

Reproductive cycle of *Stichopus herrmanni* from Kish Island, Iran

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Abstract

This study provides information on the reproductive biology of *Stichopus herrmanni*. The gonad morphology of *S. herrmanni* from Kish Island, Iran, is similar to that of other populations. Gonad colour is an unreliable characteristic for sex determination except at maturity. The sequence of gametogenesis events begins in late winter and continues until summer. The active stage of gametogenesis coincides with increasing photoperiod and temperature. Very little spawning activity out of season was noticed and a distinct peak spawning period was identified in summer. The average body length at first maturity was 310 mm and the average diameter of mature oocytes was 200 μm . Relative and absolute fecundities were around 8×10^3 oocytes.

Introduction

Several studies have been conducted on the reproductive biology of different species of the genus *Stichopus*: *Stichopus tremulus* (Jespersen 1971), *Stichopus variegatus* (= *herrmanni*) (Conand 1993a), *Stichopus japonicus* and *Stichopus chloronotus* (Uthicke 1997; Conand et al. 2002). But no published work is available on the reproductive biology of sea cucumbers from Iran. This paper details some aspects of the sexual reproduction of the sea cucumber *Stichopus herrmanni* from Kish Island, Iran, in the Persian Gulf, which was studied over a 16-month period. The results presented were obtained from histological sectioned gonads and smears.

also where the majority of sampling (by local divers using scuba) took place over a 16-month period. Altogether, 220 specimens of *Stichopus herrmanni* were collected at various depths, mainly from the east coast where reefs are denser and where most sea cucumbers occur (Fig. 1).

Macroscopic examination

In the laboratory, total length (TL), body wall wet weight (BW) and gonad wet weight (GW) were measured, and the gonads fixed in formalin (10%). Eviscerated weight (We) was also recorded. Frequency distributions and biometric relationships were determined. For each specimen, information

Materials and methods

Sampling site

Kish Island lies between 26°29' N and 26°35' N, and 53°53' E and 54°4' E, some 18 km south of Iran's mainland. Kish — a coral island with an area of 90 km² and fringing reefs — is one of the most important recreational sites along the coast and is also a free trade zone. This situation puts considerable pressure on the marine environment, and has resulted in the destruction and disappearance of many corals and coral communities over the last 10 years. Today, most of what remains are a few scattered corals reefs located on the east coast of the island where the main recreational activities occur. This is



Figure 1. Kish Island, in the Persian Gulf.

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on gonadal development, sex and colour of gonadal tubules was noted. Gonadal development was estimated by taking a small piece of gonadal tubules, using a clean glass pipette and smearing the sample on a slide. Smears were examined under a compound Zeiss microscope at $\times 400$ and/or $\times 1600$. Five stages were recognised: Stage 1: immature, Stage 2: growth, Stage 3: advanced growth, Stage 4: mature and spawning, and Stage 5: post spawning. A gonad index ($GI = GW \times 100 / BW$) was calculated each month for males and females. Fecundity was measured from mature ovaries at Stage 4. A portion of gonad tubules were extracted, weighed and fixed in Gilson fluid — 100 ml of 60% alcohol + 880 ml of distilled water + 15 ml of 80% nitric acid + 20 ml of acetic acid, Hgcl — for five hours (Conand 1990). Then it was immersed in a saturated sodic acid EDTA, which was stirred with a magnetic shaker and filtrated through a riddle to isolate the oocytes, which were later counted. Absolute fecundity (Fa) was calculated as $Fa = n (GW/g)$. Relative fecundity was calculated as $Fr = Fa/Dw$ (Conand 1990).

Histological examination of the gonads

Stichopodid gonads have two tufts of tubules located on either side of the mesentery on which saccules develop (Conand 1993b). Gonads were transferred to Bouin fixative for four weeks then dehydration was performed through a series of alcohol solutions at 30%, 50%, and 70%, allowing two hours between each change. Samples were then preserved in 70% alcohol. To prevent the loss of tubule contents during embedding, the tubule sections were cut well beyond the segment selected for sectioning (Hamel and Mercier 1996). For each individual, six 5- μ m sections were cut from tubules. The slides were stained with hematoxylin-eosin.

