

Gonad maturation of the tropical abalone *Haliotis varia* Linnaeus 1758 (Vetigastropoda: Haliotidae)

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Abstract

Gonad development of the tropical abalone *Haliotis varia* Linnaeus, 1758 from the Gulf of Mannar, southeast coast of India, was studied through the observation of gonad histology and analysis of gonadosomatic index. The sequence of gametogenesis was similar to previously described tropical haliotids. Gonad maturation stages were assigned to six categories and the details of different stages documented. Pre-vitellogenic oocytes were abundant in the early and late maturing ovary and were attached to the connective tissue tubules by their stalks. Vitellogenesis began when the primary oocytes reached 50 µm in diameter culminating in ripe oocytes of 180 ± 20 µm in diameter. All six gonad maturity stages were observed during the 14-month study period suggesting that gametogenesis was continuous throughout the year. The breeding season extended from December to February with asynchronous gonad development. Since active gametogenesis in this species was observed in all months, mature specimens could be produced throughout the year by induced maturation which would facilitate hatchery production.

Key words: gonadosomatic index, gonad histology, annual reproductive cycle, maturity stages, vitellogenic oocytes, Gulf of Mannar, gametogenesis.

Introduction

All tropical maritime countries involved with abalone fisheries have intensified abalone research owing to the rising demand for cocktail size, live tropical abalone in the international market (Chen 1989, Guo *et al.* 1999). *Haliotis varia* Linnaeus, 1758 has a limited distribution along the Indian coast and in some other Southeast Asian countries. Even though *H. varia* does not support any commercial fishery in India, the successful accomplishment of hatchery seed production of this species has opened new avenues in the field of abalone mariculture in the country (Najmudeen and Victor 2004a). Preliminary studies on the reproduction and juvenile production of *H. varia* have been carried out in recent years to elucidate its mariculture prospects (Najmudeen 2000, Najmudeen *et al.*, 2000, Najmudeen and Victor 2004a, b). In India, *H. varia* is distributed in the Gulf of Mannar, and Andaman and Nicobar Islands.

The developmental changes occurring in the germ cells of molluscs during gametogenesis have received considerable attention. Knowledge of the breeding seasons and gonad maturation process are vital in attempting captive breeding and induced maturation of cultured species. Gonad maturation stages of nearly all the commercially important temperate (e.g., Hayashi 1980, Wells and Kiesing 1989, Bang and Hahn 1993, McShane and Naylor 1996, Wood and Buxton 1996) as well as tropical (e.g., Liu *et al.* 1987, Jarayaband *et al.* 1994, Apisawetakan *et al.* 1997, Minh 1998, Capinpin *et al.* 1998, Jebreen *et al.* 2000, Counihan *et al.* 2001) abalone species have been studied and documented except that of *Haliotis varia*. Investigation of the gonad maturation process and thereby the reproductive potential of the valuable abalone resource in tropical Indo-Pacific countries may help to establish aquaculture production. The purpose of the present investigation is to describe the reproductive changes taking place within the ovary and testis

of *H. varia* in relation to gonad maturation and to find out its peak breeding period along Indian coastal waters.

Materials and Methods

Collection of samples: Samples of *Haliotis varia* were collected monthly from the intertidal rocks at Tuticorin Harbour basin of the Gulf of Mannar (08° 45' N 78° 12' E), on the southeast coast of India from January 1998 to February 1999. Here *H. varia* was distributed at a depth range of 1–3 meters. Each month, 30 specimens were shucked and dissected to examine the gonad and maturity condition. The soft body weight, digestive gland weight and gonad weight were taken on a wet weight basis.

Gonad histology: Gonads collected from *H. varia* were processed for histological assessment of gametogenesis. At each sampling, ten abalone were selected at random. Their conical appendages containing the gonad and underlying digestive gland were excised half way along their length and fixed in 10% neutral buffered formalin for 24 hours, then stored in 70% ethanol. Fixed conical appendage pieces were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Sections (7 µm) were stained with Harri's haematoxylin (Preece 1972) and eosin. The best slides were analysed and photographed.

