Reproduction and metabolism of *Turbo (Batillus) cornutus* in Chiba, Japan.

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**SUMMARY:** The reproduction and oxygen consumption of *Turbo (Batillus) cornutus* were studied in specimens collected monthly between March, 1999 and April, 2000 in Chikura, Chiba Prefecture, Japan. Gonadal histology, gonadal index and oocyte diameter were used to determine the reproductive cycles and spawning period for both sexes. Oxygen consumption and ammonia excretion were measured to estimate metabolism. The reproductive cycle was annual, with the peak activity in the late summer. Oxygen consumption and ammonia excretion increased linearly with increasing soft body weight and showed an annual cycle in connecting with gonadal maturation cycle.

**KEYWORDS:** reproductive cycle, oxygen consumption, gonad maturation, spawning, oocyte,

**INTRODUCTION**

The Japanese turban top shell, *Turbo (Batillus) cornutus*, represents one of the most common species of edible gastropods in Japan1. *T. (B) cornutus* inhabits shallow rocky coast and it is an important coastal resource in Japan1,2. Its geographical distribution is restricted only to Japanese coastal waters (except northeastern Honshu and northern and eastern Hokkaido) and the southern part of Korean Peninsula3.

Although this is an important species as fisheries resources and there is need for information on fisheries biology, few comprehensive works have been made in reproductive biology and physiology of this species1,2,4. Monitoring the maturation process is important for the management and sustainable utilization of the resources. The present study was carried out to determine the reproductive cycle of *T. cornutus* from Chikura, Chiba Prefecture. Oxygen consumption and ammonia excretion of *T. cornutus* were measured as an indicator of the metabolism and activity in relation with maturation.

**MATERIALS AND METHODS**

A total number of 525 *T. cornutus* with shell height of 63-111mm were collected monthly from March, 1999 to April, 2000 from Chikura, Chiba Prefecture. Sexes of *T. cornutus* were determined based on the colour of gonads5. The whole specimens of female and male collected every month were subjected to a chi square goodness-of-fit test to test the null hypothesis on the sex ratio is 1:1.

Gonad index (GI): The visceral coil of each individual was fixed and preserved in 10% formalin seawater. GI was determined by measuring the proportion of the area occupied by the gonad to total area of the visceral coil section1,2,4. The GI was calculated using the formula:

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GI (\%) = \frac{\text{gonad area} \times \text{total cross-sectional area}}{100}
\]

Each preserved visceral coil of the specimens was sectioned, following the method established for *T. cornutus*1. The section just behind the stomacial caecum was used for GI measurement.

Gonad histology: The gonad-digestive gland complex was dehydrated, embedded in paraffin, sectioned at 5-8 µm and stain with Harris Hematoxylin and Eosin.

Based on the histological observation of gametes, gonad maturation process was categorized according to the criteria for *T. (B) cornutus*1,2,4.

Diameters of the oocytes were measured with micrometer for 150 oocytes or ova with visible nucleus in each individual from 4-7 female histological specimens.

Oxygen consumption and ammonia excretion: Specimens of live *T. cornutus* selected from monthly samples were used for measurement of oxygen consumption and ammonia excretion. The total wet soft body weight (W) ranged from 17.1 to 72.4 g.

All the animals subjected to the experiment were in post-digestive condition. They were starved at least for 2 days and acclimated into experimental condition in which average temperature and salinity were 20°C and 35 ppt, respectively. Following the acclimation for 4-6 hrs in the respiratory chamber under flow through
condition, dissolved oxygen concentration was measured by the Winkler titration method and ammonia contents by Indophenol method.

RESULTS

Sex Ratio

Sex ratio (male to female) ranged from 0.7 to 1.3 (1.1 ± 0.2). Since the Chi-square test value did not significantly different (χ², p>0.05) from expected male to female sex ratio of 1:1, the population of _T. cornutus_ from Chiba Prefecture consists of equal number of male and female all through the year.

Gonad Index (GI)

GI in both sexes rose from March 1999 with a peak (around 66%) in August and dropped to around 40% in September (Fig.1). In October, GI declined further to about 20%, dropped to the lowest (<20%) in November and gradually increased until March 2000. In April 2000, GI was slightly lower than March. During the increasing phase in GI of both sexes from March to August 1999, GI slightly dropped from June to July before reaching the peak in August. Seasonal changes of GI in both sexes showed similar patterns (p>0.05), which indicates a strong synchronization in reproductive development between male and female throughout the year.

Oxygen consumption and ammonia excretion

_R/W_ ranged from 47.7 to 82.3 (ml O₂/kg W/hr) for _T. cornutus_ 17.1-72.4 g. Figure 3(A) shows the monthly change in mean _R/W_ (ml O₂/g W/hr) of _T. cornutus_. Monthly fluctuation in _R/W_ of male and female showed similar patterns throughout the year. High _R/W_ was recorded between August to October, while _R/W_ in other months was relatively lower and constant. During the high _R/W_ period, GI changed with a peak spawning activity. At this period, the seawater temperature was level off and remain around 25°C. Therefore, this change of _R/W_ with high value during Aug-Oct period may be resulted from the effect of maturation and spawning. However, this phenomenon needs to be further elucidated by more detailed research in future.