Environmental factors

Continuous temperature measurements at the sampling site were made throughout our study and average monthly temperatures were calculated; day lengths were obtained from the Kish Airport (see Fig. 5 c and d).

Results

Biometry

Total length, total weight, drained weight and gutted weight frequency distributions calculated from the monthly sampling are shown in Figure 2.

Gonad morphology

The gonads of *Stichopus herrmanni* consist of two tufts of tubules on which saccules develop. The

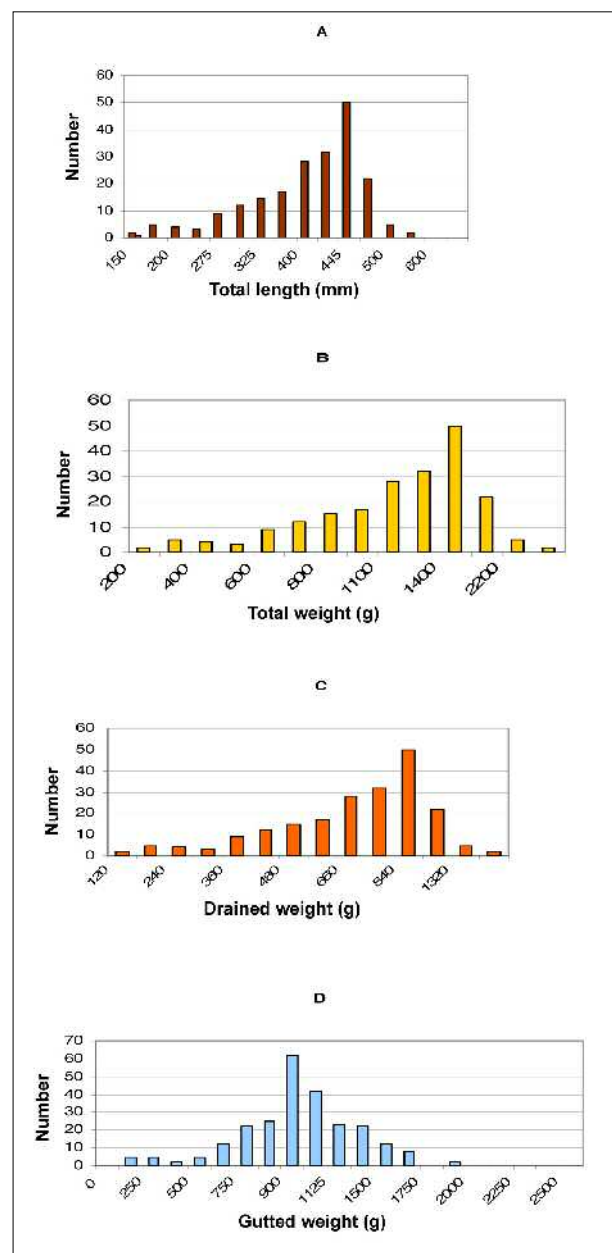


Figure 2. Biometry of *Stichopus herrmanni*. (a) total length, (b) total weight, (c) drained weight, and (d) gutted weight.