Gonadosomatic index (GSI): The use of a gonad index as a measure of the reproductive cycle is based on the assumption that maturation and breeding coincide with maximum gonad weight (LeBour 1938). The gonadosomatic index (GSI) for each abalone was calculated using the formula, $GSI = (\text{wet weight of gonad in grams} / \text{soft body weight of abalone in grams}) \times 100$ (Webber and Giese 1969). Differences in GSI for males, females and pooled sexes over time were compared using two factor analysis of variance (ANOVA) (Snedecor and Cochran 1967).

In addition to variation in gonad indices, the annual reproductive cycle of *Haliotis varia* was determined by recording the relative abundance of different gonad maturity stages each month during the study.

Results

Morphological and cellular events of gametogenesis

The germinal epithelium in the gonad of *Haliotis varia* was supported by connective tissue tubules that run perpendicular to the outer gonad wall. The testis development was classified based on the presence and relative distribution of spermatogonia, spermatocytes, spermatids and spermatozoa within the testis lumen as well as around the connective tissue tubules. The spermatogonia were approximately $5.33 \pm 0.65 \mu\text{m}$ in diameter, and developed into primary and secondary spermatocytes of 4.00 to 4.23 μm diameter and highly basophilic spermatids of between 1.23 and 3.43 μm in diameter. The spermatozoa consisted of an anterior oesnophilic conical acrosome followed by a posterior barrel-shaped basophilic nucleus of $2.80 \pm 0.7 \mu\text{m}$ in length. When fully mature, the whole testis lumen was packed with mature spermatozoa.

Pre-vitellogenic and early-vitellogenic oocytes were found clumped together in the developing ovary of *H. varia*. The pre-vitellogenic oocytes were basophilic, less than 50 μm in diameter and had a prominent spherical nucleus. Vitellogenic oocytes were classified into early-vitellogenic and late-vitellogenic oocytes. The former were 50–120 μm in diameter, moderately oesnophilic and with a clear nucleus. The latter were 120–200 μm in diameter, strongly oesnophilic and polygonal in shape. The process of vitellogenesis started when the primary oocytes reached 50 μm in diameter. Ripe ovaries were exclusively filled with vitellogenic oocytes.

The sex and the stage of maturity of *H. varia* could be identified by pulling back the foot tissue using forceps. Ripe gonads constituted about 15% of the soft body weight. Based on the size, shape, colour and texture of the gonad and its microscopic structure through histological examination, testes and ovaries at different stages of maturity were placed into six categories: early maturing/ recovering, late maturing, ripe, partially spawned, spent and immature stages. These stages were used to describe the histology of spermatogenesis and oogenesis.

Stage I: Early maturing/ recovering: The testis was flaccid, pale orange in colour and comprised only 40% of the conical appendage. In cross-section, it appeared as a thin sheath over the thick digestive gland (Fig. 1A). The lumen of the testis was traversed by branching tubes of connective tissue and mainly contained spermatogonial cells and spermatocytes. Spermatogonia and primary spermatocytes were characterised by an oval nucleus, while secondary spermatocytes had a more or less round nucleus. The ovary appeared as a thin flabby, grayish sheath and comprised 40% of the conical appendage. Early-vitellogenic oocytes, about 45 μm in diameter, were seen attached to the follicle cells by

a stalk (Fig. 2A). The oocytes under magnification appeared round, sometimes irregular and transparent. These had well defined oolemmae and prominent nuclei, and had not yet filled with yolk.

