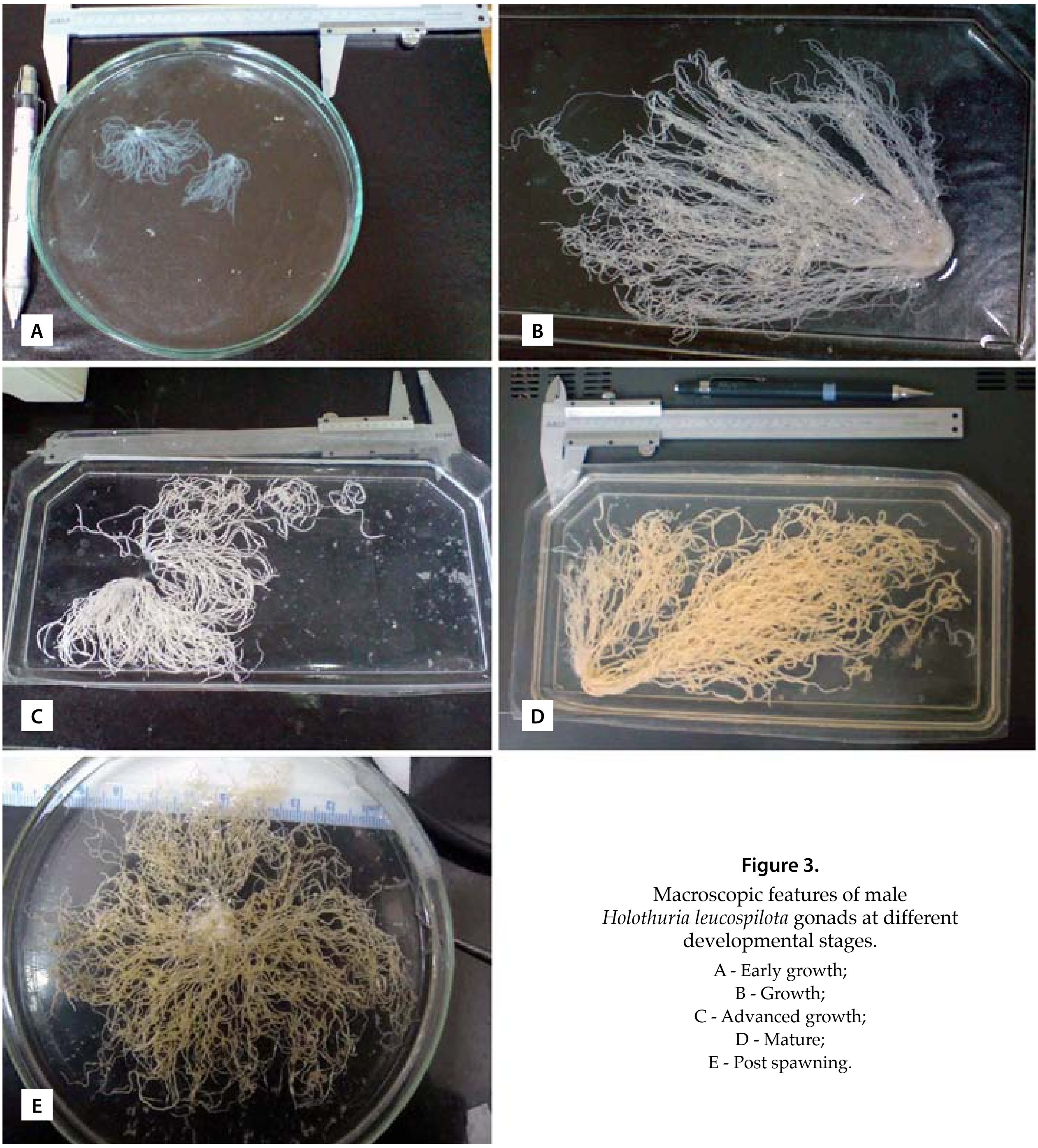

Figure 3.

Macroscopic features of male *Holothuria leucospilota* gonads at different developmental stages.

A - Early growth;
B - Growth;
C - Advanced growth;
D - Mature;
E - Post spawning.

Stage III – Advanced growth

Tubules were longer, thicker, more branched and more numerous in both sexes. Female gonads were dark pink (Fig. 2C) and male gonads were a slightly dark-cream colour (Fig. 3C).

Stage IV – Mature

Tubules were bulging, long and thick in both sexes. Female gonads changed colour from pink to red-orange (Fig. 2D) and male gonads turned to beige (Fig. 3D).