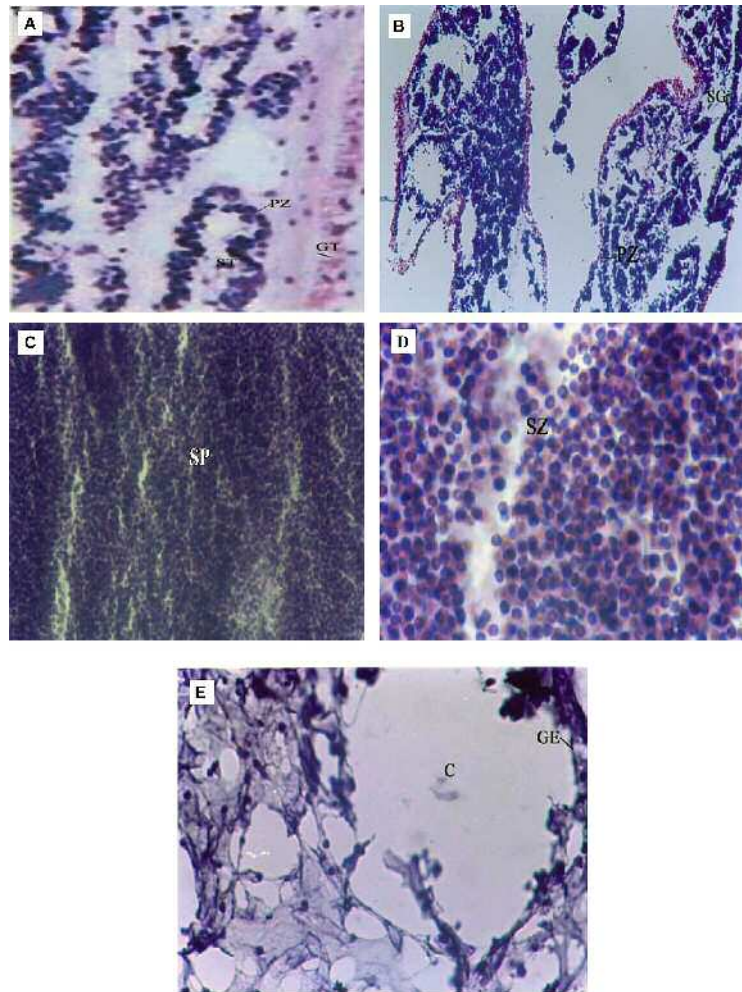
tubules join at a single gonoduct, which exits through a gonopore located between the feeding podia. The number of tubules is greater in males (z test, $p > 0.01$).

Spermatogenesis

Figure 3 shows the sequence of spermatogenesis events based on histological data from monthly preparations over a 16-month period:

- Stage 1 (immature) (Fig. 3a): The tubule wall is thick and contains small quantities of spermatozoa.

- Stage 2 (growth) (Fig. 3b): The gonadal tubule wall is beginning to decrease in thickness and spermatogonia are abundant along the germinal epithelium. There is a layer of spermatocytes in the lumen of tubules.
- Stage 3 (advanced growth) (Fig. 3c): The tubule wall is thinner and the lumen is filled with spermatozoa.
- Stage 4 (mature and spawning) (Fig. 3d): The tubules are stretched and completely filled with spermatozoa and earlier spermatogenesis stages are absent.
- Stage 5 (post spawning) (Fig. 3e): In the sections, we observed an empty area along the length of tubules.



The nuclear chromatin staining is dark in spermatogonia, which are free from the tubule wall. Primary and secondary spermatocytes can be distinguished as the primary spermatocytes undergo cytoplasmic and nuclear growth producing a darker nucleus, and suggesting meiosis-prophase in the secondary spermatocytes.

Oogenesis

The development of female gametes in *Stichopus herrmanni* is shown in Figure 4:

- Stage 1 (immature) (Fig. 4a): The gonadal tubule wall is very thick. The germinal epithelium has small oocytes.
- Stage 2 (growth) (Fig. 4b): The tubule wall is still very thick. Along the surface of the germinal epithelium, many small oocytes and some pre-vitellogenic oocytes are present.
- Stage 3 (advanced growth) (Fig. 4c): The tubule wall is thinner and the diameter of the tubules is increased. In the lumen of tubules, large pre-vitellogenic and vitellogenic oocytes are present.
- Stage 4 (mature and spawning) (Fig. 4d): The tubule wall is thin and filled with mature oocytes. Each oocyte contains a germinal vesicle; immature oocytes are absent.
- Stage 5 (post spawning) (Fig. 4e): The gonadal tubule wall is thin. Some residual oocytes are present. Empty areas are seen.

Figure 3. Light micrographs of testicular sections of the spermatogenic cycle of *Stichopus herrmanni*.

