Annual Reproductive Cycle of the Chichibu-Goby

Tridentiger obscurus*1

Toyoji KANEKO*2 and Isao HANYU*2

(Accepted March 26, 1985)

To clarify the whole aspect of the annual reproductive cycle of the chichibu-goby Tridentiger obscurus, we examined the seasonal gonadal changes of adult fish caught from its natural habitat, and the participation of underyearling fish in reproduction under laboratory conditions.

Seasonal changes in GSI and gonadal histology of adult fish indicate that the annual reproductive cycle can be divided into four phases: the resting period in September and October (phase 1), the period from November to February when yolk vesicles appear in ovaries and testes are in moderately active spermatogenesis (phase 2), the period in March and April when yolk globules are accumulated and spermatogenesis is further activated (phase 3), and the spawning period from May to August (phase 4).

The underyearling fish come to mature within the spawning period in which they have been born, only if they grow to a critical size in time. The critical total length is estimated to be 35 mm in females and 45 mm in males.

The chichibu-goby Tridentiger obscurus is a small fish, commonly found in both fresh and brakish waters almost all over Japan. The life history of the goby was already reported in the 1940s,1) and recently KISHI2) made a detailed observation on its social behavior. But little information is available of the annual reproductive cycle of the goby.

In general, gobies are hardy fish with a wide variety of breeding periods ranging from mid-winter to early autumn. So they are convenient for comparative studies on annual reproductive cycles and their environmental regulation. Seasonal changes in gonadal maturation of gobies have been reported in Gobius minutus,3) Gillichthys mirabilis,4) Chaenogobius isaza,5) Hypseleotris galii,5,6) and Chasmichthys dolichognathus.8)

In the present study, we examined seasonal gonadal changes in both sexes of the chichibu-goby caught from its natural habitat, and the participation of underyearling fish in reproduction, to clarify the whole aspect of the annual reproductive cycle of the goby. The understanding of annual reproductive cycles as they occur in nature is an essential prerequisite to laboratory studies dealing with the influence of environmental factors on reproduction.

Materials and Methods

Seasonal Gonadal Changes in Natural Fish

The chichibu-goby were collected from Lake Kasumigaura in Ibaragi Prefecture at intervals of about a month from March 1981 to March 1983. For this investigation, adult females and males more than 40 mm in total length were used. Each of the monthly samples contained from 3 to 14 females and 6 to 20 males. At the time of the samplings water temperature was measured usually between 10:00 and 12:00.

The fish were killed by decapitation on the day of the collection or on the following day. The fish with the abdominal cavities opened were fixed with Bouin's solution for 1 day, and then transferred to 70% ethanol. Later, total length, body weight and gonadal weight were measured and gonadosomatic index (GSI: gonadal weight/body weight ×100) was calculated. Gonads were embedded in paraffin for histological observation. Tissues were sectioned at 5-10 μm and stained by Mayer's hematoxilin and eosin, or AZAN stain.

Participation of Underyearling Fish in Reproduction

Juvenile chichibu-goby were collected from Lake Kasumigaura in July 1984. They were cultured in an outdoor tank on our campus under...
natural condition, and fed on commercial trout pellet and tubifex. The fish were checked for spawning every day. From the stock, sampling was made three times i.e. on July 26, August 27, and September 28, 1984. Each sample contained 20 females and 19 or 20 males. Total length, body weight and gonadal weight were measured and GSI values calculated. Gonads were also examined histologically.

**Results**

*Seasonal Gonadal Changes in Natural Fish*

1. Seasonal changes in GSI: The chichibu-goby showed distinct seasonality in GSI (Fig. 1). In female fish GSI exhibited the lowest value in September 1981 and October 1982. Subsequently GSI increased gradually and reached about 2% by February in 1982. There was a sharp increase in GSI in March, and the average GSI was kept high from April to August in 1981 and April to July in 1982. In these periods, however, GSI showed a large variation, which extended from 2% to 20%. Then GSI began to decrease and was low in September.

GSI of male fish exhibited low values less than 0.3% in October. GSI increased slightly afterward and reached a moderately high level of about 0.5% in November. This level was maintained for two or three months, and then GSI began to increase again in January. High values of GSI, ranging from 1.0% to 1.5%, were kept during the period from April to August. GSI decreased in August and was low in October.