Stage II: Late maturing: Testis was thicker and bright orange in colour and comprised about 60% of the conical appendage. It contained large numbers of spermatocytes and spermatids (Fig. 1B). Few sperm were seen with their tails facing towards the digestive gland. The spermatocytes were abundant near the walls of the testis and around the tubules (Fig. 1C). Highly basophilic spermatids were also present in the testis lumen. The ovary became bluish in colour and appeared as a thick sheath over the digestive gland. Many large vitellogenic cells, approximately $68 \pm 5 \mu\text{m}$ in diameter could be seen (Fig. 2B), mostly attached to the trabeculae by stalks and oriented towards the digestive gland.

Stage III: Ripe: The testis was turgid, cylindrical and bright cream in colour and comprised 80–90% of the conical appendage. Little or no digestive gland area could be seen in the cross section of the conical appendage. Many radially arranged spermatozoa with their tails facing the digestive gland area were seen in the ripe testis section (Fig. 1D, E). Mature spermatozoan measured $2.8 \pm 0.7 \mu\text{m}$ in length. The ovary was blue or bluish green in colour, massive and turgid with little or no digestive gland area in the cross-section of the conical appendage. The gonad covered about 80% of the conical appendage and became more cylindrical. The lumen of the ovary was filled with large number of late vitellogenic oocytes, which measured $180 \pm 20 \mu\text{m}$ in diameter (Fig. 2C). Most of the eggs were polygonal in shape, with a distinct nucleus and a round nucleolus (Fig. 2D). Oocytes closer to the periphery of the ovary were predominantly attached to the trabeculae, while the detached oocytes appeared to be undergoing maturation or ready for spawning.

Stage IV: Partially spawned: The testis was bright cream, flaccid, loose in appearance and comprised about 80% of the conical appendage. Some spermatogonial cells and spermatocytes appeared in the periphery of the testis (Fig. 1F). Phagocytes were present in the inter trabecular spaces. The ovary is flabby, thick, and bluish and comprised 80% of the conical appendage. In the lumen near the digestive gland, the eggs were fewer in number, compared to the ripe ovary (Fig. 2E). Some oocytes could be seen in a state of reabsorption. The oocyte diameters ranged between 140 and 228 μm .

Stage V: Spent: The testis was a thin gray sheath covering the digestive gland. The relative area of the digestive gland was larger than in stage IV in cross-section. Relatively few darkly stained sperm occurred within the lumen, apparently a residue from the previous spawning. The connective tissue tubes were more convoluted due to the collapsed testis (Fig. 1G). The ovary was highly shrunken and collapsed. It enclosed the digestive gland as a thin translucent and flaccid sheath, loosely packed with some primary oocytes. The trabeculae were emaciated due to the collapsed state of the lumen (Fig. 2F). Lacunae were present in the trabeculae and a few opaque, largely disintegrated oocytes were observed.

