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Reproductive Development of *Holothuria pulla* and *Holothuria coluber* (Holothuroidea: Echinodermata) in Pamilacan Island, Central Philippines

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Abstract

The reproductive cycle of the Aspidochirote holothurians, *Holothuria coluber* and *Holothuria pulla* in Pamilacan Island were studied from July 1990 to August 1991. These were deduced from gonad indices, gametogenic development of the gonads and spawning behavior of the animals. *H. coluber* and *H. pulla* manifested synchronous annual spawning in December 1990 and October 1990. The size at first sexual maturity for coluber was 13 cm in total length for the females and 15 cm in total length for the males. For *H. pulla*, the size at first sexual maturity for the females was 16 cm in total length for the females and 18 cm in total length for the males.

Introduction

Pioneering works on holothurians started during the latter half of the 16th century and were mostly taxonomic in nature. Knowledge of their distribution was gathered on the great voyages of discovery (Challenger, Albatross, Siboga). By the end of the 19th century, papers have been published on holothurian fishing for "trepang" production in various areas. The occurrence of this activity was described by Semper (1868) and Seale (1911) in the Philippines, Saville and Kent (1903) on the Australian Barrier Reef, Koningsberger (1904) in the Dutch East Indies (Indonesia) and Hornell (1917) in India. (Conand 1990).

In recent times, the worldwide growth of fishing activities on "trepang" has made effective management of this activity a necessity. There have been various indicators that holothurian fishery is experiencing difficulties. Problems encountered by Indo-Pacific "trepang" gatherers include the proliferation of commercially important "trepang" species in deeper waters and depletion of resources in traditional fishing grounds, thus forcing fishers to look for new fishing grounds. The diminishing size of the specimens of the various species

concerned and the failure of the processed product to meet the required quality criteria are also causes for concern. In the Philippines, little is known of the reproductive cycles of commercially important holothurians like coluber and pulla despite the fact that they are being gathered in many regions of the country for processing into "trepang" or beche de mer.

These two species are abundant in the shallow portion of Pamilacan Island and their biology, ecology and habitat requirements have never been studied.

This study aims to determine the spawning season and reproductive cycle of the above-mentioned species in Pamilacan Island. Results of this study could serve as a valuable contribution to the basic knowledge necessary for the possible, successful culture of these animals.

Materials and Methods

The study was conducted along the intertidal zone of Pamilacan Island, Baclayon, Bohol, Central Philippines, located at 929.7 N, 12355.4'E. (Fig 1).

Two methods were used, namely, gonad index and microscopic examination of the gonads, to investigate the reproductive cycles of the two species.

Samples of male and female sea cucumbers were collected monthly from the study area for 14 consecutive months (July 1990 to August 1991). Twenty individuals (10 males and 10 females) of each species were collected and analyzed. Total length of each individual was measured dorsally from the anus to