2. Ovarian histology: The ovaries of the goby are paired, elongate structures lying dorsally in the abdominal cavities. The development of the oocytes occurs synchronously. In the ovary of the fish just before ovulation, three groups of oocytes can be distinguished: fully ripe oocytes, yolk vesicle or early yolk globule stage oocytes, and yolkless oocytes.

In September and October, when GSI was low, ovaries were filled with early perinucleolus stage oocytes which were characterized by basophilic cytoplasm and diameters less than 80 μm. By November a part of oocytes grew into late perinucleolus stage, at which the cytoplasm became less stainable by basic dyes and cell size ranged from 80 to 130 μm (Plate I-1). From November to February, when GSI kept gradual increase, late perinucleolus stage oocytes developed into yolk vesicle stage of 120-160 μm in diameter (Plate I-2). The appearance of yolk vesicles in the peripheral regions of the cytoplasm was not necessarily synchronized between individuals. But at latest by the end of February, the oocytes grew into yolk vesicle stage in most fish. In March yolk globules began to appear in the outer half of the cytoplasm with a sharp increase in GSI (Plate I-3). Afterward yolk globule stage oocytes became even bigger and the diameter reached more than 400 μm in April (Plate I-4). During the period from May to August, various maturational stages of ovaries were observed: ovaries with empty follicles in addition to yolk vesicle stage and younger oocytes, with various sizes of yolk globule stage oocytes, and with migratory-nucleus stage oocytes or ovulated eggs. In August, atresia and absorption of yolkly oocytes occurred as GSI began to decrease. In September, the ovaries consisted of perinucleolus stage and younger oocytes.

3. Testicular histology: The testes are also paired, elongate structures. Each testis is composed of numerous seminiferous lobules, the walls of which are lined with cysts of germ cells. Within any cyst the germ cells are all at the same stage of development. Newly formed sperms are released into the lumens.

During the months of September and October, when GSI was low, seminiferous lobules stayed undeveloped. Although various stages of germ
cells were observed in the cysts, spermatogonia and primary spermatocytes were predominant (Plate I-5). In some fish a few sperms were present in the narrow lumens. In November, with a minor increase in GSI, the lobules developed moderately and grew slightly in size. The cysts of secondary spermatocytes and spermatids were commonly found in the lobules, and sperms were usually observed in the lumens. These histological conditions as well as GSI were maintained for two or three months (Plate I-6). Between January and March seminiferous lobules further developed with concomitant increase in GSI. Testes of the fish collected from April to August were in active spermatogenesis. During this period, when GSI was kept high, lobules attained the largest size, and numerous sperms were present in the swollen lumens (Plate I-7). Between August and September, testicular regression occurred in most fish as GSI decreased. Although there were numerous sperms left in the lumens, few cysts were recognized in the lobules (Plate I-8).

**Participation of Underyearling Fish in Reproduction**

1. Growth: Changes in total length and body weight of the underyearling fish from July to September 1984 are summarized in Fig. 2-A and

![Fig. 2. Changes in total length (A), body weight (B) and GSI (C) of underyearling Tridentiger obscurus from July to September 1984. Each value is the mean±S.E. Spawnings in the experimental tank were frequently observed during the period marked by arrows.](image)

![Fig. 3. Frequency distributions of GSI of underyearling Tridentiger obscurus at the time of the samplings.](image)

B. There were no differences in total length and body weight between male and female fish examined in July. Afterward, however, males grew larger than females. In September the average total length of males attained 58 mm, whereas that of females was 48 mm.

2. Changes in GSI and gonadal histology: Fig. 2-C shows changes in GSI of both sexes and occurrences of spawnings in the stock tank. Frequency distributions of GSI at the time of the samplings are summarized in Fig. 3. Spawnings in the tank were frequently observed until early September, but did not occur at all after mid-September. In the females sampled in July the average GSI was 1.6%, and in 11 out of 20 fish GSI values were more than 1%. These fish already started yolk accumulation and some of them had late yolk globule stage oocytes. The ovaries of the fish, whose GSI values were less than 1%, consisted of perinucleolus stage and younger oocytes. In August the average GSI of the females was increased and the values ranged from 1% to 16%. Yolk accumulation was in progress or had finished in all the fish examined. In contrast with this, GSI values of all the females sampled in September went down to less than 1%. Yolky oocytes were not observed in their ovaries. The change in GSI of males was similar to that of females. The male fish sampled in July, except only one, had GSI values less than 0.4%. Most of these fish were in inactive spermatogenesis, whereas in some fish sperms were observed in the
Fig. 4. Relations between total length and GSI of female and male *Tridentiger obscurus* examined in July and August 1984. Arrows indicate estimated critical sizes of both sexes.

lumens of the seminiferous lobules. In late August, the average GSI was high, and in 15 out of 19 fish examined the values exceeded 1.0%. The lumens of the seminiferous lobules in these fish were filled with numerous sperms. The rest of the males, whose GSI values were less than 0.4%, were quite immature. The average GSI of the males examined in late September was very low and individual GSI values did not exceed 0.4%. Although there were a small number of sperms left in the lumens in some fish, spermatogenesis in the testes of all the males fell into an inactive condition.