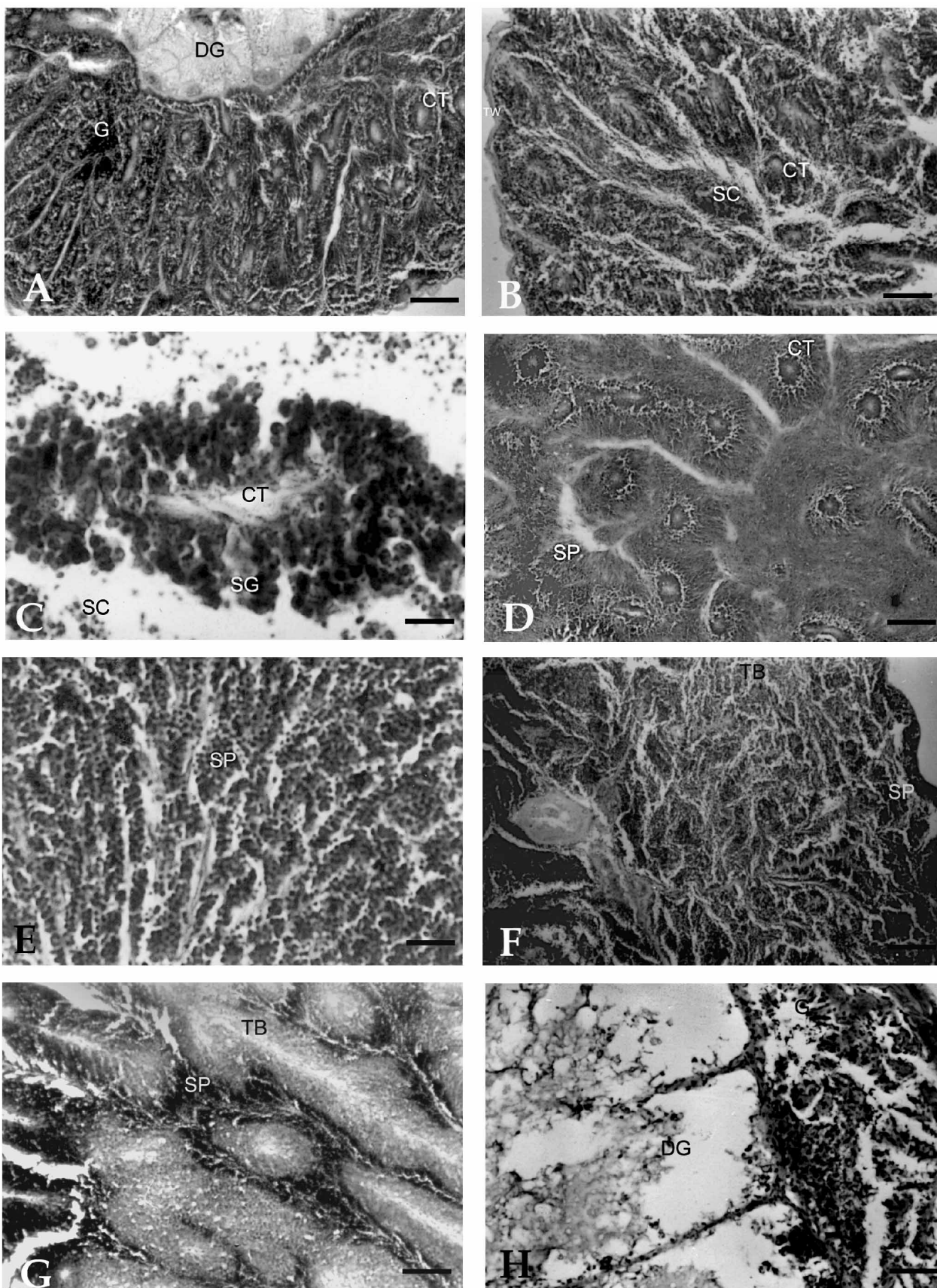


FIGURE 1. Light micrographs showing histological sections of the testes of *H. varia*. **A** Cross-section through an early maturing testis showing growing spermatogonia along the connective tissue tubules (CT). Gonad (G) forms a sheath over the digestive gland (DG) **B** Late maturing stage with more spermatocytes (SC) **C** Enlarged view of late maturing testis showing cluster of spermatogonial cells (SG) around the connective tissue tubule **D** Ripe testis filled with radially arranged spermatozoa (SP) around the connective tissue tubules **E** Enlarged view of ripe testis with spermatozoa **F** Partially spawned testis showing collapsed trabeculae (TB) **G** Spent testis with residual spermatozoa and enlarged trabeculae **H** Cross-section through an indeterminate stage gonad with very little gonad area (G) compared to the digestive gland area (DG) in the conical appendage. Scale bars: **A, B, C, D, G, H** = 80 μ m; **C, E** = 20 μ m