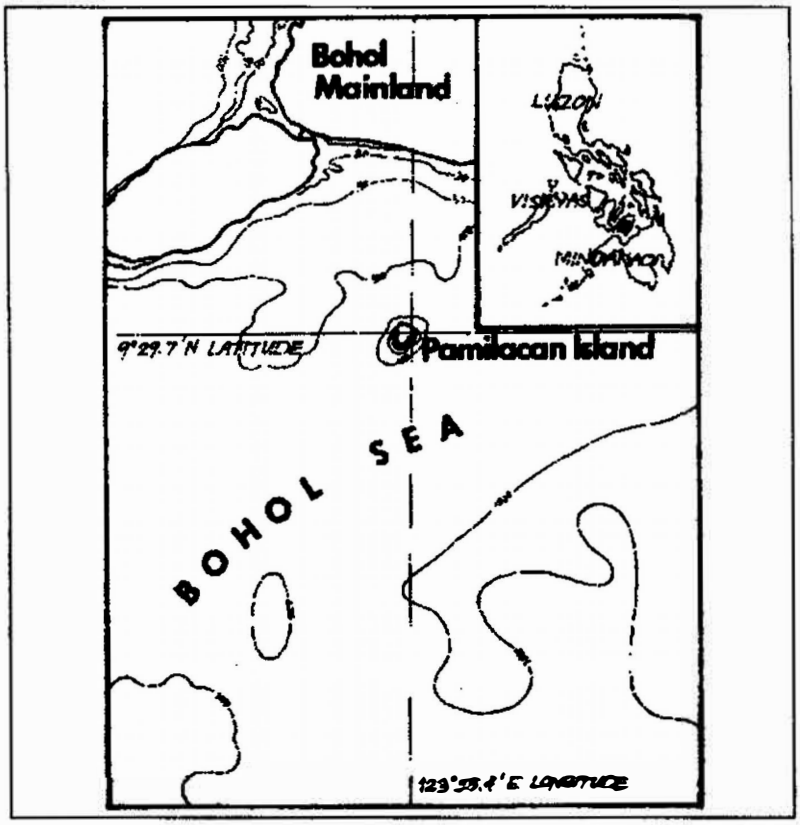

Fig. 1. Map of the study site.

the center of the tentacular crown using a flexible curve. Drained weights were also taken using a commercial weighing scale. Prior to weighing, a small slit was made on the dorsal side of each animal to drain all the fluid inside and each animal was allowed to eviscerate its internal organs. The gonad tubules were removed from the dissected animals and weighed to the nearest 0.5 grams using a triple beam balance. The color of the gonads and other characteristics were noted.

Gonad index (GI) was obtained from the ratio of fresh gonad weight to drained body weight multiplied by 100 (Conand 1990). Monthly gonad indices were taken from each animal. Mean monthly gonad indices were graphed over time to yield the annual reproductive cycle of the species.

For the microscopic examination, gonads taken from the dissected animals were fixed in Bouins Fluid. Sections of the gonads were cut 7-10 microns, embedded in paraffin and stained with hematoxylin followed by eosin. The different stages of reproductive activity for each individual were determined based on the description of Conand (1990). Spawning of *H. coluber* was observed in the field during the month of December 1990 between 2000H to 2200H three days before the full moon. Prior to spawning, the animal attached itself (at the posterior end) to the substratum and, with a "cobra-like" posture, the gametes were released into the water by rhythmic swinging of the anterior end of the body. It was further observed that the gametes were released in an intermittent manner. For *H. pulla*, spawning was observed in the field in the month of October 1990. *H. pulla*'s spawning behavior was very similar to that of *H. coluber*.

Results

The two species under study exhibited typical gonadal characteristics of Family Holothuroidea. They are dioecious but do not exhibit external dimorphism. The sex ratio was not significantly different from 1:1. In both male and female individuals, the gonads consist of a single tuft of many tubules, each ramifying into two or three branches. The gonads are attached to the dorsal mesentery at their bases or otherwise hang free in the coelom. The gonadal tubule open into a hollow base. From the common base, the gonoduct proceeds into the mesentery to the gonopore, which leads to the outside. The size of the gonads vary depending on the species and sexual maturity of the animal. The gonadal tubules vary from small, short slim threads to voluminous tubules that almost fill the entire body cavity.

In the male gonad, the diameter of a single mature tubule is irregular since there are nodules or constrictions along its entire length. In the female gonad, the diameter of a single mature gonad tubule is more or less uniform throughout its length, nodule or constrictions being absent. The gonads are whitish and transparent when immature, becoming opaque when mature.

Holothuria coluber

H. coluber is black in color with yellow spots marking tips of papillae and pedicels. Its body length is from 10-55 cm, cylindrical with the posterior part

usually larger than the anterior part. The body wall is soft and fairly thick. In Pamilacan Island, this animal inhabits sandy areas, with the posterior part of the body usually found under large rocks or coral head with the anterior part exposed and hard to pull out.

Seasonal changes in gonadal development and gonad indices for both sexes are shown in Figs. 2 and 3. Male and female individuals showed a very similar trend in gonadal development. Stage III (developing stage) individuals were dominant from July to October 1990 and June to August 1991 for both sexes. Mean gonad indices ranged from 2.4 to 4.07 in males and 2.36 to 4.09 in females. Microscopic examinations of the gonads showed developing sperms and ova in the follicle. Stage IV (mature) individuals were most dominant during the months of November and December 1990 for both sexes. These months registered the highest mean gonad indices of 5.52-5.87 in males and 6.35-6.48 in females. Microscopic examinations of the gonads showed the male tubules filled with mature sperms and the female tubules with mature ova. The mean diameter of the mature ovum was 190 microns. The mean length of the mature female tubule was 95 mm. External morphology of the male gonads showed the presence of nodules with a mean nodule length of 30 mm. January to March 1991 were post-spawning months. Mean gonad indices during these months ranged from 1.45 to 2.01 for males and 1.55 to 2.5 for females. Microscopic examination of the male gonads showed gaps in the mass of spermatozoa. In the females, it showed few oocytes scattered around the tubules. Macroscopic examination of the male gonad tubule showed disappearance of the nodules and, in both male and female, the tubules were more or less transparent.

