REPRODUCTIVE BIOLOGY OF *CHICOREUS CAPUCINUS* (LAMARCK, 1822) FROM PHUKET ISLAND, THAILAND

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ABSTRACT

Field observations on the reproduction of *Chicoreus capucinus* were carried out in the mangrove of Nam-Bor Bay during May-September 1995. The snails gathered in clusters before spawning. Clusters were found throughout the observation period. Males outnumber females in all clusters. The differences from 1:1 ratios were statistically significant ($P<0.01$). The average length of females was greater than that of males. A histological study showed active female gonads and ripe male gonads in June. Gonads from September did not differ markedly in microscopic appearance from those in June.

INTRODUCTION

Chicoreus capucinus (Lamarck, 1822) is found between 105-180°E, and 15-20°S (Houart 1992). Its general habitat is muddy sand. The ecology of the snail in the mangrove area of Nam-Bor Bay was studied by Frith *et al.* (1976) and Nielsen (1976). The species seems to be restricted to the intertidal zone. Frith *et al.* (1976) reported that the snails were found only in a narrow zone between MHWN (mean high water neap tide levels) and MLT (mid tide levels) along the Nam-Bor shore. Middelfart (pers. comm.) found egg capsules of *C. capucinus* in the mangrove during May-August.

The aim of the present study is to describe reproductive behavior, the sex and size distribution at maturity, and histological changes in both sexes during reproduction (June and September).

MATERIALS AND METHODS

Study area

The study area is located in the mangrove of Nam-Bor Bay, Phuket (Fig. 1). The mangrove forest at Nam Bor Bay is approximately two hundred meters wide from the landward to the seaward edge and is dominated by *Rhizophora apiculata* (Frith *et al.* 1976). The study area was approximately 300 m^2. The sediment is dominated by mud (mean grain size = 4.53-4.81 phi) with a sorting value of 3.86-5.54 (poorly sorted), loss of ignition is 10.37±0.87 % and the water content is 46.78±3.91 %. Soil surface temperatures in the study area and salinity in the tidal channel were monitored during the research period (see **, Fig. 1). Average temperature and total rainfall were measured from January to August 1995. Data were obtained from the Data Processing Sub-division, Climatology Division, Meteorological Department in Phuket.

Reproductive biology

Observations on mating behaviour of *C. capucinus* were recorded once a month during May-September 1995. In June snails were collected in order to study the sex ratio and size distribution. Shell length was determined by measuring the distance be-

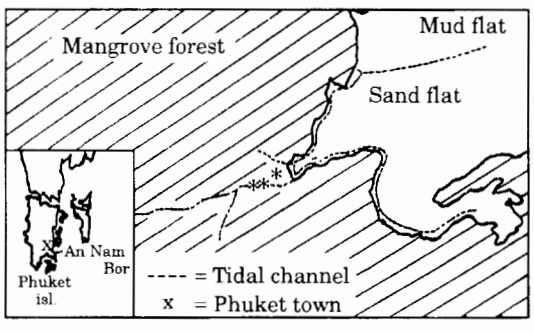

Figure 1. The study area (*) in the mangrove of Nam-Bor Bay, Phuket Island (Modified after Frith *et al.* 1976).

tween the shell apex and the tip of the anterior siphonal canal with Vernier callipers. Sex determination was carried out after removing the soft parts from the shell. Penis and the capsule gland were inspected. Sex ratio was calculated and Chi-square test used to test whether the population followed a 1:1 sex ratio. After sex determination each of 20 females and males were randomly selected for histological study of the gonad. External features like colour and texture of fresh gonad were recorded. Gonads of both sexes were cut into small pieces for transverse sectioning. The tissues were fixed in 10 % formalin. Additional 10 specimens of each sex were collected randomly in September. Whole gonad of the specimens were fixed in 10 % formalin for longitudinal sectioning. Histological examination of the gonads was performed at Life History Laboratory, Samutsakorn Coastal Aquaculture Development Center (transverse sections), and Department of Pathobiology, Mahidol University (longitudinal sections). The tissues were embedded in paraffin, sectioned at 8 µm, and stained with Mayer's haematoxylin and eosin Y.