3. Relation between body size and GSI: Fig. 4 shows the relations between total length and GSI of the female and male fish examined in July and August, when environmental conditions were considered suitable for maturation and spawning of this species. GSI was apt to be high as the size of fish became larger in both sexes. In females, GSI values of all the fish which were less than 34 mm in total length were about 1%. The fish whose GSI reached 2% first appeared in the total length of 35 mm. Females over 35 mm exhibited various values of GSI that extended from 1% to 16%. In the male fish less than 43 mm, GSI values were low and did not exceed 0.5%.

These data indicate that underyearling fish can mature within the spawning season in which they have been born only if they grow to a critical size in time, and that the critical size lies around 35 mm and 45 mm in female and male fish, respectively.

Discussion

Our findings are summarized in Fig. 5. The chichibu-goby exhibited distinct seasonality in reproductivity as evidenced by marked cyclic changes in GSI and gonadal histology. The spawning period of this species seems to extend from May to August since females with ovulated eggs or with empty follicles in their ovaries were often observed during the period, when GSI values of both sexes were kept high. This finding agrees to the report by Nakamura.1) The fish are known to spawn repeatedly during this period.2) We also made a detailed observation on the cyclic spawning of the goby.*

From our results, the annual reproductive cycle of this goby can be divided into four phases: the resting period in September and October (phase 1), the period from November to February when yolk vesicles appear in ovaries and testes are in moderately active spermatogenesis (phase 2), the period in March and April when yolk globules are accumulated and spermatogenesis is further activated (phase 3), and the spawning period from May to August (phase 4).

The prolonged phase 2 from November to February, when yolk vesicles appear in females, is

characteristic of this species as well as Acheilognathus tabira. On the other hand, Chasmichthys dolichognathus starts accumulating yolk globules as soon as the oocytes get to yolk vesicle stage.

Our investigation also indicates that underyearling fish come to mature within the spawning period in which they have been born only if they grow to the critical size in time. The critical total length was estimated to be 35 mm in females and 45 mm in males. Most probably, the fish born early in the spawning period can reach the critical size before the end of the period and then start to mature, whereas the fish born later cannot mature. On the other hand, most of the yearling fish are destined to die after the spawning period.

As described previously, the spawning period extends from May to August. But in August some of the yearling fish collected from the natural habitat had already completed spawning, while a part of underyearling fish were maturing or matured. Thus, it seems that underyearling fish become predominant over yearling fish in the late spawning period.

The male underyearling fish grew larger than the females in the experimental tank, although there was no sexual difference in size at the sampling in July. The males tended also to be bigger than the females in the natural samplings. These facts that males grow larger than females are in agreement with the previous paper.

Generally, environmental factors are believed to play important roles in regulating the reproductive cycles in various teleosts. Temperature and photoperiod are presumed to be important factors especially in temperate zones. In the chichibu-goby, changes in GSI and gonadal histology are closely related with environmental changes: GSI increases rapidly in spring when water temperature is rising (Fig. 1-A) and spawning period comes to an end as daylength becomes shorter. It suggests that environmental factors are involved in regulating the annual reproductive cycle of this species.

Acknowledgment

This study was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

References


Explanation of Plate

Gonadal histology of Tridentiger obscurus in different phases of the annual reproductive cycle.

Plate I-1~4. Ovaries. AZAN stain. ×70.
1. November. Late perinucleolus stage oocytes (L) are observed in addition to early perinucleolus stage (E) and younger oocytes.
2. January. Yolk vesicles (arrows) appear in the peripheral regions of the cytoplasm.
4. April. Large yolk globule stage oocytes are commonly observed.

Plate I-5~8. Testes. Mayer's hematoxilin and eosin. ×185.
5. October. Seminiferous lobules are undevloped.
7. May. Numerous sperms are present in the swollen lumens.
8. September. Few cysts are recognized in the lobules.