Stage V – Post-spawning

After spawning, the tubules in both sexes deteriorated and turned brown (Fig. 2E, 3E). Tubules were flaccid and more or less empty, but some parts of the tubule volume were still occupied by undischarged gametes. Undischarged oocytes at various stages of deterioration were noted in females and were likely reabsorbed.

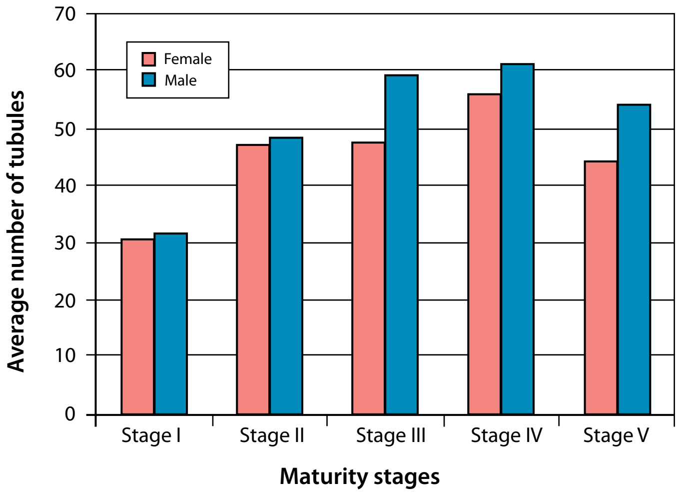

Figure 4. Average number of tubules at different stages of maturity for female and male *Holothuria leucospilota*.

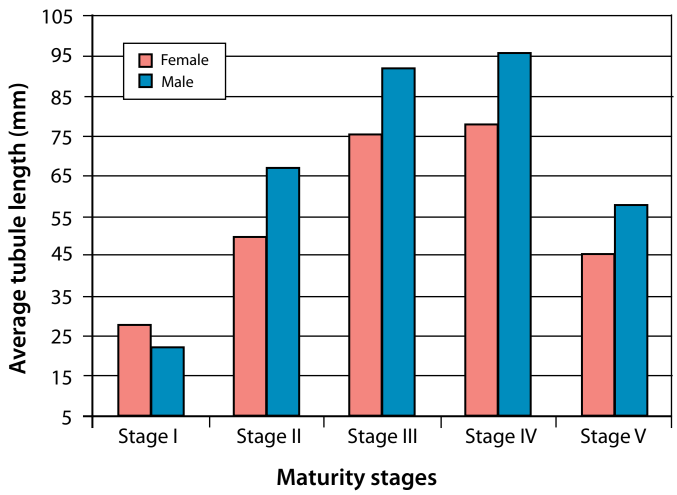

Figure 5. Average tubule length at different stages of maturity for female and male *Holothuria leucospilota*.

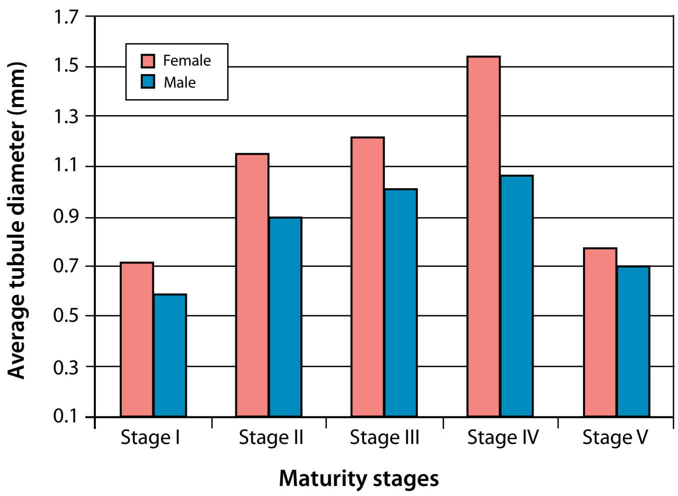

Figure 6. Average tubule diameter at different stages of maturity for female and male *Holothuria leucospilota*.

Biometry

Variations in gonad biometrical parameters, including number, length and diameter of tubules in different stages of maturity, are presented in Figures 4, 5 and 6 for both sexes. Biometrical parameters clearly show the increasing number of tubules per individual with developing maturity. The value of tubules' length and number were greater in males but the diameter was higher in females than males (t-test, $p > 0.05$).