- Immature gonad showing the germinal epithelium (GE) and the proliferation zone (PZ);
- Growth stage showing the proliferation zone (PZ) and spermatogonia (SG);
- Advanced growth stage showing a gonadal tubule and an abundant of spermatozoa (SP);
- Mature stage showing a great numbers of spermatozoa (SZ) in the lumen;
- Post spawning stage showing the germinal epithelium (GE) and channels where sperm passed during spawning and atresia in the lumen of the tubules.

Seasonal changes in gametogenesis

Tubules showed a seasonal pattern that is correlated with the gonad-index cycle (Fig. 5a and b). Following spawning, there is a period of inactivity until mid-March and a progressive increase in the frequency of the growth and advanced growth stages between May and June.

It can be seen in Figures 5 and 6 that early gametogenesis takes place during March and April,

Stage 2 occurs in May, Stage 3 in June, Stage 4 in July–August, and Stage 5 during the cold season.

During June and July, the gonads underwent a rapid transition to ripe and spawning stages. Stage 4 — throughout July and August — corresponded to the summer peak spawning and was followed by an abrupt decline in late August. In September, there was a noticeable decline in the percentage of *Stichopus herrmanni* in the spawning stages (Fig. 6 a and b).

Both indices give similar patterns to that obtained from histology and are clear enough to suggest that there is one peak spawning event in summer (July–August). Figure 5c shows the mean monthly seawater temperature. In December, January, February and March, the temperature ranges from 15–20°C. In April the temperature begins to rise, and goes above 30°C in August and drops in September.

To summarise, the reproductive cycle of *Stichopus herrmanni* is seasonal with one peak spawning period in summer (July–August), related to highest seawater temperature. The sexual stages studied from both histological preparations and from simple gonad smears showed similar annual patterns. The average diameter of mature oocytes is 200 µm. Absolute fecundity was measured at 6–10 × 10³ oocytes, and relative fecundity at 8 × 10³ oocytes per gram of drained weight.

Discussion

Gonad colour

The gonad colour of the *Stichopus herrmanni* population studied was reddish brown in females and orange in males at the mature stages, whereas immature gonads were all cream coloured. These characteristics are similar to those of other populations studied, apart from the colour of mature female gonads in New Caledonia (Conand 1993a).

Gametogenesis

A mature *Stichopus herrmanni* is identifiable by the size of its gonads and microscopically by examin-

