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Reproductive Cycles of *Moroco oxycephalus* and *M. lagowskii* in Korea

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We investigated the reproductive cycles of two freshwater fishes, *Moroco oxycephalus* and *M. lagowskii*, in Korea. Seasonal changes in gonadosomatic index (GSI) and gonads were investigated histologically from April 1998 to April 1999. The reproductive cycles of two species were not shown any differences. The reproductive cycle can be divided into 5 phases : phase I (spent phase), phase II (immature phase), phase III (early developing phase), phase IV (late developing phase), and phase V (ripe phase). In phase I, the gonads of two species began to lose distinctly their weights from mid April, and reached the lowest GSI in late July (phase II). In September, the GSI values of testis and ovary increased very slowly (phase III) and gonadal developments rested during the winter season (phase IV). In March, the GSI values of *M. oxycephalus* and *M. lagowskii* began to increase, and reached the maximum in April (phase V). From the cyclic changes in the GSI and histological analyses, the spawning period was between mid April and mid May.

Key words : *Moroco oxycephalus*, *M. lagowskii*, reproductive cycles, gonadosomatic index (GSI), histological analysis

Introduction

The Chinese minnow, *Moroco oxycephalus* and amur minnow, *M. lagowskii* (Pisces : Cyprinidae) are freshwater fishes which are found in East Asian countries such as Korea, Japan and China (Qingtai and Baoshan, 1987; Kawanabe and Mizuno, 1989; Kim, 1997).

Most freshwater fishes have their own special characteristics of reproductive patterns, and breeding habits. The reproductive biologies inform us about the role of reproduction on aspects of the survival and evolution of species (Lagler *et al.*, 1977) and they can be applied to control biological productivities. Therefore, previously there have been many studies on reproductive cycles and histomorphological changes of gonads (Yamamoto, 1955; Honma and Tamura, 1961; Malhotra, 1970; Hayashi, 1971, 1972; Van den Hurk and Peute, 1979; Love and Westphal, 1981; Hibiya, 1982; Heins and Rabito, 1986; Heins and Baker, 1989; Heins *et al.*, 1992; Chung and Kim, 1994; An, 1995; Fernandez-Delgado and Herrera, 1995; Lee *et al.*, 1995; Taylor and Burr, 1997), mating behaviors and strategic efforts in fish species (Platania and Altenbach, 1998).

Although various aspects of phenetic and genetic differences as well as the geographic distributions of these two sibling species have been reported by various authors (Uchida, 1939; Aoyagi, 1957; Chyung, 1977; Min and Yang, 1986; Yang and Min, 1988, 1989; Jeon, 1989; Kim, 1997), very little information is available on the reproductive biology of these species. With these kept in mind, we investigated the reproductive cycles of *M. oxycephalus* and *M. lagowskii* in Korea. To understand the patterns of gonadal

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development and breeding season, seasonal changes in gonadosomatic index (GSI) and histological analyses on gonads were studied from April 1998 to April 1999.

Materials and Methods

Study specimens were collected using fish-pots and fish-traps from one site of Yangpyong-gun, Kyonggi-do and two sites of Kosong-gun, Kangwon-do, Korea from April 1998 to April 1999. Collection was done monthly or biweekly except May 1998. No collection was made in January 1999 due to ice cover (Table 1). Water temperature was measured on each visit using a standard thermometer. All fish specimens were transported on dry ice (−70°C) to the lab. Since gonad size is dependent upon body size (DeVlaming *et al.*, 1982; Erickson *et al.*, 1985a), 70~110 mm sized fish samples were used to measure gonadosomatic index (GSI) according to the formula:

$$\text{GSI}(\%) = \text{Wet Weight of Gonad (g)} / \text{Wet Weight of Body (g)} \times 100$$

Table 1. Number of specimens and collection date of *M. oxycephalus* and *M. lagowskii* for gonadosomatic index (GSI) analyses

