Korean J. Ecol. 28(4): 193~198, 2005

Developmental Characteristics of Eggs and Yolk Sac Larvae of Korean Striped Bitterling, *Acheilognathus yamatsutae* (Cyprinidae), Spawning in Mussels

Song, Ho-Bok* and Yeong-Mok Son[1]

Division of Life Sciences, College of Natural Sciences, Kangwon National University, Chuncheon, 200-701, Korea

[1]Department of Science Education, Seowon University, Cheongju 361-742, Korea

ABSTRACT: This study investigated the characteristics of the eggs and yolk sac larvae of Korean striped bitterling, *Acheilognathus yamatsutae*, spawned and grown in mussels. The number of eggs in the ovary was small (358±108 SD). The eggs were oval and large, and the formation of the perivitelline space was narrow. The eggs were hatched at only 41 hours after fertilization but the hatched larvae were underdeveloped. The development of yolk projection and minute tubercles on the skin surface was notable, along with the vividly moving tail in the hatched larvae. The yolk projection and minute tubercles were disappeared upon enhancement of the motor ability of the larvae was enhanced. The formation of eyes and body pigments of the larvae was relatively delayed in comparison with that of other cyprinid larvae. After completely consuming the yolks the larvae escaped from the mussel for free swimming and exogenous feeding.

Key words: *Acheilognathus yamatsutae*, Bitterling, Early life history, Egg and larva, Host mussel

INTRODUCTION

Cyprinid bitterlings are small-sized freshwater fish comprising 60 species worldwide, and are commonly distributed throughout East Asia such as China, Korea, Japan and Vietnam with one additional species also inhabiting Europe and Russia (Nelson 1994). The Korean striped bitterling, *Acheilognathus yamatsutae*, is a Korean endemic species inhabited in the middle reaches of rivers. Bitterlings spawn in the gills of freshwater mussels (Bivalvia: Unionidae), and the Unionidae is famous for interrelationship in that the mussels allow aquatic vertebrates such as fish to attach their glochidia on the bodies (Duyvene de Wit 1966, Zeal and Neves 1982).

The male bitterlings form their own territories around freshwater mussels in the spawning season to allure females, and the female bitterlings spawn in the gills of the mussels through the exhalant siphon by using a long ovipositor developed in the season. After the spawning is over, the males ejaculate sperm nearby the inhalant siphon of the mussels. The eggs are fertilized in the gills of the mussels (Wiepkema 1961). According to studies, the eggs are developed and hatched, and the postlarvae escape from the mussels to swim in the water after the yolk sac larva stage (Heschl 1989, Reynolds *et al*. 1997, Mills and Reynolds 2002a).

The bitterlings which have evolved with host specialization of the bitterling-mussel have a peculiar spawning habit and early life history. The females are known to have developed the ability to control fecundity based on the spawning in the mussel (Aldridge 1999). Meanwhile, the embryological development of bitterlings is considerably different from that of typical cyprinid fish due to the early ontogeny in the gills of host mussels (Aldridge 1999, Mills and Reynolds 2002b). Because the host mussels always attempt to spit out the eggs and yolk sac larvae in the gills, the bitterlings have developed defenses to protect against this action (Fukuhara *et al*. 1982, Suzuki and Jeon 1987).

In this study, the authors investigated the fecundity, the number of eggs per spawning, and the morphologic, developmental and behavioral characteristics of the eggs and yolk sac larvae of *A. yamatsutae*, as the characteristics of the early ontogeny of bitterlings according to the spawning in mussels.

MATERIALS AND METHODS

Collection Site

The fish and Unionidae used in this study were collected from Uiam reservoir (127° 41′ E, 37° 52′ N), located in Chuncheon, Gangwon-do, Korea, between April and July, 2003. A trap net was used to collect the fish, while the mussels were collected by hand and collector. Uiam reservoir, constructed for production of electric power in 1967, is 80 million m^3 in maximum pondage and 15 km^2 in water area, and is inhabited by of nine families and 33 species of fish. There are four species of bitterlings living in the reservoir (*Rhodeus ocellatus*, *R. uyekii*, *A. yamatsutae* and *A. lanceolatus*), and seven species of Unionidae.