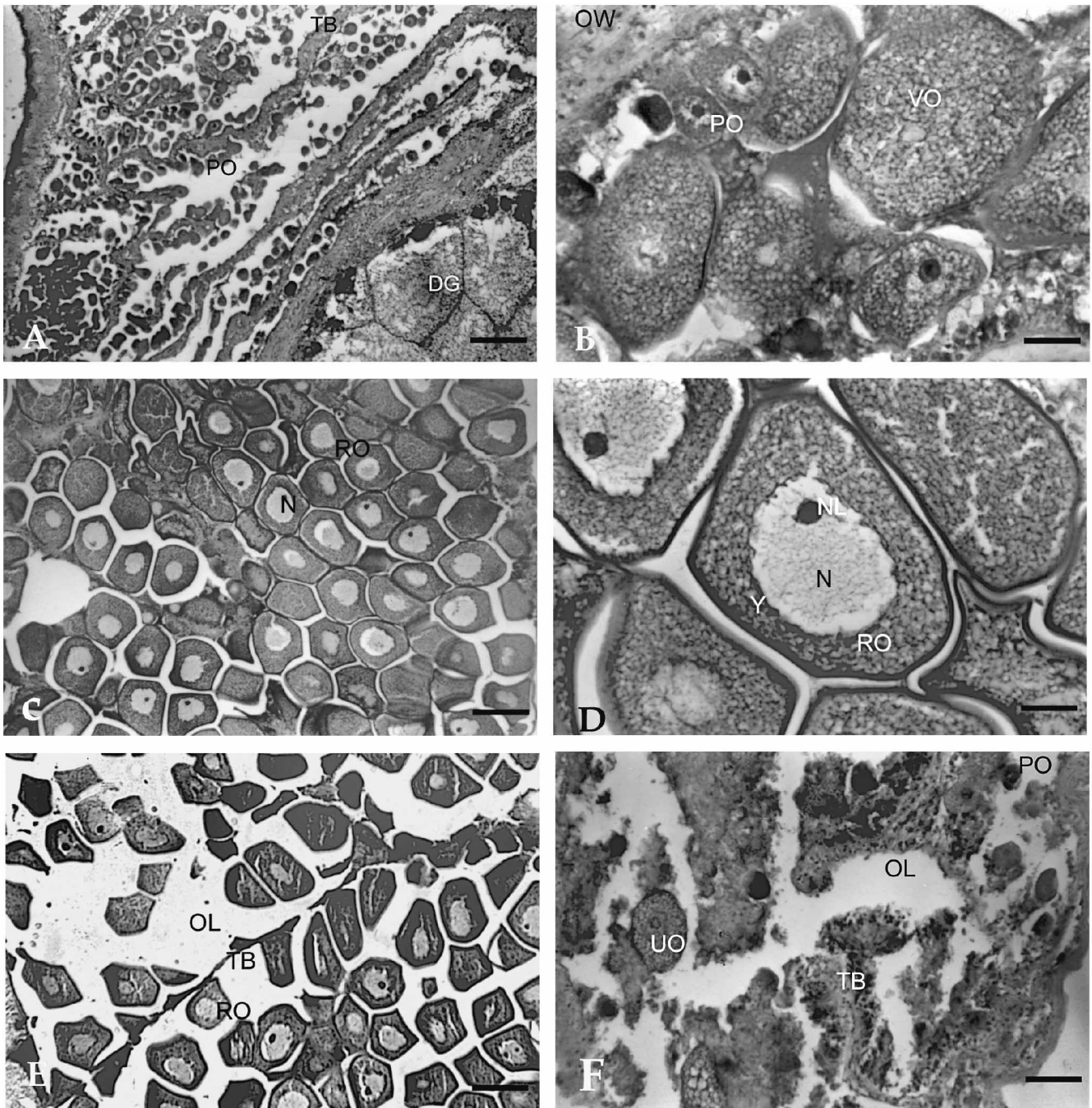


FIGURE 2. Light micrographs showing histological sections of the ovaries of *H. varia*. **A** Cross-section through the early maturing ovary with proliferating primary oocytes (PO) along the connective tissue tubules (TB) **B** Late maturing ovary with early vitellogenic oocytes (VO), OW- ovarian wall **C** Ripe female gonad with tightly packed oocytes (RO) having a prominent nucleus (N) **D** Enlarged view of the ripe oocyte with clear nucleus (N) and well developed nucleolus (NL) and filled with yolk granules (Y) in the cytoplasm **E** Partially spawned ovary with loosely packed unspawned ripe oocytes (RO) in the ovarian lumen (OL). Note the spaces vacated by partial spawning **F** Spent ovary with collapsed trabeculae, and some unspawned oocytes (UO). Scale bars: **A, F** = 80 μm ; **B, D** = 40 μm ; **C, E** = 200 μm .