Collection date	*M. oxycephalus*	*M. lagowskii*	
	Mt. Yongmun*	Br. Changshin -5*	Mt. Noin*
	N (♀ : ♂)	N (♀ : ♂)	N (♀ : ♂)
Apr. 18, 1998	6 (4 : 2)	–	8 (7 : 1)
May 1, 1998	9 (6 : 3)	10 (8 : 2)	8 (4 : 4)
May 18, 1998	11 (9 : 2)	8 (3 : 5)	18 (13 : 5)
May 31, 1998	11 (10 : 1)	6 (3 : 3)	3 (2 : 1)
June 14, 1998	28 (19 : 9)	13 (9 : 4)	27 (19 : 8)
July 8, 1998	12 (9 : 3)	14 (13 : 1)	16 (4 : 12)
July 30, 1998	20 (19 : 1)	17 (7 : 10)	17 (7 : 10)
Sept. 4, 1998	29 (12 : 17)	14 (10 : 4)	43 (32 : 11)
Oct. 9, 1998	24 (7 : 17)	24 (13 : 11)	31 (22 : 9)
Oct. 22, 1998	28 (13 : 15)	17 (5 : 12)	29 (17 : 12)
Nov. 11, 1998	32 (22 : 10)	15 (9 : 6)	33 (22 : 11)
Dec. 1, 1998	22 (17 : 5)	10 (2 : 8)	23 (12 : 11)
Feb. 10, 1999	10 (3 : 7)	12 (6 : 6)	27 (14 : 13)
Mar. 6, 1999	12 (5 : 7)	32 (18 : 14)	35 (15 : 20)
Mar. 26, 1999	23 (12 : 11)	24 (8 : 16)	12 (8 : 4)
Apr. 19, 1999	9 (7 : 2)	18 (11 : 7)	20 (12 : 8)

* Mt. Yongmun : Stream of Mountain Yongmun, Okchon-myon, Yangpyong-gun, Kyonggi-do, Korea
Br. Changshin-5 : Bridge Changshin-5, Kansong-up, Kosong-gun, Kangwon-do, Korea
Mt. Noin : Stream of Mountain Noin, Sanbuk-ri, Kojin-up, Kosong-gun, Kangwon-do, Korea

Table 2. Number of specimens and collection date of *M. oxycephalus* and *M. lagowskii* for histological analyses

Collection date	*M. oxycephalus*	*M. lagowskii*	
	Mt. Yongmun	Br. Changshin -5	Mt. Noin
	N (♀ : ♂)	N (♀ : ♂)	N (♀ : ♂)
Apr. 18, 1998	4 (2 : 2)	–	4 (3 : 1)
May 1, 1998	3 (2 : 1)	4 (2 : 2)	4 (2 : 2)
May 18, 1998	5 (3 : 2)	3 (1 : 2)	4 (2 : 2)
June 14, 1998	4 (2 : 2)	3 (2 : 1)	3 (1 : 2)
July 30, 1998	3 (2 : 1)	4 (1 : 3)	4 (2 : 2)
Oct. 22, 1998	4 (2 : 2)	4 (2 : 2)	5 (2 : 3)
Dec. 1, 1998	4 (2 : 2)	4 (2 : 2)	4 (2 : 2)
Mar. 26, 1999	6 (3 : 3)	5 (3 : 2)	5 (2 : 3)
Apr. 19, 1999	5 (3 : 2)	4 (2 : 2)	4 (2 : 2)

After GSI analysis, randomly selected ovaries and testes (Table 2) were cut into small piece and preserved in Bouin's fixative (Bouin, 1897). Tissues were sectioned by the usual paraffin method at 5~6 μm thickness and stained with Harris' Hematoxylin-eosin for histological studies.

Results

The general structure of gonads

The general morphological structure of gonads in *M. oxycephalus* and *M. lagowskii* were very similar and it was hard to distinguish their differences by morphological structure. The ovaries of *M. oxycephalus* and *M. lagowskii* were paired and elongated bodies, each suspended by a mesovarium from the ventral margins of the air bladder. As maturation progresses, their ovaries were yellowish orange in color because the oocytes were accumulated with numerous yolks. Their ovaries were round in shape. The ovaries of two species were cystovarian type which were connected with oviducts, and oviducts connected from each bilateral ovary to lead to the genital pore (Hibiya, 1982).

The testes of two species were paired and elongated structure lying on either side in the posterior half of the abdominal cavity central to the kidneys. Each had a spermatic duct running throughout its length and the two ducts united posteriorly to open by a common aperture. The testes were fused with one another along their whole length. The pigments were absent from the surface of the testis. During the breeding season, the testis was milky-white in color, and looking as finger-like projections. *M. oxyceph-*

alus and *M. lagowskii* were lobule types and their spermatogonial distributions were restricted (Hibiya, 1982).