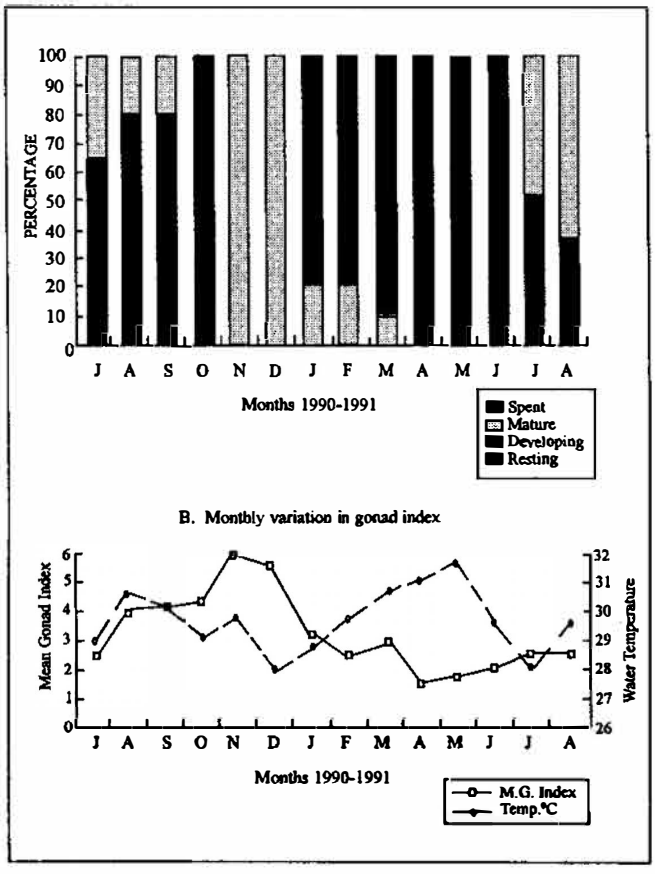

Fig. 2. Male sexual cycle of *Holothuria coluber* in Pamilacan Island.

Holothuria pulla

H. pullla is deep reddish brown/black in color with long dorsal papillae all over the body. Body length is from 24-40 cm with cuvierian organs easily released at the slightest stimulation. In Pamilacan Island, this animal inhabits sandy areas with eel grasses.

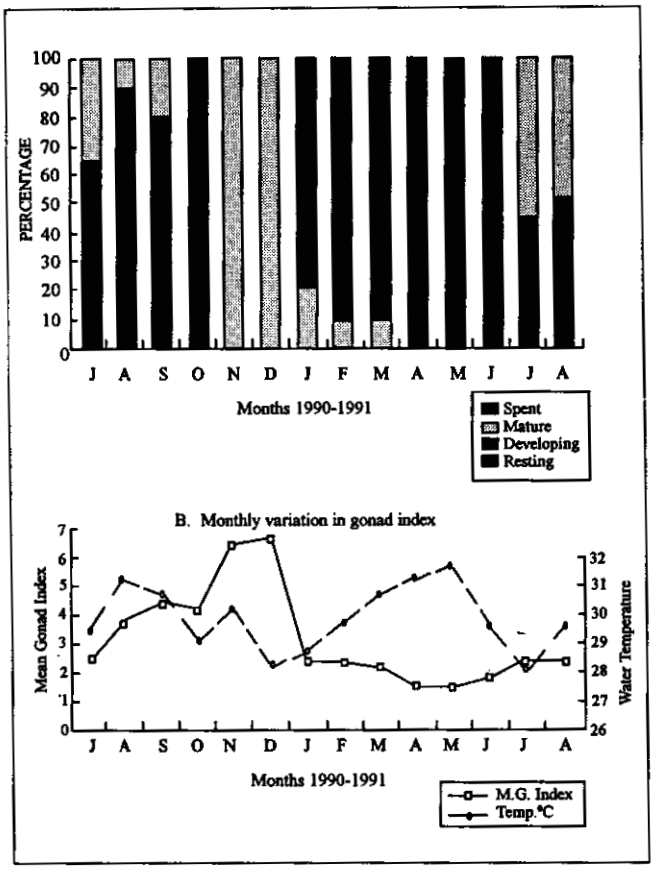

Fig. 3. Female sexual cycle of *H. coluber* in Pamilacan Island.