Examination was done under stereo- and compound microscopes. The reproductive cycle of the species was determined by monitoring changes in the gonad histology. The stages of gametogenesis were evaluated according to Feare (1970). For gonad maturation, the stages given by Giorgi & DeMartini (1977), and Hahn (1989) were applied.

RESULTS AND DISCUSSION

Environmental conditions

Soil surface temperature ranged between 26.5-30.5 °C in the study area. Salinity in the channel next to the study area was 6-17 ‰. The spawning period of *C. capucinus* was in accordance with the rainy season, when the salinity decreased as a result of precipitation and fresh water runoff (Fig. 2).

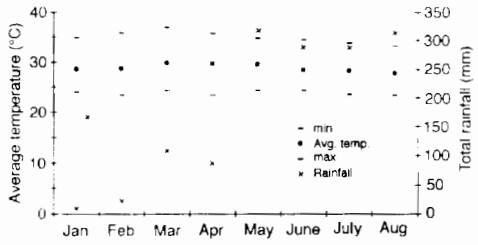

Figure 2. Monthly average temperature and total rainfall in Phuket province.

Reproductive behaviour

The snails gathered in clusters of 4-15 individuals throughout the observation period. The clusters were observed on *Rhizophora apiculata* branches and roots in contact with the mud, between *Isognomon ephippium* L., 1758, inside dead shells of *I. ephippium*, and in decomposing pieces of *R. apiculata* branches. Males outnumbered females in both mating and egg laying clusters: 18 females and 84 males in 13 mating clusters with no egg masses; 6 females and 15 males in 5 egg laying clusters. The sex ratio in mating clusters deviated significantly from the 1:1 ratio (Chi-square, $P < 0.01$). Egg masses were found from July to September.

Shell length and sex

The average length of females (45.5±2.8 mm) was greater than that of males (39.5±2.9 mm). The smaller size *C. capucinus* are almost entirely males, while the larger sizes are preponderantly females. In the range between 30 and 39 mm shell length practically all specimens examined in the present study were males, whereas between 39 and 44 mm there were at least 84 % males. On the other hand, between 44 and 47 mm there were 60 % females and all specimens having shell length up to 47 mm were females. In 13 mating clusters, the shell lengths of males were 35.6 to 47.1 mm while females ranged from 44.1 and 50.4 mm (Fig. 3).

The relationship between shell length and proportions of the two sexes is not fully understood. The phenomenon might be explained in two ways.

First, if one assumes that the rates of growth

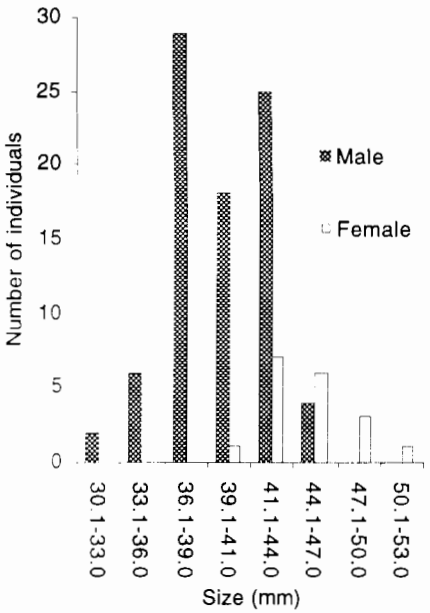

Figure 3. Size distribution of males and females of *C. capucinus*.

and development of males and females are similar, and this is certainly true for the gonad. the sex may actually change from male to female during the snail's lifetime (Coe 1936; Orton *et al.* 1956). It has been suggested that the snails start their breeding life as males, when the body of the individual has reached only a small size, but that later in life nearly all individuals become females. The male phase may be retained for several years. The change in sex is thereby postponed until the body has attained practically its full size (Coe 1936).