Reproductive cycles of *M. oxycephalus* and *M. lagowskii*

There were no differences in the seasonal changes of the histology of the gonads between *M. oxycephalus* and *M. lagowskii*. The oocytes of two species, on the basis of histomorphological changes exhibited in the nucleus and cytoplasm, and the formation of yolk eight separate stages have been distinguished to categorize the developing oocyte (Honma and Tamura, 1961; Rastogi and Saxena, 1968; Malhotra, 1970; Hayashi, 1972; Goto, 1978; Malhotra *et al.*, 1978; Hibiya, 1982), *i. e.* : chromatin nucleolus stage (stage i), peri-nucleolus stage (stage ii), yolk vesicle stage (stage iii), yolk globule stage (stage iv), migratory nucleus stage (stage v), prematuration stage (stage vi), ripe egg stage (stage vii) and discharged follicle stage (stage viii) (Fig. 3). On the other hands, germ cells in the testis were classified in five cell types, according to the degree of chromatin condensation, cell sizes and their shapes, such as spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid, and spermatozoon (Fig. 4). The GSI values of female of *M. oxycephalus* and *M. lagowskii* were always higher than that of male and the GSI cycle patterns of two species were very similar (Fig. 1, Table 3). On the basis of the GSI and the gross morphology as well as the size of germ cells from the preserved gonads, gonadal phases of *M. oxycephalus* and *M. lagowskii* could be classified successively into five phases, and these phases showed a periodicity. The criteria used in defining the categories are follows.

Phase I: Spent phase (mid April to June)

The GSI values in females and males of two species decreased distinctly from mid April when spawning occur, and reached the lowest values between June and July in *M. lagowskii*, and between late July and September in *M. oxycephalus* (Figs. 1, 2). The GSI values during phase I were the most variable than any other phases (Fig. 1, Table 3).

In females, the ovary revealed the oocytes in various stages, particularly, undischarged oocytes in the stage ii, and discharged follicles in the stage viii were remained in the ovarian lobules (Figs. 3A, B). The ovaries of two species were

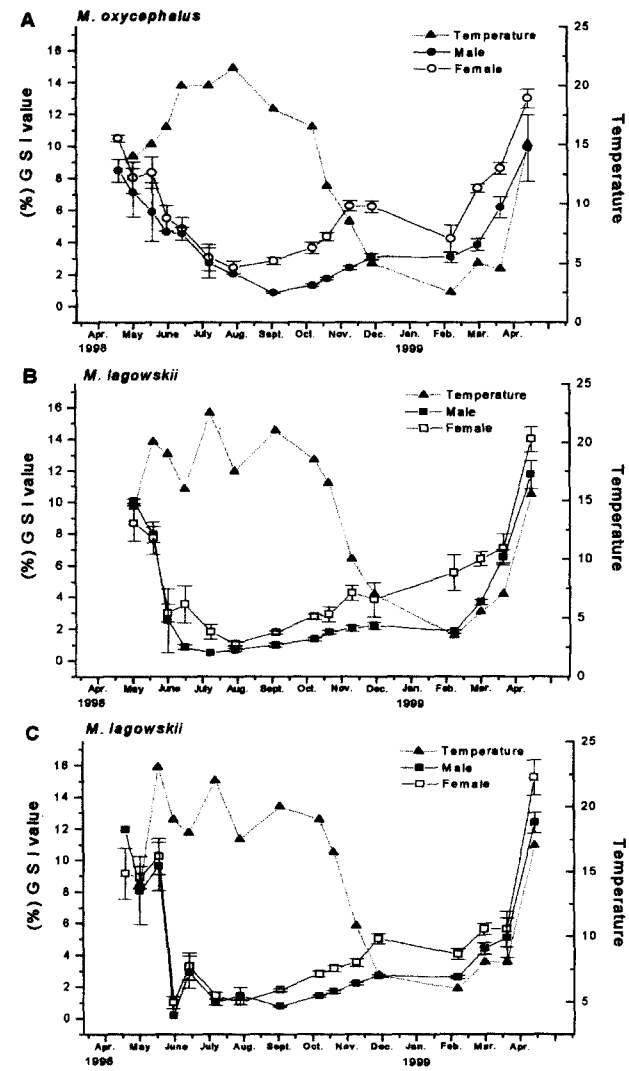

Fig. 1. GSI of *Moroco oxycephalus* and *M. lagowskii* in Korea. A: Stream of Mountain Yongmun, Okchon-myon, Yangpyong-gun, Kyonggi-do, Korea, B: Bridge Changshin-5, Kansong-up, Kosong-gun, Kangwon-do, Korea, C: Stream of Mountain Noin, Sanbuk-ri, Kojin-up, Kosong-gun, Kangwon-do, Korea.

filled with approximately 50% of stage ii and 39% of stage viii oocytes (Fig. 5). In males, all of the germ cells in testis were still stained with Hematoxylin. A few of undischarged spermatozoa and spermatids were found in each cyst of testicular louble (Fig. 4A).