Gonad Maturation and Oocyte Size Frequency

Five stages of gonad development were identified based on the histological analyses: stage 1, early active; stage 2, late active; stage 3, ripe; stage 4, partly spawn; and stage 5, spent. Gonad development in stage 3 appear from April to October, stage 4 July to December, and stage 5 from September to January. Stage 1 was present almost throughout the year except July and August, indicating that gametogenesis for next season started from September. Stage 2 for the next season appeared from January and developed into stage 3 from April. Spawning started from July and end in October.

Oocytes diameter _T. cornutus_ ranged from <10 to <260 µm (Fig.2). Large oocytes (>140 µm) predominated during a period from May to August, while small oocytes (<80 µm) predominated from October to April. Although large number of small oocytes and large oocytes (160µm< to <200µm) were present throughout the year, no oocyte larger than 200µm was observed in October and November. A decrease in the number of bigger oocytes (>160 µm) started from September onward, with increase in number of small oocytes (<100 µm) indicated that peak spawning period occurred during a period from late August to September. Oogonia and small oocytes (<40 µm) were present throughout the year with progressive increase in number starting from September to April. This indicates that gametogenesis in _T. cornutus_ is initiated on and after spawning. Size frequency distribution of oocyte (Fig.2) indicated seasonal changes of gonad maturation.
Monthly changes in $E/W$ for both sexes is showed in Fig. 3(B). $E/W$ in this study ranged from 0.05-0.43 (μg-atNH₄⁺-N/gW/hr) at the same condition and ranged of animal sizes used in $R/W$. In June and July 1999, and April 2000, $E/W$ was relatively higher compare to other months during this study. The high $E/W$ value in April, June, July coincided with the period of progress in gonad maturation while they utilised protein in high proportion in metabolism and used lipid for vitellogenesis. $E/W$ for both sexes fluctuated similarly but different pattern from $R/W$.

The O/N ratio was scattered widely during the reproductive cycle of $T. (B)$ cornutus in this study. The mean O/N ratio ranged from 24.9 in July to 54.9 in October. When the spawning season starts in July, O/N ratio was at its lowest and peak O/N ratio coincided with end of spawning season.

**DISCUSSION**

Gonad index has been used as a good indicator to estimate the reproductive maturation of gastropods. Combining the information of GI with gonad developmental stages composition and oocys size frequency distribution, the population of $T. cornutus$ in Chiba Prefecture was estimated to have a single major spawning period a year. This spawning period peaks in late August through September, which agrees with the results of previous study in Chiba. Spawning activity of wild population of $T. cornutus$ was from June to November at temperatures ranging from 20 to 25°C.

Based on the histological examination, gametogenesis in $T. cornutus$ was classified into five stages as in previous studies on the same species and generally the same process for gastropods. Two gametogenic periods occurred during the reproductive cycle of $T. (B)$ cornutus. The first period occurred during the spring and summer and correlated well with the period of large increase in gonad size (early active, late active and ripe stages); the second period occurred immediately after spawning (partially spawn and spent) and was not reflected as a large change in gonad index.

The $R$ on individual basis in the present study increases with increase in $W$ as described by $R=aW^b$, where $b$ range from 0.54 to 1.2 with a mean value of 0.9. The $b$ value reported for 15 different bivalves range from 0.6 to 0.93 with their mean of 0.72. In $Eucidaris tribuloides$, $b$ value was 0.70 during winter (20°C) and 0.65 in summer (30°C). The mean $b$ value in this study indicates that metabolism of $T. cornutus$ is almost proportional to body weight.

The $R/W$ (ml O₂/g W/hr) in the present study varied with season during the reproductive cycle and ranged from 0.80 to 1.37 mlO₂/kg W/min at average temperature of 20°C. $T. cornutus$ studied by
temperature of 20°C. *T. cornutus* studied by Yamamoto et al.\(^{16}\) reported values of 1.26 and 1.46 mLO₂/kgW/min at 23°C and 28°C, respectively. Therefore, the values of \(R/W\) in this study are comparable and within those reported for the same species\(^{16}\).

As the gonad matured towards spawning period, \(R/W\) becomes higher during the period of peak spawning activity. *Mytilus edulis* increased \(R/W\) in winter in conjunction with gametogenesis\(^{14,17}\), but no effect of gonadal condition and sex on \(R/W\) for *Eucidaris tribuloides*\(^{15}\).

\(O/N\) ratio is used as good indicator of metabolite, especially that of protein\(^{21}\). Although the \(O/N\) ratio calculated in this study was varied widely, the ratio was lower in June, July and April which indicates the use of protein at higher rate as metabolite during this period. During the spawning and late active stages and other season, the relatively high \(O/N\) ratio was retained, and this indicate that the main metabolite was lipid rather than protein. The pattern \(O/N\) ratio in this study is comparable the one has been described for bay scallop, *Argopecten irradians concentricus*\(^{18}\).

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