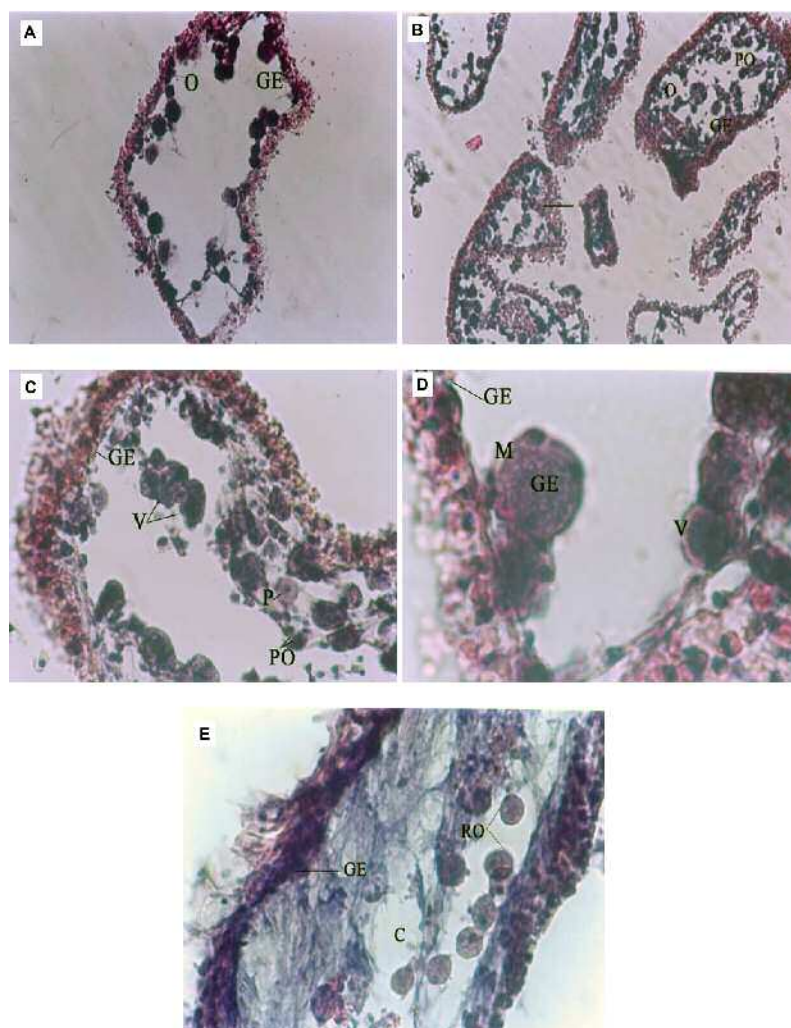


Figure 4. Light micrographs of ovarian sections showing the oogenic cycle of *Stichopus herrmanni*.

- Immature stage showing oogonia (O) and the germinal epithelium (GE);
- Growth stage showing oogonia (O) germinal epithelium (GE) and primary oocytes (PO);
- Advanced growth stage showing germinal epithelium (GE) and primary oocytes (PO) vitellogenic oocytes (V) and phagocytes (P);
- Mature stage showing large mature oocytes (M) containing germinal vesicle (GV) and vitellogenic oocytes (V);
- Post-spawning stage showing the germinal epithelium (GE) and residual oocytes (RO).

ing histological preparations of gonads. Several schemes of histological gonad indices in this study were found to be reliable.

Reproductive cycle

Conand (1993b) studied *Stichopus variegatus* (= *herrmanni*) and found that in New Caledonia, in the southern hemisphere, maturing occurs from September to November when seawater tempera-

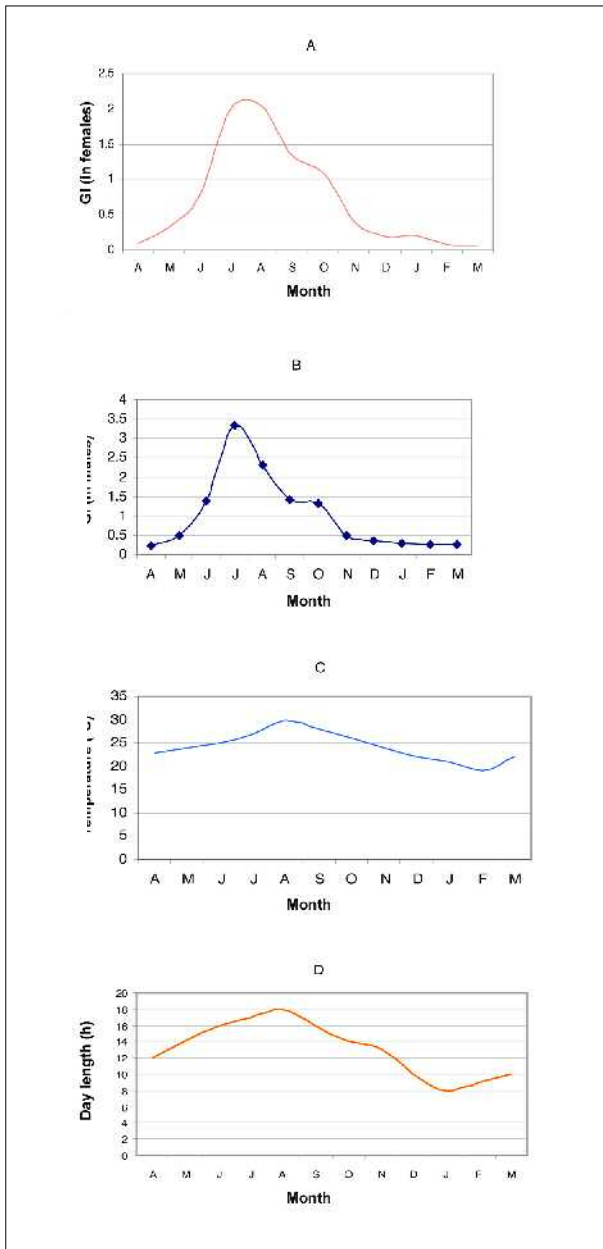


Figure 5. Monthly variations of (A) females gonadal index, (B) males gonadal index, (C) temperatures, and (D) day length.

tures increase, and spawning takes place during the warmer months of January and February.