* Corresponding author; Phone: +82-33-250-8523, Fax: +82-33-251-3990, e-mail: hoboksong@hanmail.net

Fecundity and Spawning in Mussels

In order to investigate the fecundity, 17 mature females were selected by age and the number of eggs in the ovary were counted. The individuals that were relatively high in gonadosomatic index (gonad weight / body weight × 100) immediately before spawning were selected as the study subjects. In order to observe the development of the eggs in the ovary, they were extracted, fixd by 10% neutral formalin, and tissue section preparations were made. For the egg number per single spawning, the collected mussels were dissected to count the eggs and yolk sac larvae inside the gills. The eggs and yolk sac larvae in one mussel were separately counted on the basis of the developmental stage.

Characteristics of Eggs and Yolk Sac Larvae

In other to observe the development of the eggs and the morphologic and behavioral characteristics of yolk sac larvae based on the developmental stages, artificial insemination was carried out. The place of breeding was dark in order to maintain the circumstances similar to those of the mussel gills, with the water temperature of 20~22℃.

Minute Tubercles on the Skin Surface

In order to observe the changes in minute tubercles on the skin surface based on development, the yolk sac larvae were classified into 8 groups of immediately after hatching, and on the first, 4th, 6th, 9th, 12th, 15th and 18th days of the hatching (from immediately after hatching to swimming stage). For the electron microscopy, the larvae were fixed with 1% glutaraldehyde and osmium tetroxide, were dehydrated by alcohol, underwent the addition of isoamyl acetate, and finally were dried by critical point dryer. Then, the samples were gold-coated to be photographed by scanning electron microscope (Hitachi S-4200). The remaining larvae were fixed with 10% formalin to be observed by optical microscope.

RESULTS

Fecundity and Spawning in Mussels

The mean egg number in the ovaries of 17 individuals was 358 ± 108 SD (range, 171 to 534) (Table 1, Fig. 1). As seen in Fig. 2, mature females just before spawning had eggs in various developmental stages as well as mature eggs. When the abdomens of the females who spawning was imminent were gently pressed,

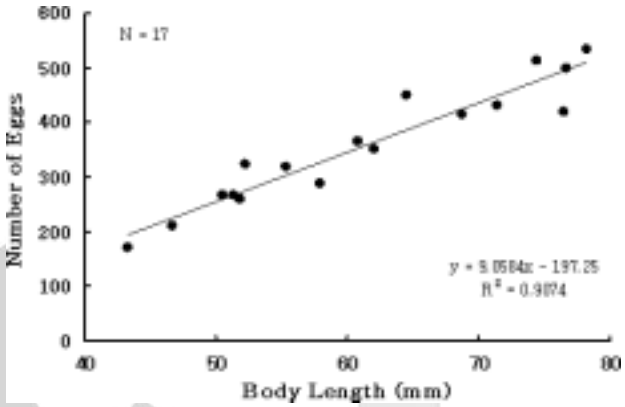

Fig. 1. Fecundity of matured *Acheilognathus yamatsutae* in spawning season.

Table 1. Comparisons of fecundity in the species of the family Cyprinidae

Species	Number of eggs in ovary	References
Cyprinidae		
Cyprininae		
Cyprinus carpio	300,000~400,000	Uchida (1939)
Carassius auratus	42,350~199,875	Uchida (1939)
Gobioninae		
Coreoleuciscus splendidus	1,132(617~1,690)	Song & Kwon (1993)
Microphysogobio longidorsalis	7,334(5,133~8,787)	Song & Son(2003)
Leuciscinae		
Rhynchocypris kumgangensis	1,616(852~2,435)	Song (2000)
Phoxinus phoxinus	1,280(789~2,011)	Song & Son (2002)
Danioninae		
Zacco platypus	920~ 2,500	Nakamura (1969)
Opsariichthys uncirostris amurensis	3,784~12,265	Nakamura(1969)
Acheilognathinae		
Acheilognathus yamatsutae	358(171~534)	Present study
Acheilognathus signifier	225(141~314)	Baek *et al*. (2003)

