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REPRODUCTIVE PERIODICITY AND GONAL DEVELOPMENT OF THE JAPANESE BUTTER FISH, *PSENOPSIS ANOMALA*

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Shyh-Bin Wang and Che-Tsung Chen (1989) Reproductive periodicity and gonal development of the Japanese butter fish, *Psenopsis anomala. Bull. Inst. Zool., Academia Sinica* **28**(3): 225-235. Seasonal changes of both macroscopic appearance and histological observations of *Psenopsis anomala* gonads and the gonosomatic index were described. Oogenesis was divided into nine cytological stages, and spermatogenesis was divided into five stages. Initial oocyte development was a continuous, asynchronous process until the beginning of the spawning. The oocytes which spawned were synchronized. The development of sperm follows the pattern of other teleosts, and the distribution of the spermatogonia in the testis conforms to that of the "unrestricted spermatogonial testis-type". The spawning seasons of *P. anomala* could be detected to extend from March to August, and seasonal changes in the histological appearance of the gonads are well correlated with seasonal changes in both GSI and macroscopic appearance.

Key words: *Psenopsis anomala*, Histology, Macroscopy, Asynchronization.

Psenopsis anomala is an important demersal fish in Taiwan. In 1985, about 4,400 tons of this species, valued at N.T.$ 2.2 million were landed (Taiwan Fisheries Yearbook, 1986). Despite its economic importance, however, comparatively little is known about its biology in Taiwan.

The reproductive biology of *P. anomala* have been studies in Kii Channels of Japan (Sakamoto and Suzuki, 1972) at some aspects such as the size-composition of spawning groups, frequency distribution of ova diameter and seasonal changes of gonad weight to determine the spawning seasons and the size at maturity... etc. To date, no detailed description of the gonads and their annual cycle has been investigated.

The purpose of this research was to examine seasonal changes of the gonads in both the macroscopic and histological appearances and the gonosomatic index (GSI). The relationship among these three catagories were also discussed.

MATERIALS AND METHODS

Samples were randomly selected at approximately 15 days intervals from Dec. 1983 through Dec. 1984 (Table 1) from the catches of several baby trawl fishing vessels which were operated around the waters of Guei Shan Island, Taiwan (Fig. 1).

The sex, fork length, total weight, somatic weight, gonad weight and liver weight of each fish were recorded. The

Table 1
Samples used in this study

Date	Number of specmiens	Range of fork length (mm)
Dec. 8, 1983	18	143-186
23, 1983	29	153-200
Jan. 9, 1984	35	145-195
23, 1984	39	149-201
Feb. 21, 1984	62	147-201
Mar. 7, 1984	33	172-203
17, 1984	22	164-202
24, 1984	38	163-203
Apr. 10, 1984	30	134-218
16, 1984	18	182-202
21, 1984	41	164-209
May 8, 1984	33	167-210
16, 1984	18	156-204
28, 1984	23	168-202
June 20, 1984	8	145-196
29, 1984	18	150-198
July 11, 1984	8	159-208
22, 1984	4	174-208
Aug. 17, 1984	18	168-206
29, 1984	9	181-201
Sept. 18, 1984	22	161-202
27, 1984	15	173-207
Oct. 17, 1984	15	168-209
24, 1984	5	177-201
Nov. 3, 1984	21	155-212
17, 1984	11	166-201
26, 1984	30	163-204
Dec. 5, 1984	47	155-190
Total	670	134-218

gonads were fixed in 10% formalin, embedded in paraffin, sectioned at 8 μm-20 μm, and stained with routine haematoxylin and eosin stain. The gonosomatic index (GSI) were expressed as: GSI= (Gonad weight/Somatic weight) $\times$100.

RESULTS

Macroscopic structure of gonads

The paired gonads of *P. anomala* are almost equal in size, each covered with a thin muscular tunica and attached separately to the dorsal wall of the coelmic cavity with a thick mesovarium. Ovaries of immature specimens were small, slender, pinkish and generally translucent, but those of matured individuals were very swollen and occupied most of the ovarian cavity. Immature testes were thin, thread-like tubes and not easy to depart from visceral tissue, but matured testes were rather flattened and heavily vascularized in their inside surface.