In Iran, one distinct peak spawning period was identified in summer (July and August) and gametogenesis took place in spring, which corresponds to that of many tropical aspidochirotetes (Harriot 1985; Franklin 1980; Conand 1981, 1993a, b).

Our study lasted 16 months and covered all the variations of the sexual stages and the gonad index. We suggest that seawater temperature is the major trigger for the summer peak spawning. But,

the only notable environmental change in late March, when *S. herrmanni* gametogenesis begins, is the return to increasing photoperiod, as the sea temperature has not yet begun to rise. Therefore, we suspect that photoperiod may also play a role in gametogenesis. Experiments with sea urchins have shown a correlation between gametogenesis and photoperiod (Pearse et al. 1986).

In *Stichopus herrmanni*, oogenesis begins in January with the production of stem cells in the gonadal tubules. Throughout the winter and spring these cells are transformed into oogonia and primary oocytes, and in summer large oocytes migrate into the lumen during maturation. Finally, these large oocytes are released during spawning in mid-summer. Our study also showed a spermatogenesis as long as the oogenesis, and beginning with the production of stem cells in the gonadal tubules in mid-winter. In late winter and spring, spermatogonia, spermatocytes and spermatids accumulate in the tubules. In summer, the production of spermatozoa increases, until spawning.

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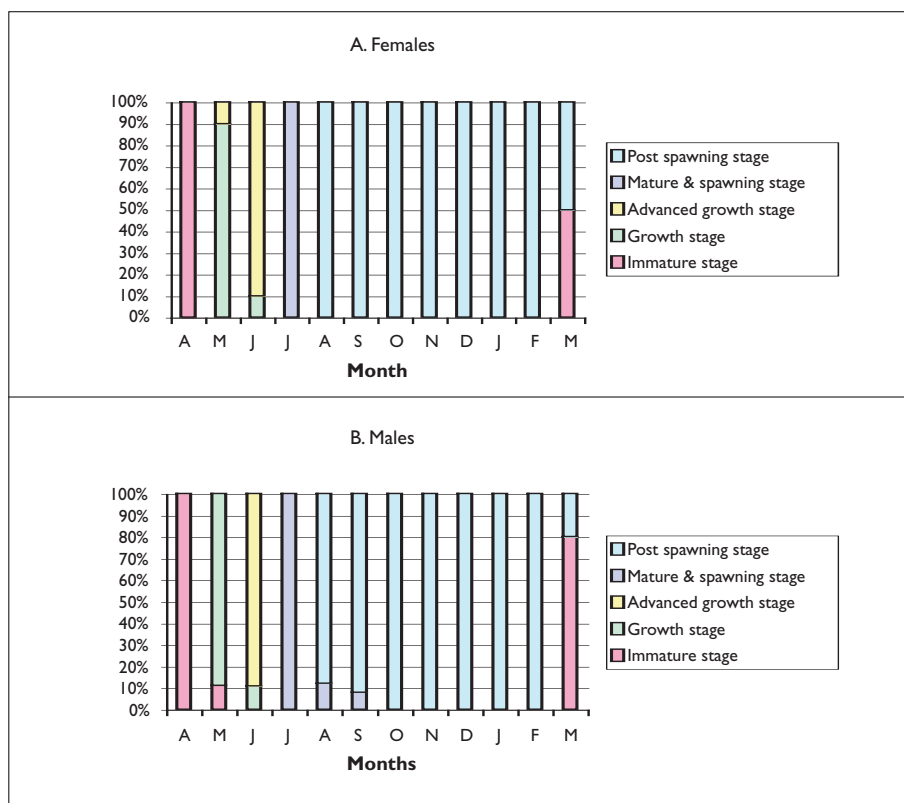


Figure 6. Reproductive cycle of *Stichopus herrmanni*.

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