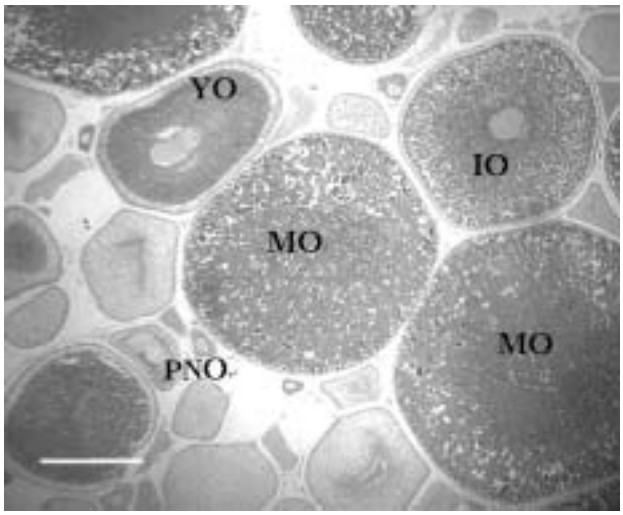

Fig. 2. Showing the various developmental stages of eggs in ovary of *Acheilognathus yamatsutae* in spawning season. Abbreviations: PNO, perinucleolus oocyte; YO, yolk stage oocyte; IO, immature oocytes; MO, mature oocyte. Scale bar = 0.5 mm.

10 to 50 mature eggs were discharged from the ovipositors (n = 20). The number of eggs or yolk sac larvae of *A. yamatsutae* found in 244 mussels ranged from 1 to 20, and 31% (76/244) of them had only one egg or yolk sac larva (Fig. 3).

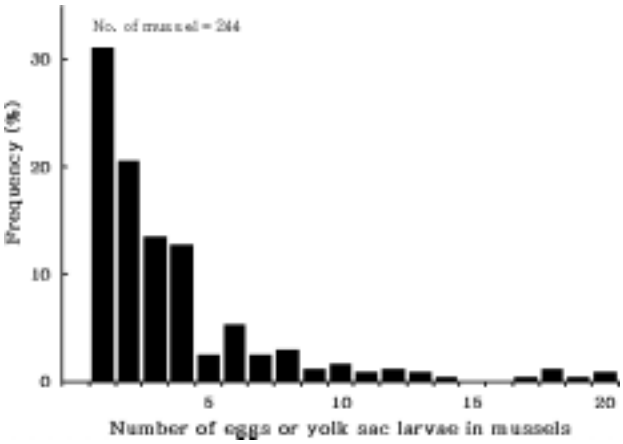

Fig. 3. The frequency and number of eggs or yolk sac larvae of *Acheilognathus yamatsutae* in host mussels.

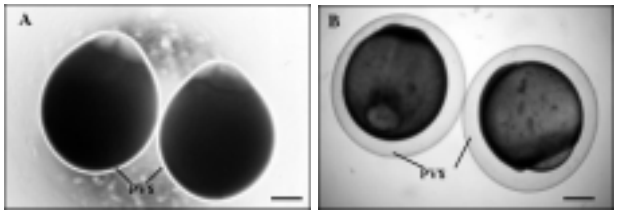

Fig. 4. Comparison of perivitelline space (PVS) in egg of *Acheilognathus yamatsutae* (A) and *Rhynchocypris kumgangensis* (B). Scale bars = 0.5 mm.

Characteristics of Eggs and Yolk Sac Larvae

The eggs of *A. yamatsutae* were oval and demersal, with a size of 1.98 ± 0.09 × 1.74 ± 0.08 SD mm (n = 30). As seen in Table 2 and Fig. 4A, the peri-vitelline space of the egg was remarkably narrow (0.03 ± 0.002 SD mm, n = 25).

The eggs were hatched at 41 hours after fertilization, and the newly hatched larvae were 3.43 ± 0.06 SD mm (n = 25) long. In the newly hatched larvae, yolk sac and yolk projection were prominent, but organs were not formed except otoliths and minute tubercles on the skin surface of microscopic size (Fig. 5A).