Second, if one assumes that the rate of growth and development can differ markedly in the two sexes, the preponderance of females in the larger size-groups can be explained by differential mortality (Coe 1936; Orton *et al.* 1956). The males might have a higher mortality rate (less than 10 % of the males reach a shell length of 47 mm) due to less resistance to unfavourable environments than the females (Coe 1936). The males have a shorter life span and disappear more rapidly than the members of the other sex.

External features and histology of the gonad

Male. In June the testis was seen as an orange or cream-coloured opaque mass which spread over a brown or greyish mass of the digestive gland. Nearly half of the volume in all specimens was visible testis. Under the microscope the testis was full of developing seminiferous tubules. Spermatogonia were layered on the wall of the tubule only whereas spermatocytes and spermatids proliferated deeply towards the centre of the tubules (Fig. 4, a). Spermatids were slightly smaller than spermatocytes and stained more distinctly with haematoxylin than did spermatocytes. Spermatozoans were about 30 μm in total length, their heads attached to epithelial walls and tails extending into the lumina. There was also a short eosinophilic mass at the posterior end of the spermatozoa.

In September, the ratio of volume of gonad to digestive gland varied among the specimens. Thirty percent of the testis became greatly reduced in size (reduction in the orange or cream-coloured opaque areas on the columellar side of the whorl), compared to conditions in June. According to Hahn (1989) the gonad should be classified as spent. Microscopic examination showed dark blue patches of ripe seminiferous tubules and tubules free of gametes, tinged with eosin (Fig. 4, c). Patches of developing seminiferous tubules were observed as well. Only 50 % of the testis was filled with developing seminiferous tubules (Fig. 4, b). Masses of ripe spermatozoa which were ready to be discharged, were packed in tubules in proximity to the kidney. Ten percent of the specimens had invisible testis, and only the greyish mass of the digestive gland could be observed. Examination of sections revealed no germinal epithelium between the outer epidermis and the digestive gland. The loop surrounding the seminiferous tubules, stained densely with haematoxylin (Fig. 4, d). This might be evidense of necrosis of unspawned spermatocytes. Necrosis is believed to be autolysis and degeneration of