Phase II: Immature phase (July to September)

The GSI values in females and males of *M. oxycephalus* and *M. lagowskii* were the lowest from July to September (Figs. 1, 2, Table 3).

Table 3. Collection date and gonadosomatic index (GSI) of adult males and females of *M. oxycephalus* and *M. lagowskii* examined in this study

Collection date	*M. oxycephalus*		*M. lagowskii*			
	Mt. Yongmun		Br. Changshin-5		Mt. Noin	
	♀ GSI (M±SE)*	♂ GSI (M±SE)	♀ GSI (M±SE)	♂ GSI (M±SE)	♀ GSI (M±SE)	♂ GSI (M±SE)
Apr. 18, 1998	10.05~10.90 (10.49±0.22)	7.76~9.17 (8.47±0.71)	–	–	3.62~15.10 (9.15±1.63)	11.94
May 1, 1998	5.56~11.30 (8.32±0.88)	4.84~9.95 (7.11±1.50)	4.22~12.17 (10.08±0.14)	9.94~10.21 (8.68±1.12)	7.14~10.58 (8.92±0.70)	2.10~11.97 (8.09±2.18)
May 18, 1998	4.32~13.38 (8.34±0.98)	4.07~7.71 (5.89±1.82)	5.81~9.38 (7.74±1.04)	7.07~ 9.89 (7.95±0.51)	5.71~17.89 (10.25±1.16)	6.54~15.03 (9.62±1.54)
May 31, 1998	2.71~ 9.51 (5.50±0.80)	4.64	2.42~4.12 (3.01±0.56)	0.29~ 6.55 (2.54±2.01)	0.62~ 1.39 (1.01±0.39)	0.19
June 14, 1998	2.21~10.59 (4.82±0.60)	2.16~ 6.07 (4.55±0.40)	0.54~11.02 (3.58±1.16)	0.46~1.16 (0.89±0.16)	0.56~12.46 (3.28±0.85)	0.49~6.62 (2.94±1.00)
July 8, 1998	1.21~8.79 (3.05±0.82)	0.83~ 3.79 (2.72±0.95)	0.71~5.08 (1.82±0.46)	0.49	1.07~1.96 (1.44±0.22)	0.64~3.00 (1.03±0.19)
July 30, 1998	1.06~6.59 (2.91±0.49)	2.02	0.42~1.37 (1.08±0.12)	0.26~ 1.29 (0.69±0.10)	0.43~1.61 (1.04±0.15)	0.36~4.78 (1.43±0.54)
Sept. 4, 1998	1.57~4.14 (2.86±0.21)	0.46~2.62 (0.96±0.11)	1.26~2.95 (1.80±0.09)	0.76~ 1.20 (1.00±0.12)	1.02~2.66 (1.80±0.07)	0.61~1.03 (0.78±0.03)
Oct. 9, 1998	2.39~5.20 (3.66±0.35)	0.89~2.51 (1.31±0.09)	2.50~ 3.92 (2.78±0.18)	1.01~ 1.83 (1.35±0.08)	1.33~4.80 (2.80±0.17)	1.12~1.69 (1.42±0.07)
Oct. 22, 1998	3.00~5.45 (4.36±0.25)	1.29~2.35 (1.74±0.08)	1.38~4.26 (2.93±0.47)	1.26~ 2.36 (1.81±0.10)	1.99~4.59 (3.16±0.19)	1.12~2.07 (1.70±0.10)
Nov. 11, 1998	4.17~8.34 (6.18±0.30)	1.73~2.94 (2.41±0.13)	2.46~7.53 (4.26±0.49)	1.30~ 2.48 (2.04±0.17)	1.64~5.14 (3.51±0.25)	1.68~2.50 (2.20±0.08)
Dec. 1, 1998	4.44~8.89 (6.11±0.32)	2.48~3.54 (3.11±0.19)	2.77~ 4.94 (3.85±1.09)	1.86~ 3.60 (2.22±0.21)	2.81~6.39 (5.02±0.31)	2.34~3.27 (2.69±0.09)
Feb. 10, 1999	2.51~5.11 (4.21±0.85)	1.49~4.01 (3.08±0.33)	1.90~8.87 (5.54±1.14)	1.52~4.39 (1.85±0.12)	1.90~6.24 (4.03±0.34)	2.14~3.56 (2.61±0.12)
Mar. 6, 1999	6.73~8.05 (7.37±0.26)	2.97~5.99 (3.85±0.38)	4.41~10.95 (6.41±0.45)	3.15~10.03 (3.69±0.16)	3.40~7.92 (5.62±0.37)	2.26~ 9.70 (4.39±0.37)
Mar. 26, 1999	6.96~10.84 (8.61±0.37)	3.23~9.58 (6.17±0.66)	3.82~10.38 (7.07±0.92)	3.52~9.49 (6.50±0.48)	1.21~10.72 (5.60±1.14)	2.90~7.76 (5.07±1.25)
Apr. 19, 1999	12.00~14.56 (12.96±0.60)	7.78~11.94 (9.86±2.08)	10.01~18.02 (13.99±0.79)	7.54~13.94 (11.73±0.89)	10.07~22.65 (15.22±1.11)	9.99~16.09 (12.38±0.65)