The tail movement of the newly hatched larvae was very vivid, and the larvae could move forward by four hours after hatching. Eyes were formed and blood circulation began on the 2nd day of

Table 2. Comparison of early ontogenic stages of Korean striped bitterling, *Acheilognathus yamatsutae* and crucian carp, *Carassius auratus*

Characters	*A. yamatsutae*	*C. auratus*
Developmental temperature	20~22℃	17.0~21.3℃
Egg shape	Elliptical	Spherical
Egg size before water absorption	1.95×1.71 mm	1.10~1.30 mm*
Egg size with peri-vitelline space	1.98×1.74 mm	1.50~1.70 mm*
Hatching	41 hours (3.43 mm in TL)	130~158 hours (4.7 mm)
First locomotion	45 hours (3.44 mm)	130~158 hours (4.7 mm)
Eyes first pigmented	137 hours (6.78 mm)	72 hours
First melanophore in body	185 hours (7.35 mm)	72 hours
Free swimming	473 hours (9.59 mm)	250~278 hours (5.8 mm)
Yolk sac resorption	473 hours (9.59 mm)	250~278 hours (5.8mm)
Exogenous feeding	473 hours (9.59 mm)	250~278 hours (5.8 mm)

Hours for each developmental stage represent the time after fertilization; TL, total length;
Data on *C. auratus* are from Uchida (1939)* and Nakamura (1969).

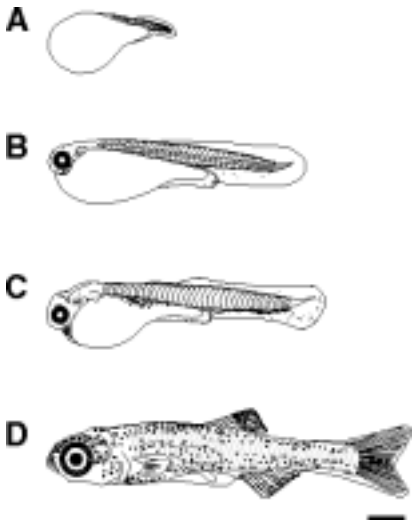

Fig. 5. Developmental stages of yolk sac larvae of *Acheilognathus yamatsutae*. A, immediately after hatching, 41 hours after fertilization; B, pigments in the eyes, the 4th day after hatching; C, melanophores in the body, the 6th day after hatching; D, yolk consumption and swimming stage, the 18th day after hatching. Scale bar = 1 mm.

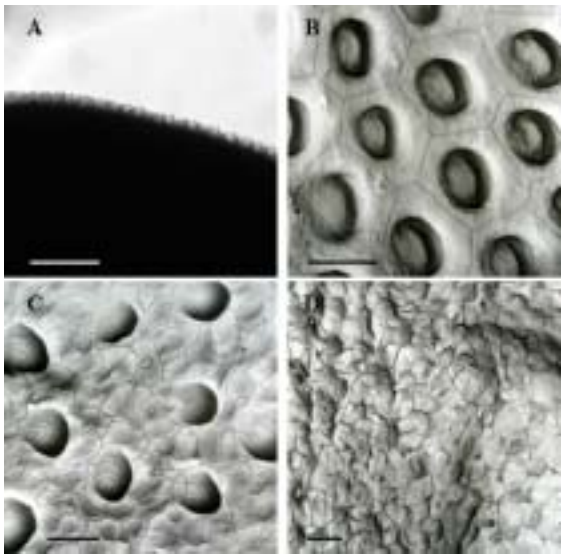

Fig. 6. Change of the minute tubercle on the skin surface of yolk sac larvae. A and B, immediately after hatching; C, the 12th day after hatching; D, the 18th day after hatching (photograph A is by optical microscope and B, C and D are by scanning electron microscope). Scale bars = A, 0.3 mm; B and C, 30 μm; D, 50 μm.

hatching and pigment in the eyes was observed on the 4th day (Fig. 5B). However, melanophore of body was shown on the 6th day (Fig. 5C). On the 18th day the yolks were completely consumed, and the larvae reached 9.59 ± 0.11 SD mm (n = 25) long, were in the free-swimming stage, and began exogenous feeding (Table 2, Fig. 5D). The larvae escaped from the host mussel in this stage.