Stage VI: Indeterminate: This stage was common for both male and female individuals. The gonad was indistinguishable even in histological examination. The gonad was a dark, grayish thin sheath comprising 30% of the conical appendage. The gonad lumen was almost filled with branches of trabeculae, which were attached to both the outer gonadal wall and the digestive gland (Fig. 1H).

Gametogenic cycle

The gametogenic cycle of *Haliotis varia* at the study

area showed a distinct temporal pattern in the relative abundance of the different gonad maturity stages (Fig. 3). Almost all the gonad maturity stages were present in each month throughout the year. Early maturing/ recovering gonads were present in high numbers for the six months of the year from June to October, indicating active gametogenesis. Partially spawned gonads were 33% of the September sample demonstrating some spawning mainly by males. In December, the number of partially spawned and spent stage gonads increased (69%), indicating the main

onset of spawning. The percentage of ripe specimens was highest in February (88%) and in January (81%). In February 1999, all the females sampled were ripe, this period representing the active breeding season. Some animals showed immediate recovery after spawning (25%) in March. The GSI values varied significantly between months throughout the study period. The highest GSIs were observed in January 1999 followed by February 1999 and February 1998. High values were maintained from January to April

(Fig. 4). A gradual decrease was evident from March to June, which indicated the completion of most spawning in March. The lowest GSIs were in August 1998 and October 1998. The standard deviation for the monthly values of GSI was high, indicating asynchronous spawning behaviour between individuals.

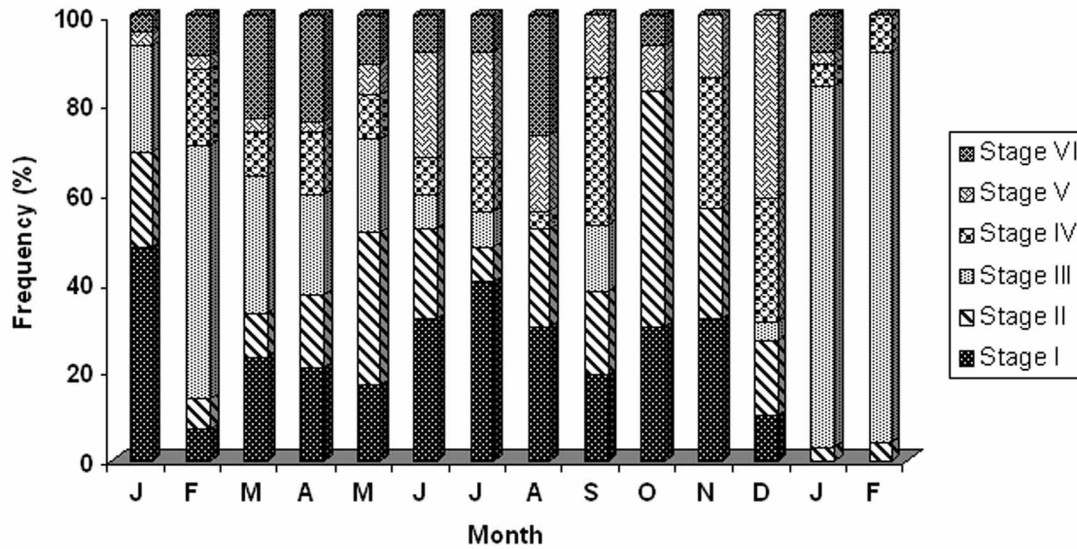


FIGURE 3. Annual gametogenic cycle of *H. varia* in the Gulf of Mannar. Histograms show the relative abundance of different maturity stages of the gonad.