* Mean±standard error

In females, the ovarian loubles were empty, contracted and degenerated. There were only remaining follicles, degenerating previtellogenic oocytes and early vitellogenic oocytes in the ovarian loubles. Oocytes were composed of about 10% of stage i, 70% stage ii and 6% of stages iii and iv (Figs. 3C, 5). In males, the testes were thin, and had only spermatogonia. Testicular loubles were vacant compared with histological views of phase I, however, the spaces were filled with connective tissues and a few interstitial cells (Fig. 4B).

Phase III: Early developing phase (October to December)

The GSI values of females and males of two species gradually increased from October to December (Fig. 1). In females, the ovarian loubles of the ovary were filled with previtellogenic oocytes and a few vitellogenic oocytes (Figs. 3D, 5). In males, a number of spermatogonia and spermatocytes were found in the cyst of the testicular louble (Fig. 4C).

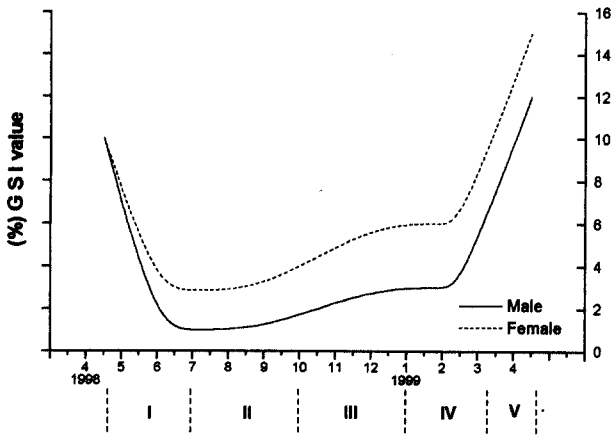

Fig. 2. Reproductive cycles of *M. oxycephalus* and *M. lagowskii* in Korea. I: Phase I (spent phase), II: Phase II (immature phase), III: Phase III (early developing phase), IV: Phase IV (late developing phase), V: Phase V (ripe phase).

Phase IV: Late developing phase (January to early March)

The GSI values of female and male of two species gradually increased between February and March (Figs. 1, 2). In females, the ovarian loubles were filled with oocytes in the stage iii, iv, v, and vi (Figs. 3E, 5). In males, the testicular loubles of the testis were filled with spermatocytes, numerous spermatids during spermatogenesis in each cyst (Fig. 4D).

Phase V: Ripe phase (late March to mid April)

The GSI values of *M. oxycephalus* and *M. lagowskii* were rapidly increased in March and reached the maximum in mid April 1999 when water temperatures increasing (Figs. 1, 2, Table 3). In females, most oocytes completed vitellogenesis during this phase. Approximately 51% of oocytes were ripe eggs (stage vii), and percentages of oocytes in the stage iv, v, vi were about 1, 3 and 5%, respectively, in the ovary (Figs. 3F, 5). In males, a few spermatocytes lying at the periphery of the cysts were also visible near the spermatids and numerous spermatozoa in the cysts of the testicular louble. The cysts in the testicular loubles were filled with spermatids, and spermatozoa (Fig. 4E).