Minute Tubercles on the Skin Surface

The round-shaped minute tubercles on the skin surface inclined toward the posterior portion were already arranged on the whole surface of the body at hatching (Fig. 6A, B). The diameter of the minute tubercles was 20~30 μm. The minute tubercles began to disappear on the 6th day of hatching, half of them had disappeared on the 12th day (Fig. 6C), and all had disappeared on the 18th day, when the larvae were in the swimming stage and had escaped from the mussels (Fig. 6D).

DISCUSSION

Fecundity and Spawning in Mussels

In general, the egg number in the ovary of cyprinid fish is relatively high: 200,000~300,000 for *Cyprinus carpio* (Nakamura 1969), 42,350~199,875 for *Carassius auratus* (Uchida 1939), 5,133~8,787 for *Microphysogobio longidorsalis*, 852~2,435 for *Rhynchocypris kumgangensis* (Song 2000) and 920~2,500 for *Zaccoplatypus* (Nakamura 1969). However, the egg number of bitterling in this study might be remarkably low (Table 1) because the larvae spend their early developmental stages in the gills of the mussel and escape from the mussel after learning how to swim and because the mortality in the early life history of this species is relatively lower than that of other species. The fish that perform parental care or entrust the eggs and larvae to hosts are considered to have a lower rate of early mortality, a smaller egg number, and a bigger egg size than those that do not (Welcomme 1967). The large production of eggs can increase the clutch size to induce intensive competition. The spawning habit of bitterlings can decrease the egg number, allow extra energy to be invested in enlarging the egg size, and reduce the mortality in early developmental stages (Ware 1975).

It is well known that bitterlings spawn a few eggs on several occasions (Suzuki and Jeon 1989, 1990, Mill and Reynolds 2000a, Baek *et al*. 2003). The eggs in the ovary show various developmental stages even in the spawning season, indicating that once mature eggs are spawned on several occasions, premature eggs become mature to enter the next developmental stage. Aldridge (1999) reported that bitterlings spawn their eggs only a few at a time in many mussels as a bet-hedging strategy to minimize the loss of eggs due to mussel vomiting or death.

Characteristics of Eggs and Larvae

Because bitterlings spend their egg and yolk sac larvae stages in the gills of mussels, they develop in a limited oxygen environment.

Therefore, the perivitelline space in the egg is hardly formed and the relatively shorter egg period, compared to that of other cyprinid fish, is a method to increase the efficiency of oxygen absorption by quickly removing the chorion that offers little protection for the embryo and that prevents oxygen absorption (Tables 2 and 3, Fig. 4). In addition, the oval shape of the bitterling egg is advantageous in oxygen diffusion because this shape provides larger ratio of surface area to volume than the round shape does (Table 2) (Aldridge 1999).

While most cyprinid fish spend a long development period as eggs and their hatched larvae have developed internal and external organs, bitterlings hatch swiftly with the barely-developed organs in order to pass through the egg stage, during which there is no movement and a high risk of being vomited, as quickly as possible. Meanwhile, the bitterling larvae have an extremely vivid tail movement after hatching in order to avoid the risk of being vomited (Table 2) (Song 1994).

Although the development of eyes and the cryptic coloring of body pigments are essential for other larvae in the swimming stage, the bitterlings are relatively slow in the development of eyes and body pigments because the yolk sac larvae grow inside the mussel without direct threat from predators (Aldridge 1999).

The bitterlings reach the swimming stage after completely consuming the yolk. The other typical cyprinid fish must acquire the swimming ability as soon as possible after hatching in order to escape the threat from predators, while the bitterling yolk sac larvae inside the mussel do not have to swim until they have completely consumed the yolk and thereafter swim out of the mussel for exogenous feeding (Table 2).

