Annual Reproductive Cycle and Spawning Characteristics of the Female Kichiji Rockfish *Sebastolobus macrochir*

Yasunori Koya, Tomonori Hamatsu, and Takahiro Matsubara
Hokkaido National Fisheries Research Institute, Fisheries Agency of Japan, Katsurakoi, Kushiro, Hokkaido 085, Japan

(Received June 24, 1994)

The ovarian morphology, annual reproductive cycle and spawning characteristics of the kichiji rockfish *Sebastolobus macrochir* collected from the Pacific coast of southeastern Hokkaido, was examined on the basis of histological observation. The ovaries of this fish are categorized as cystovarian type. The ovarian parenchyma, stroma, and blood vessels run longitudinally through the center of each ovary, suggesting a specialized structure for formation of gelatinous egg masses.

The female kichiji rockfish begins vitellogenesis from August to October, and continues yolk accumulation until March. Post-ovulatory follicles or oocytes at final maturation stage were observed in March and April, suggesting that spawning was carried out mainly in this period. During the spawning season, there were two clutches of developing oocyte, or post-ovulatory follicle and one clutch of oocyte in the ovary. Therefore, females seem to spawn twice within a single spawning season. Fish which finished spawning rapidly form yolk vesicles for the next reproduction, and maintain a resting condition for several months.

Gonad somatic index (GSI) corresponds clearly to ovarian maturity, and could be divided into immature (GSI < 2), spent (2 < GSI < 3), and vitellogenic or maturation (GSI > 3) during the spawning season. This indicates that ovarian maturity can be evaluated on the basis of GSI. By this method, the minimum maturation size of the female kichiji rockfish was estimated to be 151 to 175 mm in standard length.

Key words: rockfish, reproductive cycle, oogenesis, ovary, maturity, spawning

Materials and Methods

Fish
The kichiji rockfish used in the present study were caught by bottom trawl net on the Pacific coast of eastern Hokkaido between December 1992 and October 1993. A total of 364 females were measured for standard length (length between the tip of the upper jaw and base of the caudal fin), body weight (BW) and gonadal weight (GW). The gonad somatic index (GSI) was calculated by the expression $GSI = 100 \times GW / BW$.

Histology
For histological observation, the ovaries of 60 females were removed, fixed in Bouin’s solution, embedded in paraffin, sectioned at 5 μm, and stained with Derafield’s haematoxylin and eosin. To identify yolk vesicles, some of the sections were treated with periodic acid Schiff (PAS) reagent.

Measurement of Oocyte Diameter
For measurement of oocyte diameter, part of the ovarian tissue was fixed with 10% formalin. Then, each oocyte was isolated and the diameter was measured by a profile projector. Since the oocytes prior to or during final maturation had a long globular shape, the long axis was measured. The diameter of fresh oocytes was also measured to
indicate the range of live oocyte size at each development stage.

Results

General Anatomy of the Ovary
The paired ovaries of the kichiji rockfish are nearly equal in size and are located in the posterio-dorsal part of the abdominal cavity (Fig. 1A). Each ovarian lobe fuses caudally following a united oviduct. Figure 1B shows a cross section of the mid-ovarian lobe during the spawning season. Ovarian stroma and blood vessels run longitudinally through the center of each ovary (Fig. 1B). Each ovarian follicle projects radially from the central stroma. The ovarian stroma are connected to the ovarian wall only at the rostral part of the ovarian lobe. Therefore, the ovarian cavity situated between the surrounding ovarian wall and ovarian follicles radiates in all directions (Fig. 1B, 6A). The inner surface of the ovarian wall facing the ovarian cavity is covered with a simple columnar epithelial layer (Fig. 1C). There is a gelatinous substance in the ovarian cavity during the spawning season. The gelatinous substance was divided into two distinct layers based on its stainability with haematoxylin. The outer layer consisted of fibrous material which was stained faintly with haematoxylin, and the inner layer was a homogeneous material which was stained deeply with haematoxylin (Fig. 1C). Both layers were showed PAS-positive reaction (Fig. 1D), suggesting that these substances contained polysaccharide.