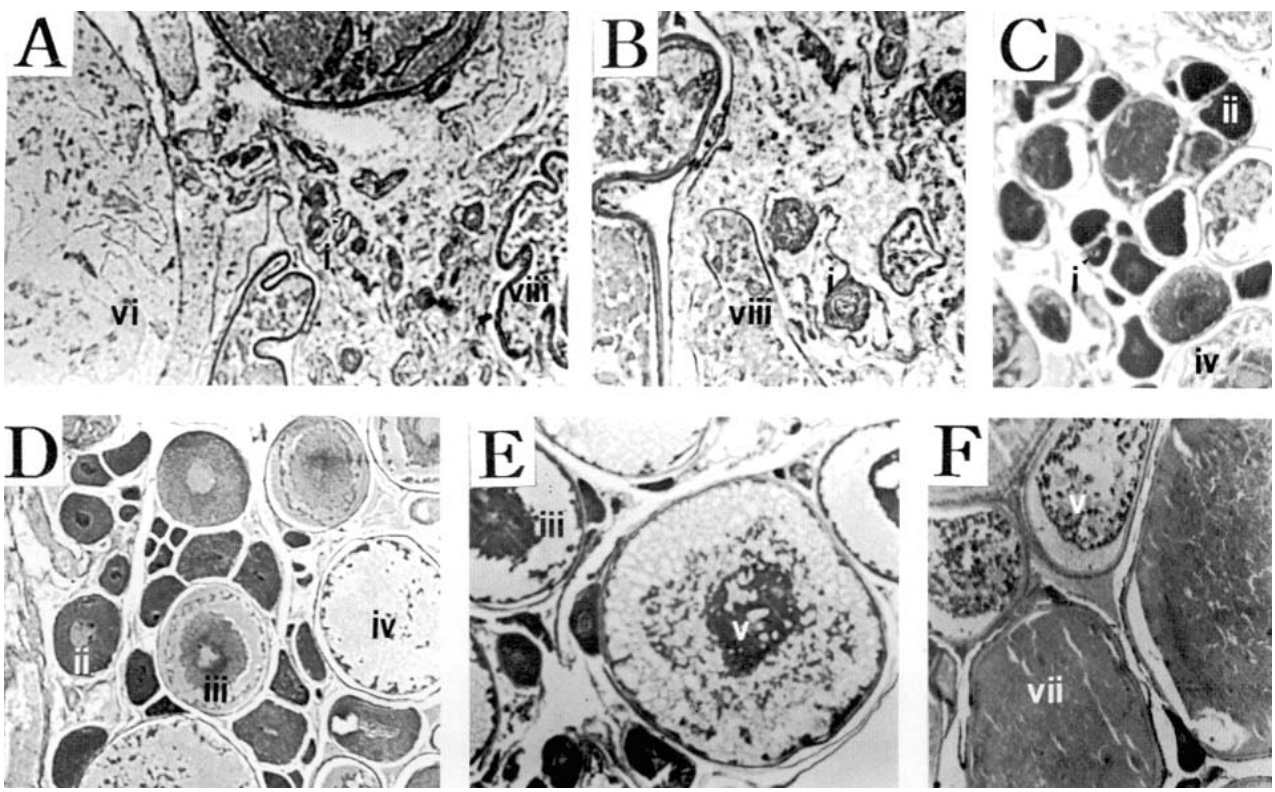

Fig. 3. Photomicrographs of cross sections of ovaries of *M. oxycephalus* and *M. lagowskii*. A: Phase I, showing stage i, vi, viii oocytes. ×100, B: same views of A. ×500, C: Phase II, showing stage i, ii, iv oocytes. ×250, D: Phase III, showing stage ii, iii, iv oocytes. ×100, E: Phase IV, showing stage iii, v oocytes. ×250, F: In phase V, showing stage v, vii oocytes. ×100.