Gonosomatic index

Figure 2 shows the monthly changes of the gonosomatic index in both male and female *P. anomala*. The GSI of females were low (0.4-0.85) from October through February, rose gradually in March, and reaching their highest point (6.8) by following May. For four months from May to August, the GSI remained at relatively high values, then fell sharply to the low point (2.0) by late September.

Similarly, the GSI values of male were increasing from March (0.83), and reached their hightest point in April (1.43), then remained at relatively high values from April through August, and sharply decreased by the following September (0.22).

Histology of the ovaries

Oogenesis was divided into nine developmental stages based on the cytological characteristics of the cells. They were defined as follows:

1) *Chromatin-nucleolus stage*

Very small oocytes, generally spherical in shape and about 0.02 mm-0.05 mm in diameter, were present throughout the year in the ovaries. They had a large nucleus, 7 μm-15 μm, which was surrounded with a thin layer of the cytoplasm. The nuclues and cytoplasm were densely stained by haematoxylin (Fig. 3).

2) *Peri-nucleolus stage*

Oocytes were 70 μm-100 μm in diameter and angular to round in shape. The

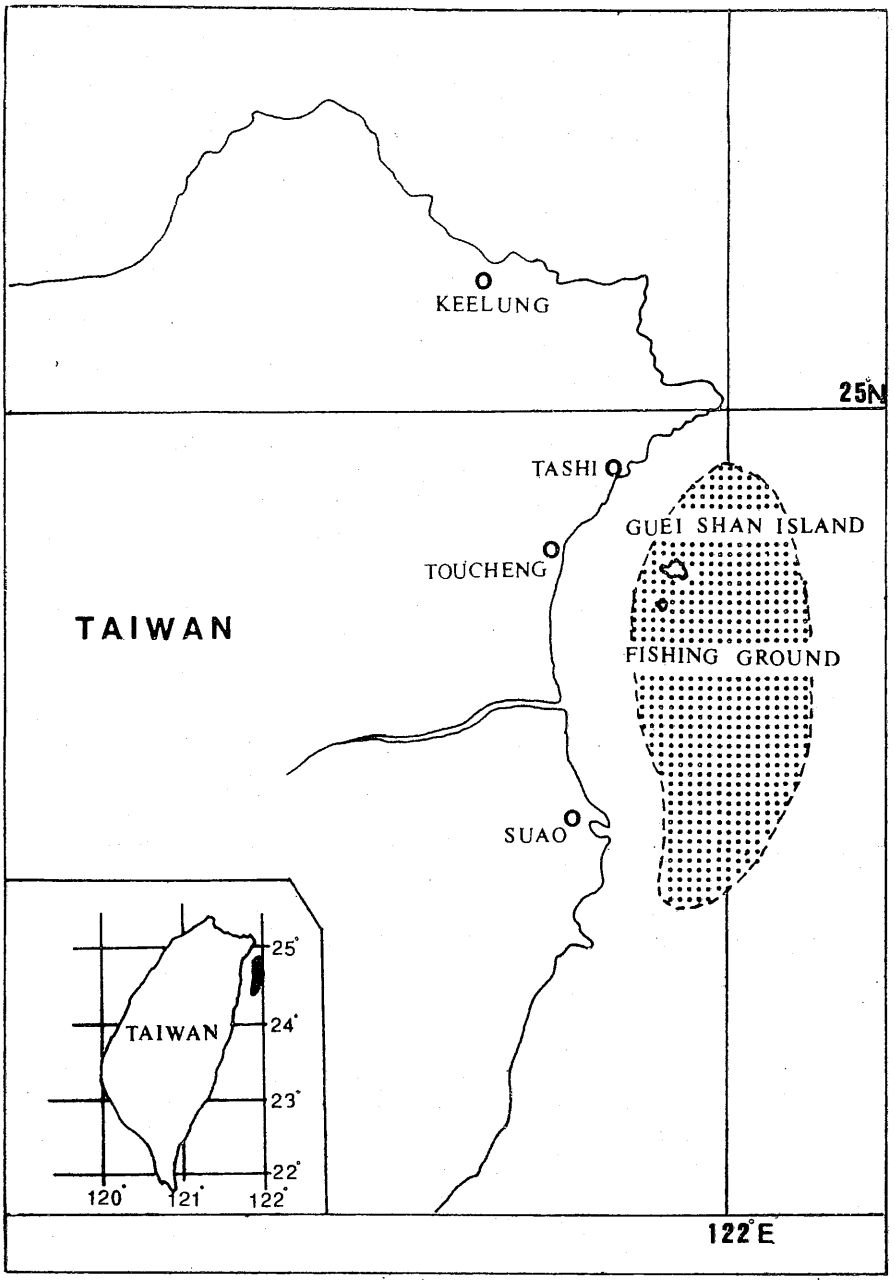


Fig. 1. Map of sampling area.