Histology of Developing Oocytes
The morphological changes of the developing oocytes of kichiji rockfish were divided into eight stages following the classification of Yamamoto.4
1) Early peri-nucleolus stage (Fig. 2A): Oocytes are 40 to 140 μm in diameter and the ooplasm is stained deeply by haematoxylin. Chromonemata are scattered throughout the nucleus, and several nucleoli are distributed along the nuclear membrane.
2) Late peri-nucleolus stage (Fig. 2B): Oocytes are 110 to 200 μm in diameter. The ooplasm becomes slightly less stainable by haematoxylin compared to the early peri-nucleolus stage. Yolk nucleus is often visible in ooplasm. Oil droplet-like vacuoles appear in the ooplasm.
3) Yolk vesicle stage (Fig. 2C, D): Oocytes are 140 to 300 μm in diameter. The yolk vesicles are stained by eosin and PAS-positive reactions appear in the ooplasm.
4) Early yolk globule stage (Fig. 2E, F): Oocytes are 240 to 360 μm in diameter. Yolk globules stained by eosin appear and are gradually accumulated in ooplasm. The yolk vesicles are scattered throughout the ooplasm.
5) Late yolk globule stage (Fig. 2G, H): Oocytes are 360 to 500 μm in diameter (550 to 700 μm when fresh), easily visible to the naked eye. The yolk globules fill the entire ooplasm, and the yolk vesicles line the cortical part of the ooplasm. The oil droplet-like vacuoles merge with each other to form several larger vacuoles.
6) Migratory nucleus stage (Fig. 2I): Oocytes become long and globular in shape, and have a long axis of 400 to 750 μm (650 to 950 μm when fresh). The germinal vesicle migrates to the cortical part of the ooplasm. The yolk globules begin to fuse to each other. A large vacuole which seems to be an oil droplet is visible in the center of the ooplasm.
7) Maturation stage (Fig. 2J): Oocytes have a long axis of 640 to 760 μm (800 to 1100 μm when fresh). The germi-

Fig. 1. Ovary of the kichiji rockfish.

nal vesicle breaks down. The yolk globules fuse to each other, and oocytes become transparent.

8) Ripe egg stage: The ovulated eggs are colorless and transparent. The long and short axis of the eggs fixed with 10% formalin are about 1300 µm and 850 µm, respectively. The ovulated eggs are maintained in the ovarian cavity, embedded in the gelatinous substance.

**Maturity of the Ovary**

Ovarian maturity of kichiji rockfish was divided into four phases as follows on the basis of the frequency of each oocyte stage.

I) Immature phase: Ovarian follicles consist of oocytes at the stage prior to the yolk vesicle stage. Distinct post-ovulatory follicles are not observed in the ovary.

II) Vitellogenic phase: Clutches of developing oocytes are in the early or late yolk globule stage. Distinct post-ovulatory follicles are not observed in the ovary.

III) Maturation phase: The most developed clutch of oocytes is in the migratory nucleus stage, maturation stage, or ripe egg stage. Post-ovulatory follicles are often observed.

IV) Spent phase: Post-ovulatory follicles exist in the ovary. Ovarian follicles consist of oocytes at the pre-vitellogenic stage. Oocytes at the late yolk globule stage or migratory nucleus stage, which seem to be the residue of spawned clutches, are infrequently observed.

<table>
<thead>
<tr>
<th>Table 1. Appearance of each ovarian maturity in the female kichiji rockfish sampled each month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
</tr>
<tr>
<td>Vitellogenic</td>
</tr>
<tr>
<td>Maturation</td>
</tr>
<tr>
<td>Spent</td>
</tr>
</tbody>
</table>

Numerals indicate the numbers of fish.

**Seasonal Changes in Ovarian Maturity**

Ovarian maturity in each month is shown in Table 1. In December and February, the ovarian condition was immature phase or vitellogenic phase. In March, in addition to the immature and vitellogenic phase, females in the maturation phase and spent phase appeared. Therefore, some fish have already finished spawning by late March. In April, all fish observed were maturation phase, suggesting this to be the spawning season. In May, six of eight were spent phase (migratory nucleus or late yolk globule stage oocytes in Table 2 were judged reserve fund oocytes), indicating the termination of spawning. Fish entering the vitellogenic phase appeared in August and October.

From August to February, the atresia of oocytes were often observed. These atresia occurred mostly on a part of vitellogenic oocytes.
Spawning Season and the Number of Spawning Times

The composition of oocyte stages in each month is shown in Table 2. The females having post-ovulatory follicles appeared from March to May, indicating that this period is the spawning season of this species. In April, several specimens had post-ovulatory follicles in spite of having maturing oocytes. This indicates that this species is a multiple spawner which spawns more than twice during a single spawning season. Furthermore, except for one specimen, all specimens had two clutches of developing oocytes with no post-ovulatory follicles. This suggests that this species spawns twice during a spawning season.

In order to confirm the above, the composition of the diameter of the developing oocyte was examined. The two distinct clutches of oocytes diameter were shown in three of thirteen individuals which had two clutches of developing oocytes. In the three specimens, two distinct clutches were divided at 750 μm diameter (Fig. 3). The numbers of larger oocytes and smaller oocytes were 167 and 172 (Fig. 3A), 116 and 95 (Fig. 3B), and 75 and 53 (Fig. 3C), at a ratio of about 1:1. This result strongly suggests that this species ovulates and spawns twice during a single spawning season.