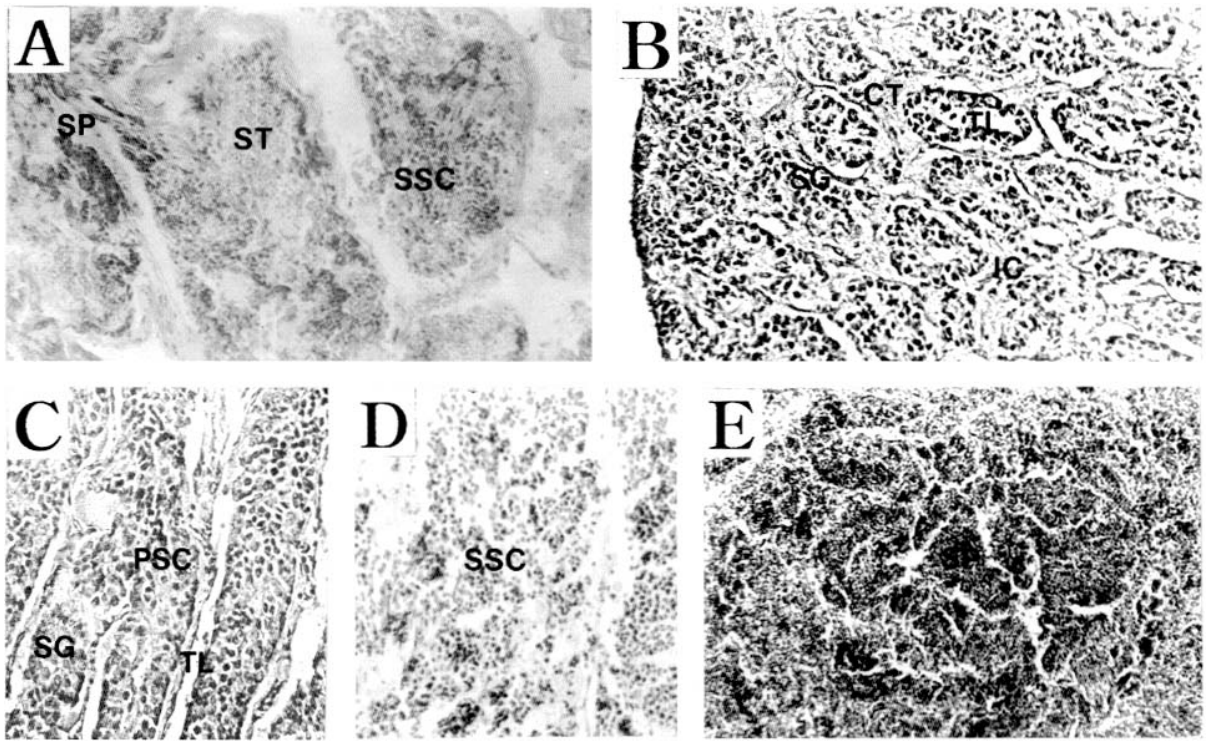

Fig. 4. Photomicrographs of cross sections of *M. oxycephalus* and *M. lagowskii*. A: Phase I. ×400, B: Phase II. 400, C: Phase III. 400, D: Phase IV. ×400, E: Phase V. ×400. SG: spermatogonium, PSC: primary spermatocyte, SSC: secondary spermatocyte, ST: spermatid, SP: spermatozoon, CT: connective tissue, IC: interstitial cell, TL: testicular louble.

Discussion

Monthly changes in the GSI were not distinctly different between the two species during the period of April 1998 to April 1999. The reproductive cycles of *M. oxycephalus* and *M. lagowskii* can be divided into 5 phases by several authors (Baglin, 1982; Erickson *et al.*, 1985b): phase I (spent phase), phase II (immature phase), phase III (early developing phase), phase IV (late developing phase) and phase V (ripe phase). Each phases were characterized by their own histological features as well as GSI values. Previtellogenic oocytes in stage i, ii on the ovarian lamella and spermatogonia, primary spermatocytes were observed throughout the year. Discharged follicles and peri-nucleolus oocytes dominated in phase I, previtellogenic oocytes and spermatogonia dominated in phase II. In phase III and phase IV, most of the oocytes were in vitellogenic stages of iii, iv, v, while most of germ cells were secondary spermatocytes and spermatids. Furthermore, ripe eggs and spermatozoa commanded overwhelming majorities in phase V.

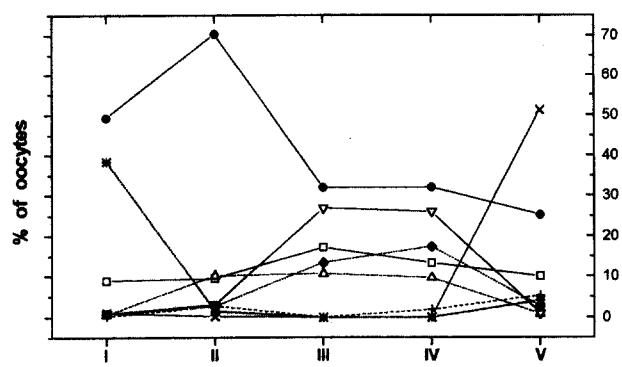

Fig. 5. Seasonal fluctuations in the percental occurrence of difference of different stages of oocytes of *M. oxycephalus* and *M. lagowskii*. I: Phase I, II: Phase II, III: Phase III, IV: Phase IV, V: Phase V. Plot symbols of open square : stage i, solid circle : stage ii, open up-triangle : stage iii, open-down triangle : stage iv, solid diamond : stage v, solid cross (+) : stage vi, solid cross (x) : stage vii, solid star (*) : stage viii.