nucleus was spherical and large. Ten to 15 deeply basophilic nucleoli were located near the periphery of the nucleus. The nucleoplasm was slightly basophilic. The cytoplasmic volume was increased and stained deeply by haematoxylin (Fig. 4).

3) *Early phase of yolk vesicle stage*

The oocytes were about 0.1 mm–0.2 mm in diameter and became irregular in outline. The nucleus was large and spherical. Ten to 20 basophilic nucleoli were arranged peripherally, just inside the nuclear envelope. The cytoplasm contained many yolk vesicles which increased in number and size as maturation progressed (Fig. 5).

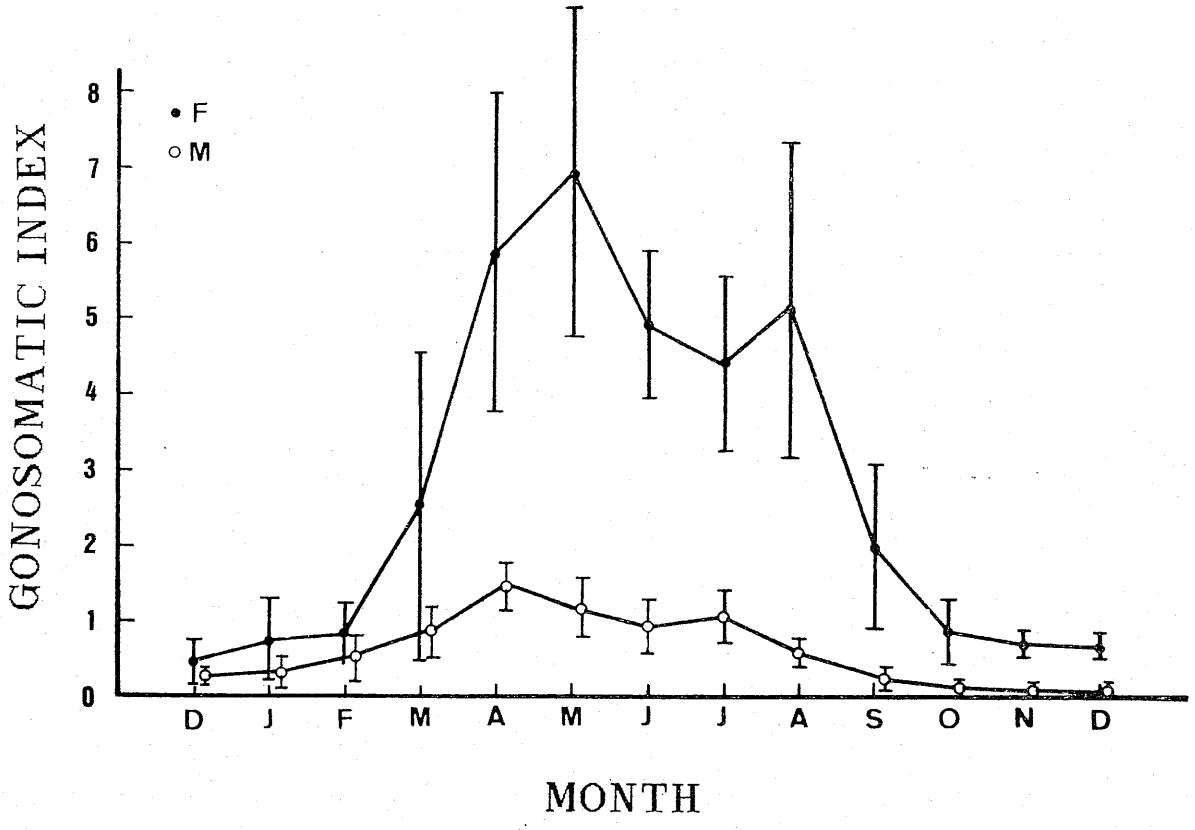


Fig. 2. Monthly changes of gonosomatic index ($\bar{X}$+1SD).