The relationship between gonad weight ($GW_{(f=female)}$, $GW_{(m=male)}$) and number of tubules ($y_{(f)}$, $y_{(m)}$) gives a high correlation coefficient (R^2) for both sexes and is expressed by the following equations (Table 1).

Table 1. Relationship between gonad weight (GW) and number of tubules (y)

Female	Male
$y_{(f)} = 34.081\ GW^{0.2121}_{(f)}$	$y_{(m)} = 37.045\ GW^{0.2467}_{(m)}$
$R^2 = 0.803$	$R^2 = 0.737$

Microscopic features of female gonad maturity stages

Stage I – Early growth

The tubule lumen area was empty of gametic cells, and some pre-vitellogenic oocytes lined the germinal epithelium of the ovary (Fig. 7A).

Stage II – Growth

Active vitellogenesis, with oocytes growing from early to later stages of vitellogenesis, was observed. Many small oocytes and pre-vitellogenic oocytes along germinal epithelium were observed (Fig. 7B).

Stage III – Advanced growth

Small oocytes were observed along the germinal epithelium and lumen, mostly occupied by large mature oocytes (Fig. 7C).

Stage IV – Mature

Lumen were packed with mature oocytes and no small oocytes were present along the germinal epithelium. Each oocyte has a well determined germinal vesicle (Fig. 7D).

Stage V – Post-spawnimg

Residual oocytes at various stages of deterioration were observed in lumen. Some nutritive phagocytes begin to appear. Elongated empty areas were seen in tubules, suggesting the passage of oocytes along the tubule during spawning (Fig. 7E).