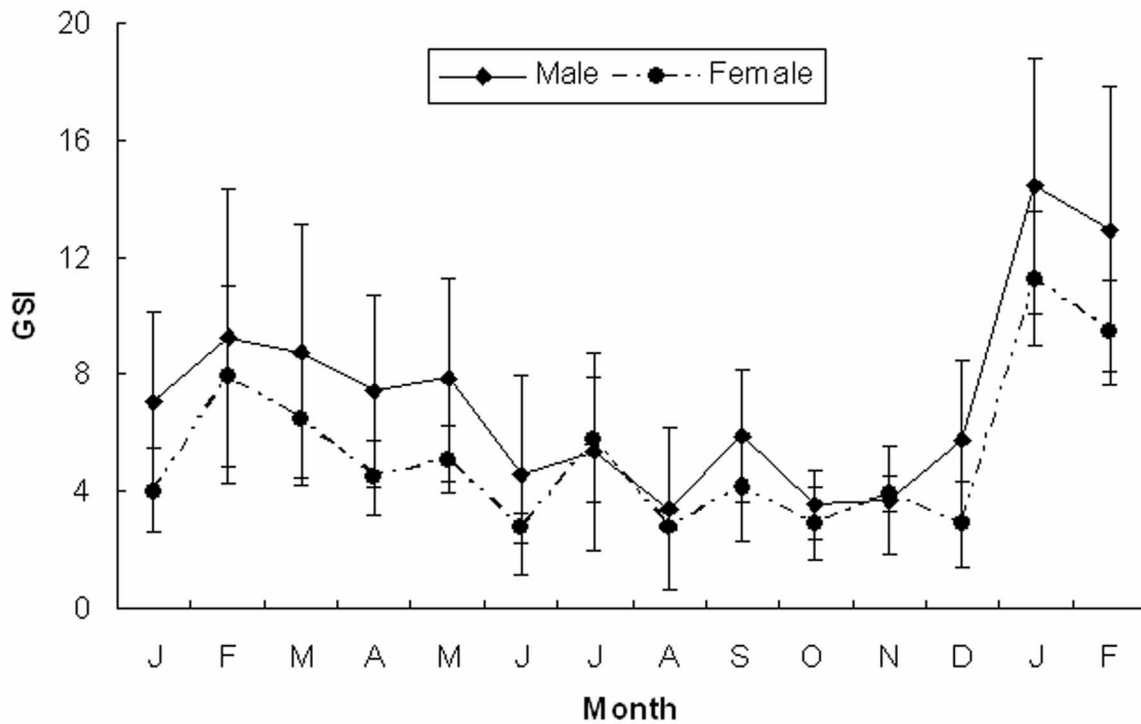


FIGURE 4. Reproductive cycle of *H. varia* in the Gulf of Mannar from January 1998 to February 1999. Mean GSI (\pm SD) for male and female.

Discussion

Different criteria such as percentage of gonad covering the digestive gland, colour and shape of gonad, gonadosomatic index and ova diameter have been employed by various workers in order to classify the stages of maturity for abalone (Tomita 1967, Giorgi and DeMartini 1977, Ault 1985). In the present study, the ovary and testis of *Haliotis varia* were classified into six stages based on all the above-mentioned criteria. This was similar to the classification used by Wood and Buxton (1996) for *H. midae* Linnaeus, 1758. The sequence of gametogenesis was similar to those described for other tropical haliotids (Apisawetakan *et al.* 1997, Minh 1998, Capinpin *et al.* 1998, Jebreen *et al.* 2000, Counihan *et al.* 2001), except in the number of maturity stages assigned. Oogenesis started with primary germ cells that developed through several vitellogenic stages, and finally to ova. Spermatogenesis commenced with the formation of spermatogonial cells from the germinal epithelium and proceeded with various cell types to mature sperm (Webber and Giese 1969, Jebreen *et al.* 2000). In *H. varia*, the early vitellogenic oocytes measured about 50 µm in diameter. In *H. midae* also, vitellogenesis started when the oocytes had a diameter of 50 µm (Newman 1967, Wood and Buxton 1996). In most broadcast fertilisers, after spawning, the gonad enters a 'resting stage' in which gametes are absent (Loosanoff 1962). Temperate species, in particular, show the resting phase in winter, when gametogenic activity is suspended (Giese and Pearse 1977). However, a period of gonad quiescence has not been reported in some other temperate species like *H. cracherodii* (Webber and Giese 1969). For *H. varia*, a resting phase was found but only for a short period and immediately after that gametogenesis was initiated.