In our investigations of reproductive cycles, the gonads of two species had the two periods of gonadal developing phases, *i. e.*, phase III (early developing phase) and phase IV (late developing phase). In these phases, germ cells were immatured conditions at yolk loading stages iii, iv, v, and primary, secondary spermatocytes or spermatids, moreover, GSI values were lower than that of phase V (ripe phase). While in phase V, the gonadal weights of two species were the maximum, and the gonads were filled with ripe eggs, and spermatozoa. Therefore, it is presumed that they start breeding after the phase V is reached and flourish in mid April to mid May, during the period of phase I. In phase I, the GSI values decreased rapidly because of the release of eggs and spermatozoa. The GSI values are hightly variable due to the coexistence of early and late spawners at this phase.

To assure reproductive success within the numerous environments, teleost fishes have become adapted in a variety of ways of reproductive strategies (Wallace and Selman, 1981). The maximum GSI values of *M. oxycephalus* and *M. lagowskii* males were about 10~12% while 13~15% in females. In general, the GSI value of male is lower than that of female in fish species. The maximum GSI value of male of *Chromis motatus* (Lee and Lee, 1987) and Verasper variegatus (Kim *et al.*, 1998) were 6.1% and 1.7% respectively. These various GSI values in teleost fish species were considered as a results of different spawning behaviors (Billard, 1986). Suquet *et al.* (1994) suggest a hypothesis that fish species having low GSI in males spawn in couples, while fish species of high GSI in males spawn in shoals. *M. oxycephalus* and *M. lagowskii* reveal spawning behavior in shoals where several tens of males following up a female (Kawanabe and Mizuno, 1989). Accordingly, the high GSI values in males of two species regarded as selection of reproductive strategic efforts corresponding to the Suquet's hypothesis. Furthermore, Wallace and Selman (1981) referred a useful classification of ovarian types provided by Marza (1938) in their *dynamic aspects of oocyte growth* section. The ovarian spawning types were recognized by three basic patterns, *i. e.* : synchronous, group-synchronous and asynchronous. According to our histological investigations, *M. oxycephalus* and *M. lagowskii* were considered as group-synchronous types.

At least two populations with sizes of oocytes can be distinguished; a fairly synchronous population having larger oocytes and a more heterogeneous population having smaller oocytes from which the clutch is recruited. These recruitment phenomenon can be associated with an event occurring in a clutch of growing oocytes, particularly among teleosts which spawn several times during the breeding season (Wallace and Selman, 1981). Therefore, whether *M. oxycephalus* and *M. lagowskii* are multiple spawners or not, additional investigations should be carried out as Fernandez-Delgado and Herrera (1995), Taylor and Burr (1997) and Kim *et al.* (1998) have conducted.

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한국산 버들치와 버들개의 생식 주기에 관한 연구

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한국산 담수어류인 버들치와 버들개의 생식주기 연구를 위해 버들치 1개 지역, 버들개 2개 지역에서 1998년 4월부터 1999년 4월까지 채집된 암, 수 개체의 생식소중량지수(GSI: gonado-somatic index)의 변화 주기와 각 발달 시기별 생식소의 조직학적 연구를 수행하였다. 두 종은 거의 생식소 발달 단계상의 차이를 보이지 않아 이를 제1단계(방출기), 제2단계(미숙기), 제3단계(초기 발달기), 제4단계(후기 발달기), 그리고 제5단계(완숙기)의 5단계로 나눌 수 있었다. 1998년 4월 중반부터 6월까지의 제1단계 중, 두 종의 생식소는 현저한 무게감소를 보여 제2단계인 7월말에 이르러 최소값의 GSI를 기록하였다. 1998년 9월 이후 이후 두 종의 난소와 정소는 난자형성과정과 정자형성과정의 발달로 서서히 비대해져 GSI의 증가곡선을 보였으나 동절기인 12월 이후부터의 낮은 증가율로 인해 거의 일정한 상태의 GSI값이 1999년 2월까지 유지되었다(제4단계). 1999년 3월 이후의 본격적인 난황축적과정과 정자형성과정으로 인해 GSI는 다시 빠른 속도로 증가하여 4월 경 완전히 성숙된 난소와 정소가 되었다. 일년간 조사된 GSI값의 변화와 조직학적 결과로 확인된 버들치와 버들개의 산란기는 4월 중순~5월 중순이었다.