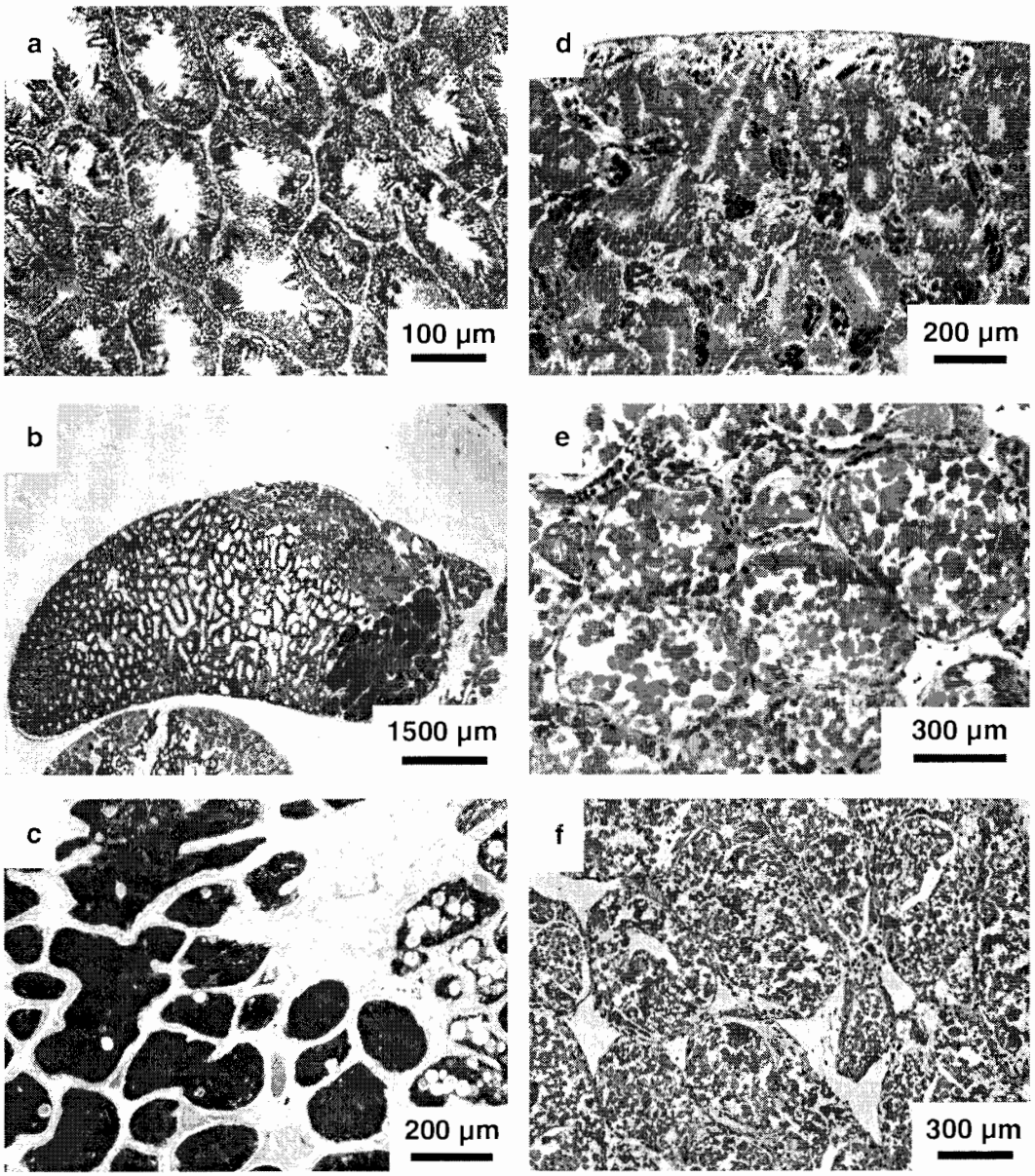

Figure 4. Development of the gonad. (a) Transverse section. Male, June 1995. Developing seminiferous tubules. (b) Longitudinal section. Male, September 1995. Ripe spermatozoa ready to be discharged. (c) Longitudinal section. Male, September 1995. Tubules full of ripe spermatozoa and tubules free of gametes. (d) Longitudinal section. Male, September 1995. Seminiferous tubules reduced in size and digestive loop visible through most of the sectional area. (e) Longitudinal section. Female, September 1995. An active germinal epithelium. (f) Longitudinal section. Female, September 1995. Post-vitellogenic ova and a reduced germinal epithelium.

gametes that have remained after the spawning period (Giorgi & DeMartini 1977). The quantity of necrotic spermatocytes present in the testis was used to classify the individuals to a spawning type for the previous spawning period (Giorgi & DeMartini 1977). The present result shows that the gonad is in a stage of partial spawning. Futhermore, a drop in the gonad area with a corresponding increase in digestive gland area will pinpoint the spawning period (Hahn 1989). The spawning season of the species, therefore, is expected to finish right after this.

Female. The female gonad of *C. capucinus* was orange to yellowish-white, spreading over the surface of the digestive gland. Nearly half the volume in all specimens was visible ovary tissue. The external features of the gonads (volume and colour) did not differ greatly when June was compared with September. Examination of sections through gonads showed that an active germinal epithelium was present in female gonads in June as well as September. Twenty percent of the gonads in June, and 50 % of gonads in the later collection, were full of active germinal epithelium, staining deeply with haematoxylin (Fig. 4, e). Pre-vitellogenic and intermediate ova were still attached to the follicular epithelium. Seventy percent of gonadal sections in June and 20 % in September showed reduction of germinal epithelium, and presented post-vitellogenic ova (Fig. 4, f). Nuclei of post-vitellogenic oocytes (about 0.015-0.020 mm in diameter) were packed with eosinophilic globules. For the remaining 10 % in June and 30 % in September, examination of sections showed that most of the follicles had increased in size, and the connective wall was sharply defined and thin. Post-vitellogenic oocytes were frequent in the follicles.

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