Figs. 3-12. Histological appearance of ovaries of *Psenopsis anomala.*

Fig. 3. Chromatin-nucleolus stage (Cn), 200×, from a fish, 167 mm in fork length (F. L.), 0.77 in gonosomatic index (GSI), on February 21. Scale=0.02 mm.

Fig. 4. Peri-nucleolus stage (Pn), 200×, from a fish, 191 mm in F. L., 0.84 in GSI, on March 3. Scale=0.02 mm.

Fig. 5. Early phase of yolk vesicle stage (Yv1), 100×, from a fish, 178 mm in F. L., 1.42 in GSI, on March 24. Scale=0.02 mm.

Fig. 6. Late phase of yolk vesicle stage (Yv2), 50×, from a fish, 178 mm in F. L., 2.20 in GSI, on March 24. Scale=0.05 mm.

Fig. 7. Primary yolk stage (Py), 50×, from a fish, 178 mm in F. L., 4.38 in GSI, on June 21. Scale=0.05 mm.

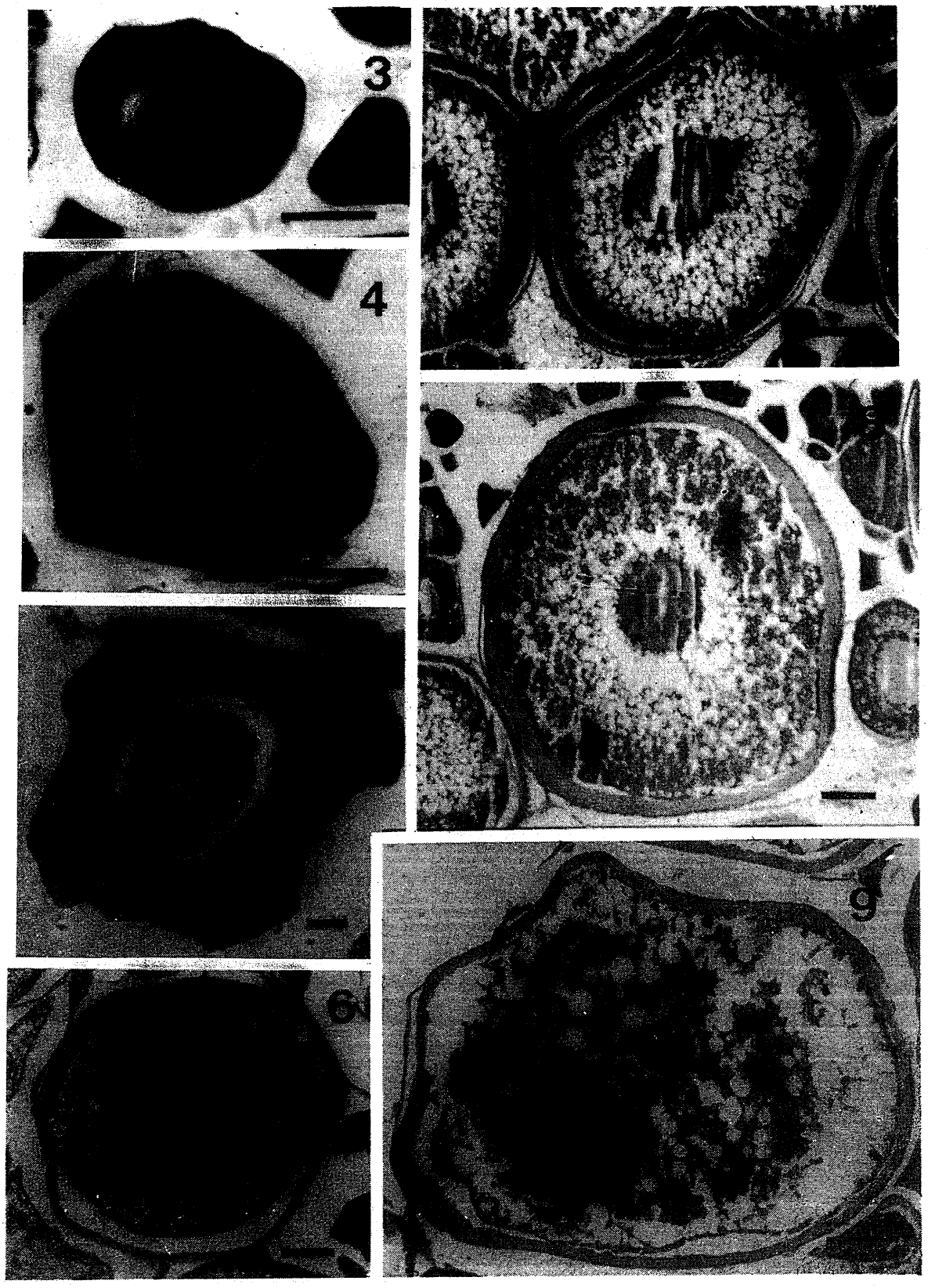
Fig. 8. Secondary yolk stage (Sy), 50×, from a fish, 191 mm in F. L., 5.21 in GSI, on March 17. Scale=0.05 mm.