Relation between Ovarian Maturity and Gonad Somatic Index

Gonad somatic indices (GSI) of each maturity stage are compared in March (Table 3). The results show that the GSI range of immature phase (0.54–0.76%), vitellogenic and maturation phase (3.33–5.44%), and the spent phase (2.21–2.97%) were clearly separated at between 2% and 3%. This indicates that it is possible to determine ovarian maturity from the GSI during the spawning season.

Female Maturation Size

Figure 4 shows the relationship between standard length and.

Table 2. Changes in stage composition of oocytes in the female kichiji rockfish

<table>
<thead>
<tr>
<th>Oocyte Stage</th>
<th>March '93</th>
<th>April '93</th>
<th>May '93</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-ovulatory follicle</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ovulated egg</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ripe egg</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Migratory nucleus</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Late yolk globule</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Early yolk globule</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Yolk vesicle</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Late peri-nucleolus</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Early peri-nucleolus</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Number of fish 1 1 3 1 1 2 1 1 4 5 1 1 5 1 2 2 3 1 1 1 2 1 7 1 4 1
Sampling date 2, Dec. '92 17, Feb. '93 23, Mar. '93 22, Apr. '93 31, May '93 3, Aug. '93 14, Oct. '93

Symbols indicate the occurrence of oocytes, categorized as few (no mark), some (+), and many (++).
Fig. 4. Standard length and gonad somatic index (GSI) of females in the spawning season.
GSI was divided into three groups; immature (less than 2), spent (more than 2 to less than 3), and mature (more than 3). Closed symbols indicate the specimens whose maturity was confirmed histologically. Samples were obtained in March (A), and in April (B).

Fig. 5. Female maturation rate for different standard lengths in the spawning season.
Numerals in parentheses indicate the number of fish examined.

Fig. 6. Schematic illustration of the formation of gelatinous egg masses in the kichiji rockfish.
Ovary at the pre-ovulatory period (A), post-ovulatory period (B), spawning (C), and gelatinous egg mass spawned (D).
fish in order to clarify the process of ovarian development, which we determined was as follows.

Female kichiji rockfish seem to begin vitellogenesis from August to October and continue accumulation of yolk globules for five to seven months. During this period, oocyte diameter increases from 240 μm to 500 μm. From March to April, oocytes at final maturation, ovulated eggs or post-ovulatory follicles were often observed. In May, post-ovulatory follicles were observed, but not vitellogenic oocytes, and the post-spawning females appeared in large numbers. Therefore, the spawning season of this species seems to be mainly March and April. Fish which finished spawning seemed to rapidly form yolk vesicles for the next reproductive phase, and to maintain a resting condition for several months until the beginning of vitellogenesis.

The spawning season of kichiji rockfish has been estimated as being from March to June based on field sampling data of egg masses, from January to April based on morphological changes in the ovaries and the oocytes, or from February to May based on histological examination of the testes. The present results agree approximately with all previous results.

**Number of Spawning Times**

The kichiji rockfish has been thought to be a single spawner based on the results of naked eye observation of the mature ovary. However, the present study strongly suggests that this species is a multiple spawner. The vitellogenic oocytes developed almost synchronously until the late yolk globule stage. When the leading clutch oocytes began final maturation, two clutches which consisted of oocytes at the late yolk globule stage and the maturation stage were clearly distinguishable. Furthermore, the ratio of both clutches was about 1:1 in three specimens. These results strongly suggest that this species spawns twice during a single spawning season.

The spawning interval could not be clarified in the present study. However, it is estimated that the interval between spawning was less than one month, because spent females appeared late in March, and the pre-spawning females and those which had completed the first spawning seemed to rapidly form yolkk vesicles for the next reproductive phase. Therefore, the spawning season of this species seems to be mainly March and April. Fish which finished spawning seemed to rapidly form yolk vesicles for the next reproductive phase, and to maintain a resting condition for several months until the beginning of vitellogenesis.

The spawning season of kichiji rockfish has been estimated as being from March to June based on field sampling data of egg masses, from January to April based on morphological changes in the ovaries and the oocytes, or from February to May based on histological examination of the testes. The present results agree approximately with all previous results.

**Acknowledgements**

The authors wish to thank Dr. K. Watanabe, Dr. T. Wada, and Mr. K. Yabuki, Hokkaido National Fisheries Research Institute, for their useful suggestions. For technical help, we are greatly indebted to the staff of the Demersal Fish Resource Laboratory and Pelagic Fish Resource Laboratory, Hokkaido National Fisheries Research Institute.

**References**