Seasonal changes in gonadal development and mean gonad indices for both sexes are shown in Figs. 4 and 5. Male and female populations of *H. pulla* showed a trend in gonadal development which was very similar to that of *H. coluber*.

Highest mean gonad indices were registered in the month of August 1990 (4.5-7.45 in males; 4.6-7.24 in females). Microscopic examination of the gonads showed that the individuals were in Stage IV (mature). The tubule in both sexes were filled with mature sperms and ova. The mean diameter of the mature ova is 200 microns. As with *H. coluber*, external characteristics of the mature male gonads showed nodules or swellings with an average nodule length of 35 mm. The mean length of the mature female tubule was 100 mm. During the months of November and December 1990 and January 1991, mean gonad indices were 2.44-2.66 in males and 2.53-2.72 in females. Microscopic examination of the gonads showed nearly empty tubules with very little ova and sperms left. External examination of the male gonad showed the nodules disappearing. In both sexes, nodules appear to be less opaque.

Discussion

This study on the reproductive cycles of *H. pulla* and *H. coluber* is a pioneering one as there have been no studies conducted by local and foreign researchers on these two species. In this study, it was found out that *H. pulla*

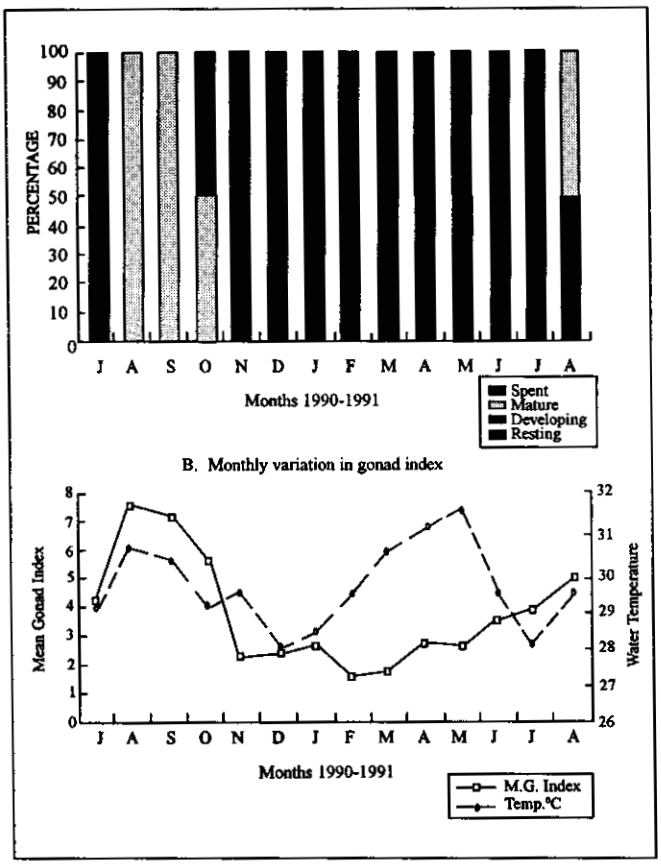

Fig. 4. Male sexual cycle of *Holothuria pulla* in Pamilacan Island.

spawned during the month of October 1990 and *H. coluber* spawned during the month of December 1990, based on field observation and microscopic examination of the gonads. The increasing values of the mean gonad indices denoted a period of gonadal growth and accumulation of ripe gametes. These took place during the month of November 1990 for *H. coluber* (5.52-5.86 in males; 6.35-6.48 in females); and during the month of August 1990 for *H. pulla* (4.5-7.45 in males; 4.6-7.4 in females). During these months, the gonads in both sexes were filled with mature gametes ready to be spawned in the succeeding months.