Fig. 9. Tertiary yolk stage (Ty) 50×, from a fish, 195 mm in F. L., 5.67 in GSI, on April 21. Scale=0.05 mm.

Fig. 10. Tertiary yolk stage (Ty), 50×, from a fish, 203 mm in F. L., 6.93 in GSI, on May 16. Scale=0.05 mm.

Fig. 11. Migratory nucleus stage (Mn), 50×, from a fish, 195 mm in F. L., 8.67 in GSI, on May 16. Scale=0.05 mm.

Fig. 12. Mature stage (M), 50×, from a fish, 192 mm in F. L., 10.38 in GSI, on April 10. Scale= 0.05 mm.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6
9

4) *Late phase of yolk vesicle stage*

Oocytes were 0.2 mm–0.35 mm in diameter and spherical in shape. The nucleus became elliptical form. The cytoplasm was full of yolk vesicles and oil globules (Fig. 6). Then, the yolk vesicle accumulated and centripetal gradually as maturation progressed.

5) *Primary-yolk stage*

Oocytes were 0.2 mm–0.45 mm in diameter and spherical in shape. Oil globules and yolk vesicles increased in number and size and accumulated gradually in the inner part of cytoplasm. Some yolk globules made their first appearance in the cortex of the cytoplasm. The globules were minute and spherical in shape. In this stage, the nuclear envelope was indistinct, and the nucleus was irregularly shaped. Nucleoli were distributed at random in the nucleus (Fig. 7).

6) *Secondary-yolk stage*

Oocytes were 0.45 mm–0.55 mm in diameter and yolk globules accumulated centripetally and very rapidly in cytoplasm. This accumulation results in the rapid growth of oocytes. The egg membrane also increased in thickness. The nucleus recovered its round shape. Nucleoli were almost the same in form, size and location as in the previous stage (Fig. 8).

7) *Tertiary yolk stage*

As yolk globules increased further in number and size, oocytes became larger, measuring 0.55 mm–0.7 mm in diameter. In this stage, several large oil globules were recognized in the oocytes. Soon afterwards the oil globules shift inward, formed a ring around the uncleus, and then the yolk globules began to break up, allowing the yolk to coalesce. The interior of the cell appeared as a homogeneous mass with no apparent yolk globules (Figs. 9, 10).

8) *Migratory nucleus stage*

Oocytes were 0.7 mm–0.95 mm in diameter, the oil globules were the same size as those in the previous stage. The yolk appeared as a homogeneous mass filling the interior of the oocytes. The nucleus was moving toward the animal pole of the egg; it was nearly circular with a smooth contour. When the nucleus arrived at the animal pole, the nuclear membrane disappeared. However, the clear nucleoplasm was discernible in the ooplasm. In this stage, eggs were spherical in form, white in colour, translucent and always collapse in histological processing, thereby making them look irregular in histological observations and easily identifiable (Fig. 11).

9) *Ripe egg stage*

The oocytes were the same size or slightly larger as the previous stage (0.85 mm–1.1 mm). A single yolk mass, originated from yolk globules, existed and the oil globules which have fused into larger ones were observed. The eggs were also spherical in form, white in

Figs. 13–17. Histological appearance of testes of *Psenopsis anomala*.