Minute Tubercles on the Skin Surface

It is known that the yolk sac larvae move to the end of the mussel's water tube by vivid tail movement after hatching and attach to the wall of the mussel's water tube by using the minute tubercles on their skin surface and the wedge-like yolk projection to prevent from being vomited from the host (Nakamura 1969, Fukuhara *et al*. 1982, Suzuki and Jeon 1990).

Suzuki and Jeon (1987) reported that the number of minute tubercles on the skin surface of *A. yamatsutae* began to decrease on the 4th day of hatching when the temperature during development was 22±1℃. Meanwhile, the tubercles in this study began to be reduced on the 6th day due to the difference in temperature. Therefore, it might be safe to stay that the minute tubercles on the skin surface begin to disappear when the motor ability of the yolk sac larvae has commenced gradual development, and completely disappear when the yolk sac larvae pass through to the swimming stage and are able to escape from the host easily.

LITERATURE CITED

Aldridge, D.C. 1999. Development of European bitterling in the gills of freshwater mussels. J. Fish Biol. 54: 138-151.

Baek, H.M., H.B. Song and O.K. Kwon. 2003. Sexual maturation and the spawning season of the Korean bitterling, *Acheilognathus signifer* in upper reachs of the Hongcheon river. Korean J. Ichthyol. 15: 278-288.

Duyvene de Wit, J.J. 1966. Some observations on the European bitterling (*Rhodeus amarus*). Suid-Afrikaanse J. van Wetenskap 51: 249-251.

Fukuhara, S., Y. Nagata and W. Maekawa. 1982. Minute scaly tubercles on the yolksac of *Rhodeine cyprinid* fishes in prolarval stages. Japan J. Ichthyol. 29: 232-236.

Heschl, A. 1989. Integration of 'innate' and 'learned' components within the IRME for mussel recognition in the European bitterling

Table 3. Hatching time after fertilization of Cyprinidae and Acheilognathinae

Species	Water temperature (℃)	Hatching	References
Cyprinidae			
Cyprinus carpio	18~22	84~144 hours	Uchida (1939)
Carassius auratus	20	120 hours	Uchida (1939)
Pseudolasbora parva	15~22	228 hours	Nakamura (1969)
Pseudopungtungia nigra	20± 2	178 hours	Kim *et al*. (1991)
Rhynchocypris kumgangensis	16± 1	120 hours	Song & Choi (1997)
Acheilognathinae			
Rhodeus ocellatus	22± 1	39 hours	Suzuki & Jeon (1988a)
R. notatus	22± 1	53 hours	Suzuki & Jeon (1988b)
Acheilognathus signifer	22± 1	53 hours	Suzuki & Jeon (1988c)
A. yamatsutae	20~22	41 hours	Present study
A. koreensis	22± 1	45 hours	Suzuki & Jeon (1988d)
Acantorhodeus gracilis	22± 1	38 hours	Suzuki & Jeon (1990)

Rhodeus amarus (Bloch). Ethol. 81: 193-208.

Kim, J.K., Y.P. Hong, K.G. An and S.S. Choi. 1991. Studies on early embryonic development of *Pseudopungtungia nigra*, Korean endemic species. Korean J. Limnol. 24: 129-136.

Mills, S.C. and J.D. Reynolds. 2002a. Host species preferences by bitterling, *Rhodeus sericeus*, spawning in freshwater mussels and consequences for offspring survival. Animal Behaviour 63: 1029-1036.

Mills, S.C. and J.D. Reynolds. 2002b. Mussel ventilation rates as a proximate cue for host selection by bitterling, *Rhodeus sericeus*. Oecologia 131: 473-478.

Nakamura, M. 1969. Cyprinid fishes of Japan, Studies on the life history of cyprinid fishes of Japan. Res. Inst. for Natural Resources, Tyoko, pp. 5-99.

Nelson, J.S. 1994. Fishes of the World. 3rd Ed., Wiley, New Yolk, pp. 1-600.

Reynolds, J.D., V.J. Debuse and D.C. Aldridge. 1997. Host specialization in an unusual symbiosis: European bitterlings spawning in freshwater mussels. Oikos 78: 539-545.