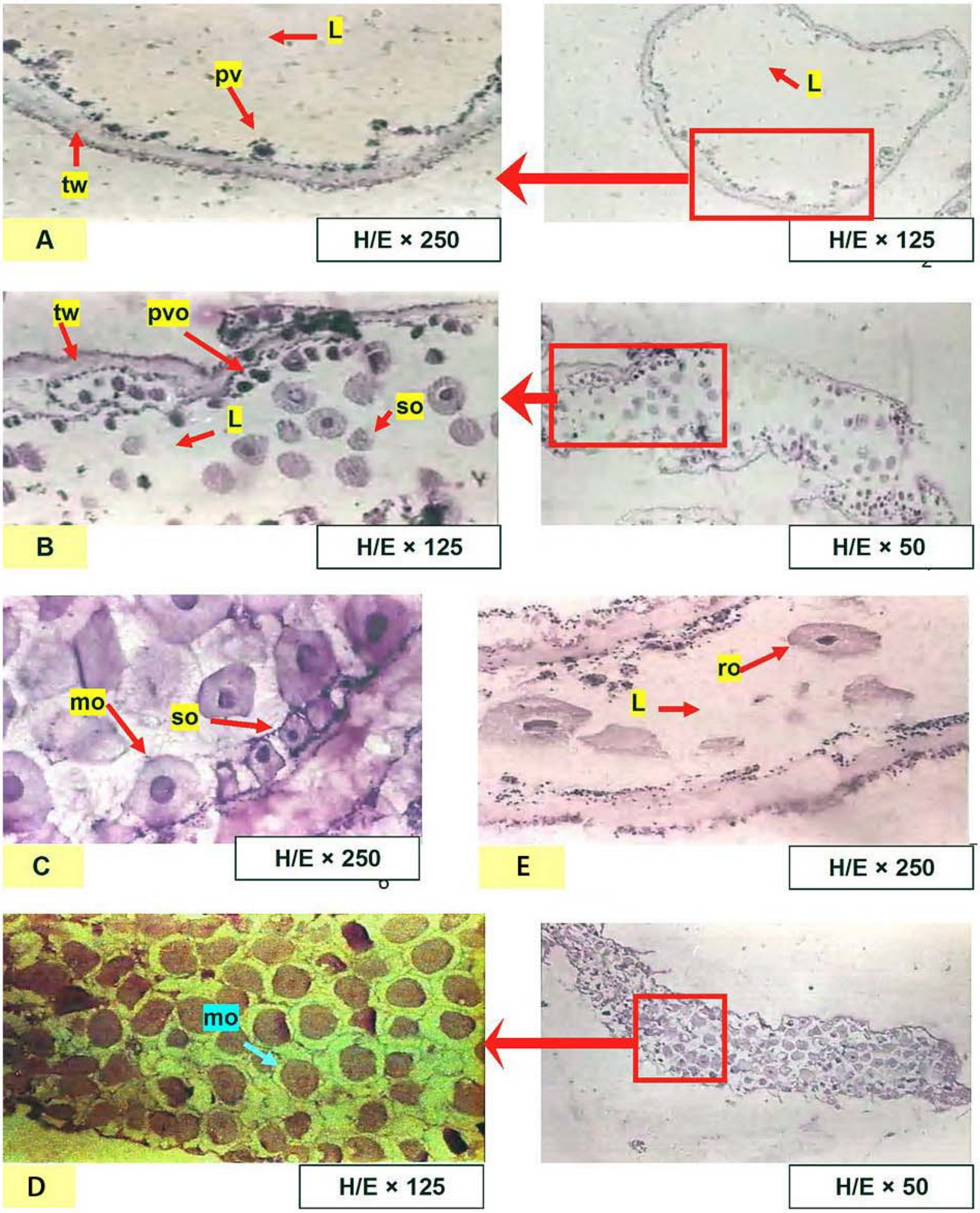

Figure 7.

Microscopic features of female *Holothuria leucospilota* gonads at different developmental stages.
L: lumen area; **mo:** mature oocyte; **pvo:** previtelogenic oocyt; **ro:** residual oocyte; **so:** small oocyte; **tw:** tubule wall.
A - early growth; B - growth; C - advanced growth; D - mature; E – post-spawning.