It was also observed that both species exhibited synchronous gonadal development and spawning for both sexes. Synchronous gonadal development and spawning are crucial to *H. coluber* and *H. pulla*, whose spawning seasons are restricted to about one month a year for both sexes. These are important to ensure fertilization of gametes as the two species are non-brooding holothurians.

Synchronous gonadal development does not occur in all holothurians. In some species, like Thelenota ananas in Bolinao, Pangasinan, asynchronous gonadal development was observed by Leonardo and Binohlan (1986) at the start of the reproductive cycle, with the female taking the lead in gonadal development. The males caught up with the females two months thereafter. The initial time lag seemed to be a successful reproductive strategy in which a high degree of synchrony in spawning between both sexes was ultimately achieved.

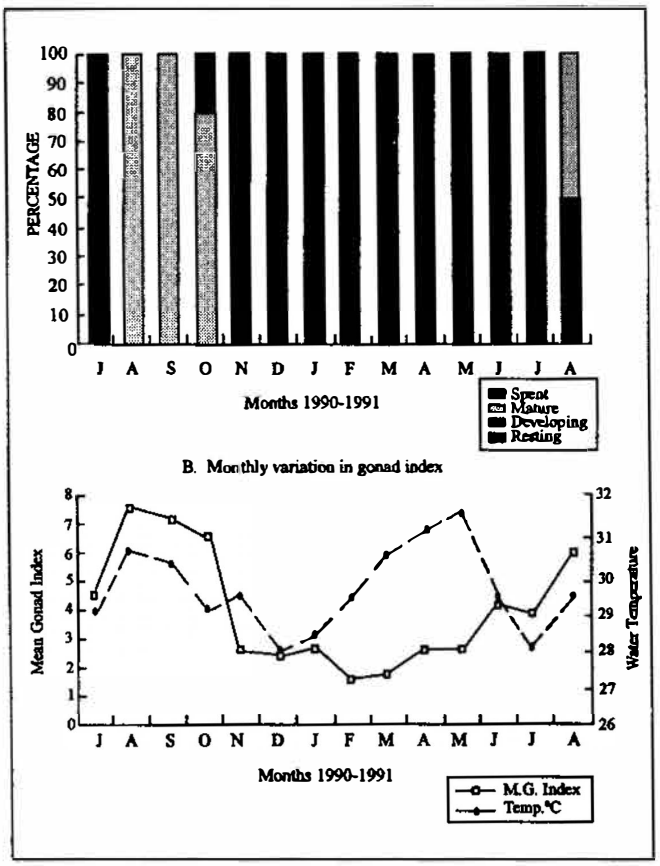

Fig. 5. Female sexual cycle of *H. pulla* in Pamilacan Island.

In Pamilacan Island, temperature, monsoon, lunar phases and chemical exudes from males and females of the same species are the important exogenous factors that control the spawning of these two species. The months of October to December are characterized by temperatures ranging from 28°C-30°C and very calm seas which are very conducive to larval development and juvenile settlement due to the presence of abundant food. Orton (1920) proposed that temperature is the most important factor in the regulation of breeding in marine animals. One of the possible reasons suggested by Giese and Pearse (1974) why synchronous spawning of invertebrates occur in the tropics despite the availability of food during the entire year is the presence of chemical exudes from the males and females of the same species as well as lunar phases. These are applicable to the species under study as supported by actual observation in the field of the animals spawning three days before full moon. The two species under study are oviparous and gametes are shed directly into the water. There must be some "chemical" that is released into the water by both male and female members of the species which trigger the animals to spawn. This is the only way by which fertilization can be effected. The post-spawning months for *H. coluber* and *H. pulla* is from January to March and November to January, respectively. The decline of the mean gonad indices for both species would suggest that these months are the right time for the harvest of the species for "trepang" production.

For *H. coluber*, the size at first sexual maturity is 13 cm in total length for females and 15 cm in total length for males. For *H. pulla*, the size at first sexual maturity was 16 cm in total length for females and 18 cm in total length for males. These were based on the smallest animals caught with mature gonads. In conclusion, with the reproductive cycle of *H. coluber* and *H. pulla* being determined, the results of this study can greatly help in the proper management of the resource and in formulating ordinances on the close and open season in the harvest of these two species in Pamilacan Island. However, it would be more interesting to do further research to find out whether continuous or discontinuous reproduction occurs for the two species in the more temperate area of their range.

Acknowledgment

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