Song, H.B. 1994. Ecological studies on the bitterling, *Acheilognathus yamatsutae* Mori (Cyprinidae) in Korea. Ph. D. thesis, Kangwon Nat. Univ., Chuncheon, 181 p.

Song, H.B. 2000. Population ecology of fatminnow, *Rhynchocypris kumgangensis* (Cyprinidae) in Korea. Korean J. Ichthyol. 12: 101-110.

Song, H.B. and O.K. Kwon. 1993. Ecological study of *Coreoleuciscus splendidus* Mori (Cyprinidae) in Hongchon River, Korea. Korean J. Limnol. 26: 235-244.

Song, H.B. and S.S. Choi. 1997. Developmental of eggs and larvae of Korean fatminnow, *Moroco kumgangensis* (Cyprinidae). Korean J. Limnol. 30: 67-74.

Song, H.B. and Y.M. Son. 2002. Maturity and reproductive ecology of the minnow, *Phoxinus phoxinus* (Cyprinidae) in the upper South Han River, Korea. Korean J. Ichthyol. 14: 262-268.

Song, H.B. and Y.M. Son. 2003. Population ecology of *Microphysogobio longidorsalis* (Cyprinidae) from Korea. Korean J. Ichthyol. 15: 303-310.

Suzuki, N. and S.R. Jeon. 1987. Development of the bitterling, *Acheilognathus yamatsutae* (Cyprinidae), with notes on minute tubercles on the skin surface and pharyngeal apparatus. Korean J. Limnol. 20: 229-241.

Suzuki, N. and S.R. Jeon. 1988a. Development of eggs, larvae and juveniles of *Rhodeus ocellatus* from Ansong river, Korea (Pisces: Cyprinidae), with a note on minute tubercles on the skin surface. Korean J. Limnol. 21: 1-15.

Suzuki, N. and S.R. Jeon. 1988b. Hybridization experiments in Acheilognathinae fishes (Cyprinidae), an intrageneric hybrid between *Rhodeus suigensis* and *R. uyekii*. Korean J. Limnol. 21: 57-78.

Suzuki, N. and S.R. Jeon. 1988c. Development of the bitterling, *Acheilognathus signifer* (Cyprinidae), with a note on minute tubercles on the skin surface. Korean J. Limnol. 21: 165-179.

Suzuki, N. and S.R. Jeon. 1988d. Development of the bitterling, *Acheilognathus limbata* (Cyprinidae) from Korea and Japan, with notes on minutes tubercles on the skin surface and on the genetic implication in hybrid embryos. Korean J. Limnol. 21: 211-229.

Suzuki, N. and S.R. Jeon. 1989. Development of the bitterling, *Acanthorhodeus asmussi* (Cyprinidae), with note on minute tubercles on the skin surface. Korean J. Ichthyol. 1: 73-82.

Suzuki, N. and S.R. Jeon. 1990. Development of the bitterling, *Acanthorhodeus gracilis* (Cyprinidae), with a note on minute tubercles on the skin surface. Korean J. Ichthyol. 2: 169-181.

Uchida, K. 1939. The Fishes of Tyosen (Korea), Part Ⅱ, Nematognathi, Eventognathi. Bulletin of the Fisheries Experiment Station of the Government-General of Tyosen, No 6. Husan, pp. 1-458.

Ware, D.M. 1975. Relation between egg size, grow and natural mortality of larval fish. J. Fish Res. Bd. Can. 32: 2503-2512.

Welcomme, R.L. 1967. The relationship between fecundity and fertility in the mouthbrooding cichlid fish, *Tilapia leucosticta*. J. Zool. Lond. 151: 453-468.

Wiepkema, P.R. 1961. An ethological analysis of the reproductive behaviour of the bitterling (*Rhodeus amarus* Bloch). Arch. Neerl. Zool. 14: 103-199.

Zeal, A.V. and R. Neves. 1982. Fish host of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginina. Can. J. Zool. 60: 2535-2542.

(*Received June 21, 2005; Accepted August 16, 2005*)