The reproductive cycle is reflected by prominent changes in gonad size. The monthly variation in GSI has frequently been used in species with gonads that are easy to separate from the rest of the soma (Grant and Tyler 1983). In *Haliotis cracherodii* Leach, 1814, Webber and Giese (1969) calculated the GSI as the weight of gonad relative to the weight of the soft body parts. Boolootian *et al.* (1962) found that monthly samples of gonad indices of black abalone showed seasonal variations in the Pacific Grove area of California. High values coincided with the breeding season. Temperate species of abalones generally have distinct annual spawning seasons, with some spawning once or twice a year, whereas tropical species appear capable of spawning all year round (Singhagraiwan and Doi 1992, Jarayabhand and Paphavasit 1996). In the present study, *H. varia* appeared capable of spawning in most months of the year. Though continuous gonad development was observed year round, the peak spawning period was restricted to three to four months in a year. Bussarawit *et al.* (1990) suggested that there was a greater gametogenic activity in tropical than in temperate species, based on their observations of a rapid post spawning recovery in *H. varia* at Phuket, Thailand. The continuous presence of ripe gametes in both the sexes and the ability to reproduce at any time of the year is unusual for the majority

of the temperate abalone species. Webber and Giese (1969) reported that *H. cracherodii* spawned only during a six week period during the year. However, some temperate abalones are also capable of spawning throughout the year, e.g., the temperate Australian *H. roei* Gray, 1826 (Shepherd and Laws 1974). The presence of ripe males in most of the months was evident in *H. varia* during the present investigation. According to Martel *et al.* (1986), some marine gastropod species have a completely inverse timing in the development of the testis and ovary. However, such separated periods of spermatogenesis and oogenesis were not found in this study of *H. varia*. As noted by Capinpin *et al.* (1998) in *H. asinina* Linnaeus, 1758, the ranges of GSI were broad, indicating asynchronous spawning by the individuals. Similar observations were made in the trochid *Trochus niloticus* Linnaeus, 1758 by Hahn (1993).

As maturation of gametes in molluscs is reported to be initiated by annual temperature fluctuations (e.g., Fretter and Graham 1964), it should be possible to obtain mature *Haliotis varia* throughout the year in the laboratory by regulating temperature with an adequate supply of algal food. Induced maturation of many commercially important bivalve species has been achieved in India by using the above techniques (Pillai *et al.* 2001). Since spawning inducement and juvenile production in this species have been achieved in controlled conditions (Najmudeen and Victor 2004a), the availability of ripe individuals throughout the year would facilitate the establishment of abalone hatchery operations to augment production through aquaculture.

Acknowledgements

I thank the Director, Central Marine Fisheries Research Institute, Cochin for providing necessary facilities. Thanks are also due to the SCUBA divers at the Tuticorin and Mandapam research centres of CMFRI, who helped me during the collection of the abalone used in this study, and Dr. A.C.C. Victor, former Principal Scientist, CMFRI for his guidance in conducting the work. I also thank the two anonymous reviewers and the editor, Dr. Winston Ponder, for their valuable suggestions and critical comments, which helped to improve the manuscript. The study was financed by the Indian Council of Agricultural Research, New Delhi.

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