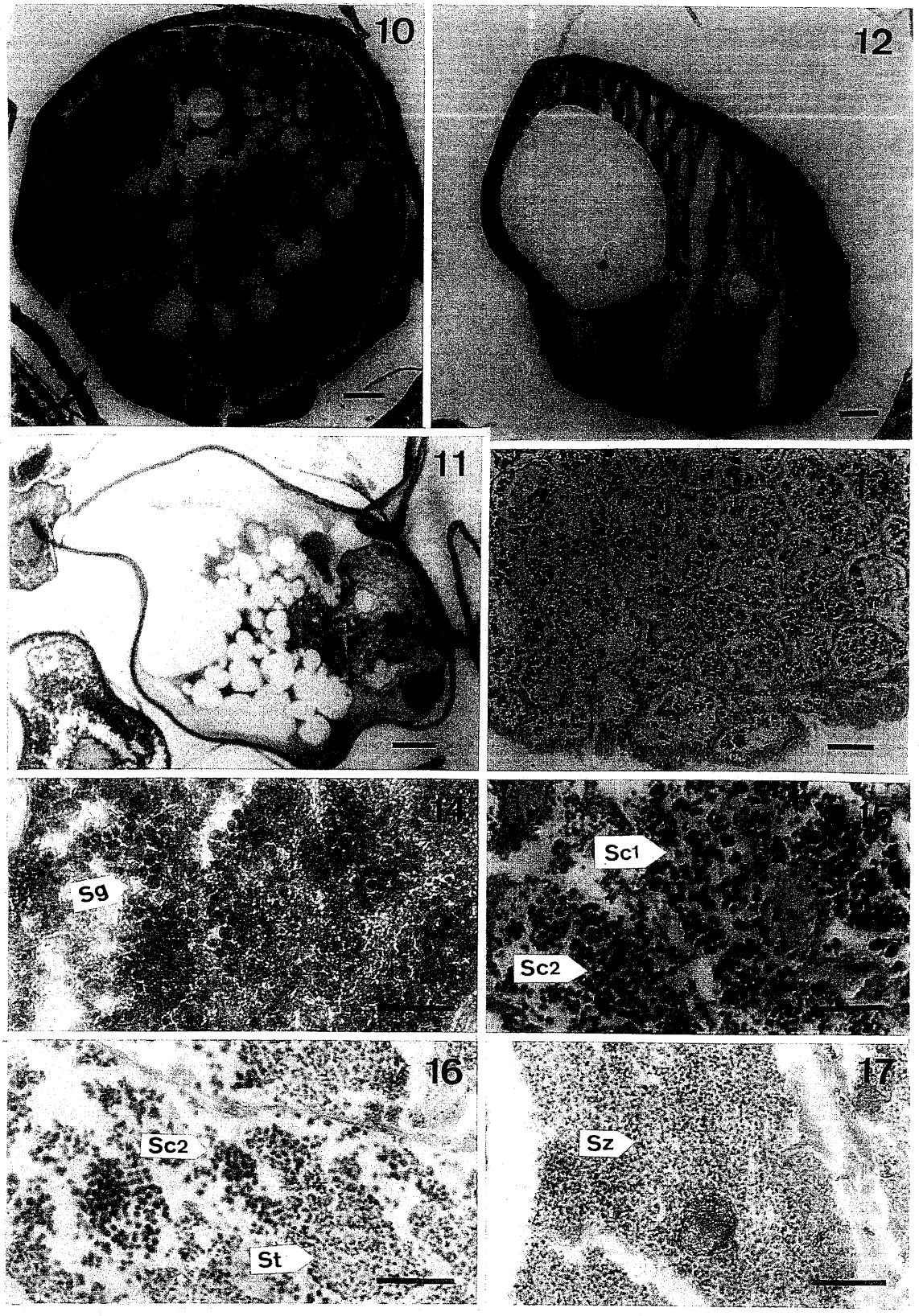
Fig. 13. Cross section of developing testes, 50×, from a fish, 171 mm in F. L., 0.85 in GSI, on March 17. Scale=50 μm.

Fig. 14. Spermatogonia (Sg), 200×, from a fish, 163 mm in F. L., 0.3 in GSI, on March 24. Scale =20 μm.

Fig. 15. Primary spermatocytes (Sc1) and secondary spermatocytes (Sc2), 200×, from a fish, 173 mm in F. L., 0.52 in GSI, on June 21. Scale=20 μm.