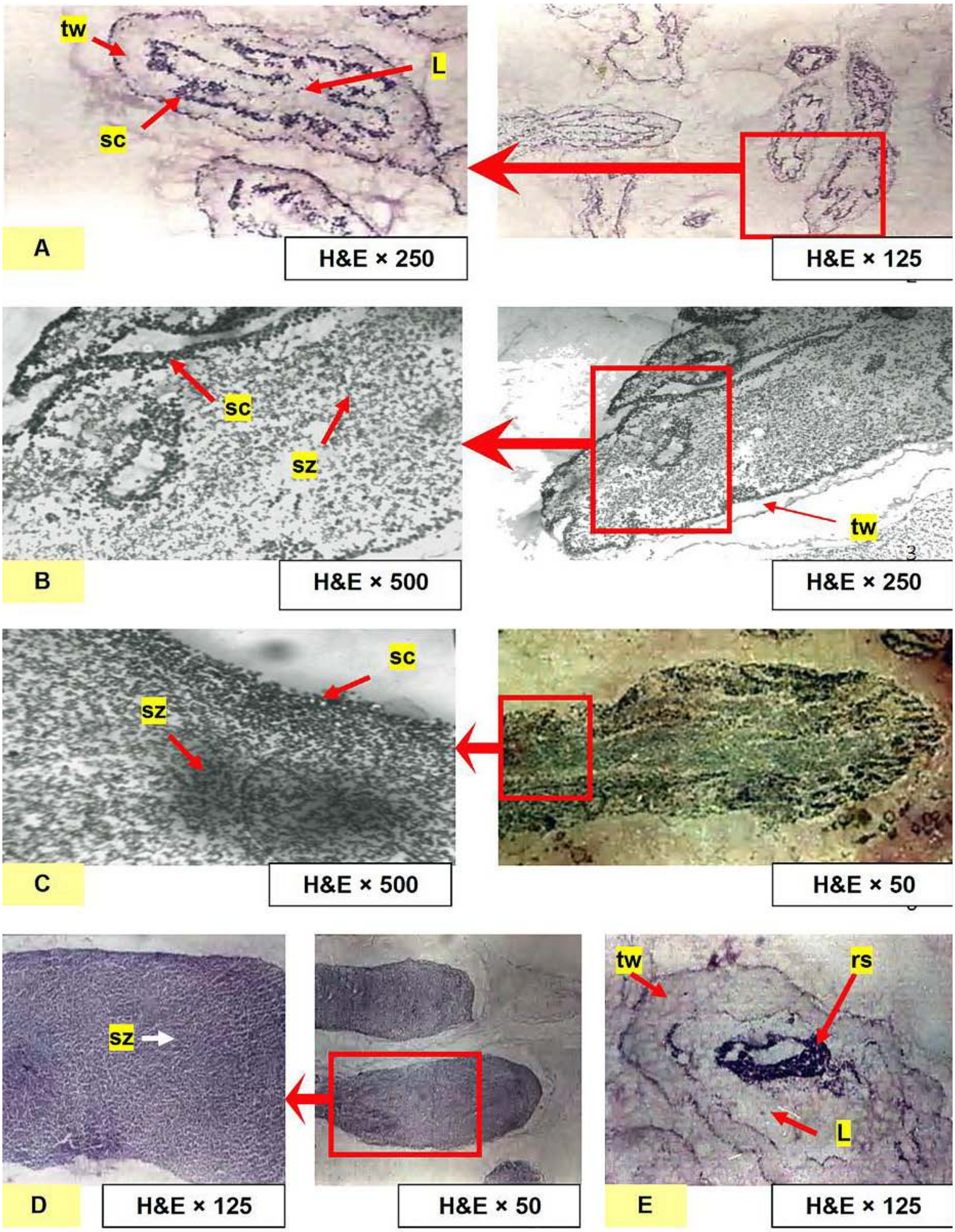

Figure 8.

Microscopic features of male *Holothuria leucospilota* gonads at different developmental stages.
L: lumen area; **rs:** residual spermatozoa; **sc:** spermatocyte; **sz:** spermatozoa; **tw:** tubule wall.

A - early growth; B - growth; C - advanced growth; D - mature; E – post-spawning.

Microscopic features of male gonad maturity stages

Stage I – Early growth

The tubules in this stage were characterised by the empty lumen area and presence of spermatocytes along the germinal epithelium (Fig. 8A).

Stage II – Growth

In this stage, tubules were seen with numerous infolds of the germinal epithelium with columns of spermatocytes along it and some spermatozoa in lumen area (Fig. 8B).

Stage III – Advanced growth

Tubules with a smooth wall were observed. Spermatocytes were very reduced and lumen were filled with spermatozoa (Fig. 8C).

Stage IV – Mature

Tubules' lumina were fully packed with spermatozoa and had very thin and smooth tubule walls. No proliferating zone with earlier stages along the germinal epithelium were seen (Fig. 8D).

Stage V – Post-spawning

Tubules in this stage showed elongated empty passages in the lumen following spawning, and a few residual spermatozoa. No proliferating zone along the germinal epithelium containing spermatocytes was seen (Fig. E)

Discussion

Development of gonadal tubules

Individuals of *Holothuria leucospilota* on the Bustaneh coast of the Persian Gulf in Iran, possess a single tuft of gonadal tubules. A large number of keys for sea cucumber maturity stages have been recommended by Chao et al. (1994), Conand (1993), Drumm and Lonergan (2005), Howaida et al. (2004), Lee et al (2008), Purwati and Luong-van (2003), Rasolofonirina et al. (2005), Shiell and Uthicke (2006), Thierry and Conand (2001). The present study shows that the ovarian tubules were shorter and wider, but the number of tubules in testes was higher than that of the ovaries. Similar results were obtained on *Actinopyga echinites* in New Caledonia where it was explained that testicular tubules were slightly longer and narrower than ovarian ones (Conand 1982). Yet, in *Stichopus herrmanni* from Kish Island, Iran, it has been reported that the number of gonadal tubules in males is higher than that of females (Tehranifard et al. 2006). In another study in New Caledonia, three species of sea cucumbers, Conand (1981) reported that: 1) in the post-spawning stage, the gonads of *Thelenota ananas* are deflated and more limp, and residual ripe oocytes or spermatozoa may be observed as well as signs of atresia and reabsorption of germinal cells by phagocytic cells; 2) *Holothuria nobilis* gonads are sexually dimorphic: in females the tubules are shorter and wider; 3) *H. fuscogilva* gonads are not sexually dimorphic.

In the present study, with the continuation of maturity in both sexes, from the early to fecund stage, the value of the biometric parameters increased. In the last stage of maturity (post-spawning), the measured parameters of gonad decreased in male and female individuals. Most likely, this situation is due to the gonad regression after spawning. Purwati and Luong-van (2003), in their study on *Holothuria leucospilota* in Darwin, Australia, explained that this species has the capability of "reabsorbing" its gonads after spawning. In a study on *H. leucospilota* in Hong Kong (Ong-Che 1990), the same results with regard to tubule dimension in males and females were reported. The presence of individuals without gonads in the resting phase was explained by Conand (1981) in research conducted in New Caledonia with three species of sea cucumbers, which showed that the gonad of those species increased in dimension from the early to fecund stage, and decreased after spawning, in the post-spawning stage.

In this study, the relationship between gonad weight and number of tubules gave a high correlation coefficient (R^2) for both sexes. Rasolofonirina et al. (2005) made the same observation for *Holothuria scabra* in the southwestern Indian Ocean.

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