Fig. 16. Secondary spermatocytes (Sc2) and spermatids (St), 200×, from a fish, 175 mm in F. L., 1.48 in GSI, on April 10. Scale=20 μm.

Fig. 17. Spermatozoa (Sz), 200×, from a fish 191 mm in F. L., 1.71 in GSI, on May 8. Scale= 20 μm.


10
12
11
13
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Sg
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Sc1
Sc2
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Sc2
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colour, somewhat and translucent (Fig. 12).

Histology of the testis

The testes of *P. anomala* were elongate, paired structures covered in a delicated peritonium. Internally, the testis consists of a network of organized lobules which contained the germ cells at different stages of maturation and separated by connective tissue of interstitial cells. Sperm ducts collecting spermatozoa from the lobules passed into the major sperm duct running most of the length of the testis.

Typical cross sections of testis were shown in Fig. 13. The development of the testis was divided into five stages: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa.

1) *Spermatogonia*

Spermatogonia were found either singly or in small groups (nests) at all times. The cells, 10 μm-12 μm in diameter, were roughly spherical in outline. The nuclei were densely stained and the cytoplasmic area was small. In this stage, the lobules were about 40 μm-60 μm in diameter and 80 μm-120 μm in length in sections (Fig. 14).

2) *Primary spermatocytes*

Primary spermatocytes occurred in groups and were characterized by densely staining nuclei, often lacking a clear

Table 2
Macroscopic and microscopic characteristics of the different maturity stage in female *P. anomala*

Maturity stage	External appearance	GSI	Egg diameter (mm)	Histological appearance
Immature A	Ovaries small, slender, conical, pinkish and generally translucent. No oocytes visible to the naked eyes.	<1.5	<0.35	Oocytes at chromatin-nucleolus, peri-nucleolus stage, some yolk vesicle oocytes are also present.
Immature B	Ovaries relatively small, yellowish in color, oocytes gradually visible to the naked eyes.	1.5-3.0	<0.45	Oocytes predominantly of yolk vesicle stage, primary and secondary yolk stage.
Maturing C	Ovaries larger in size, vascularization in the back of them and occupying most of ovarian cavity. Visible oocytes large and yellowish in color.	3.0-8.0	<0.70	Oocytes predominantly of yolk stage, mainly secondary and tertiary yolk oocytes.
Matured D	Ovaries very swollen, pinkish and translucent. Vascularization heavy in the back of ovaries. Ovarian wall thin. Ova run from vent upon slight pressure.	>8.0	0.7-1.1	Oocytes predominantly of migratory nucleus stage and ripe egg stage.
Spent E	Ovaries flaccid.	—	—	—

Table 3
Macroscopic and microscopic characteristics of the different maturity stage in male *P. anomala*

Maturity stage	External appearance	GSI	Histological appearance
Immature A	Testis very small, thread-like elongated grayish in color and not easy to separate from viscera.	<0.4	Lobules predominantly of spermatogonia (10-15 μm) and primary spermatocytes (6-10 μm).
Maturing B	Testis larger in size, whitish in color and vascularization slight in the back of them.	0.4-0.9	Lobules predominantly of spermatocytes (4-10 μm) and spermatid (3-4 μm).
Matured C	Testis very large, whitish in color and vascularization heavy in the back of them. Milt run from vent upon slight pressure.	>1.0	Lobules predominantly of spermatid (3-4 μm) and spermmatozoa (1.5-2.0 μm).

membrane (Fig. 15). The diameter of primary spermatocytes ranged from 6 μm-10 μm in this stage, and were smaller than spermatogonia. The lobular wall was now thinner due to the distention caused by spermatocytes at various stages of development.

3) *Secondary spermatocytes*

Secondary spermatocytes, also occurring in groups, contained a dense nucleus and no visible cytoplasm. They were 4 μm-6 μm in diameter and smaller than primary spermatocytes (Figs. 15, 16).

4) *Spermatids*

The development of spermatids was accompanied by a further reduction in size. The cells were 3 μm-4 μm in diameter and the outline was not very clear. They were also occurring in groups, and were densely stained by haematoxylin (Fig. 16).

5) *Spermatozoa*

At the beginning of this stage, the spermatozoa rapidly increased in number; the cells were 1.5 μm-2.0 μm in diameter and also stained intensely. As maturation progressed, the lobules enlarged, slenderized and were gorged with spermatozoa (Fig. 17).

Based on the histological observations, size of eggs, GSI and macroscopic appearance of the gonads, maturity stages of ovaries of *P. anomala* could be placed in five stages and the testes in three (Tables 2, 3).

DISCUSSION

The developmental events of the oocytes of *P. anomala* are very similar to those of most teleosts described (Alekseyeva & Tormosova, 1980; Robb, 1982). Vitellogenesis, once initiated, proceeds very rapidly, and the oocyte development is a continuous, asynchronous process, until mature stage, when the first batch of eggs is due to be released.

Dipper & Pullin (1979) have described that the completion development of the yolk forms a homogeneous mass, but there are exceptions, for example, in *Carassius auratus*, the yolk of ripe eggs remains granular in appearance with no formation of a homogeneous mass (Yamamoto and Yamazaki, 1961). In our investigation, this does not taken place, and the completion development was occurred just prior to spawning.

The testes of *P. anomala* belongs to the radial type with lobules converging

on the dorso-medial sperm duct and are similar to those of most teleosts (Cyrus and Blaber, 1984).

The sperm development of *P. anomala* also followed the pattern of development of other teleosts, and the distribution of the spermatogonia in the testis conforms to that of the "unrestricted spermatogonial testis-type" (Grier, 1981). Spermatogonia are not restricted to the tip of the lobules, but are found along their length.

In our studies, many ripe testes are found, but none of them are spent. Spent testes with some residual sperm (Davis, 1977) are usually larger and more irregular in texture than those of immature testes (Cyrus and Blaber, 1984). In addition, spent testes show the following histological peculiarities: (a) presence of a large number of migrating cells structurally and tinctorially different from all other cell types, and (b) the beginning of pycnotic degeneration or remnant primary spermatocytes and later generations of secondary spermatogonia (Ahsan, 1966). None of these features are noted in the histological sections of *P. anomala* testes. Thus, no information is available on the texture of spent testes.

The events described in the process of gonad maturation fit closely with seasonal changes in both GSI and macroscopic appearance of the gonads. Immature (maturity stage A, B) ovaries occur before March. In this period, GSI values are very low. The ovaries are small, slender, and no visible oocytes. Most of the oocytes are at perinucleolus stage and yolk vesicle stage. Some of the ovaries of adult might develop into the early yolk stage.

From March entering the spawning season, the oocytes grow very rapidly, ovaries examined are mostly at yolk stage and some develop into the migratory nucleus stage and ripe stage. Due to the presence of large maturing and matured oocytes, the ovaries are enlarged, yellowish or pinkish in color and granulated. As the percentage of fish in this maturity stage (stage C, D) increased from March to May, mean GSI values increase and reach its hightest point, then remain fairly constant through August.

By the end of August most of fish have spawned, the mean egg diameter decrease, and the oocytes are mostly converted into resting stage, thus, GSI are declined to low values.

The testes have similar changes. Before February, the testes are small, thread-like and grayish in color. Immature (stage A) testes are seen in this period, where the cells are mostly at spermatogonia and early spermatocytes stage, and mean GSI values are very low. After March, the maturation proceeds very rapidly, the testes are large in size, whitish in colour, with some heavy vascularization in the inside surface of them. The sections are mostly at late spermatocytes or spermatid stage, and some developed into spermatozoa stage. Due to the presence of maturing and matured testes, mean GSI values increase suddenly and reach a maximum level in late April.

By the end of August, most of fish have spawned, thus, cells are mostly transformed into resting or recovering stages, and mean GSI values decrease to their low levels again.

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瓜子鯧生殖周期暨生殖腺組織學之研究

王世斌　陳哲聰

本研究探討瓜子鯧之生殖週期，生殖腺細胞組織外觀及生殖腺指數之季節變化情形。將卵細胞之發育過程區分爲九個階段，精細胞區分爲五個階段。

卵細胞之發育初期爲連續，非同時發生型，直到產卵前（D階段），即將產出之一羣成爲同時成熟發育型；而精細胞之發育情形則類似於一般硬骨魚類。

生殖腺組織之季節變化與生殖腺指數及其外觀之季節變化具有極爲密切之相關性。