Morphometrical Changes on Korean Rose Bitterling, *Rhodeus uyekii*, in Early Growth Period

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ABSTRACT: We investigated the process of yolk absorption in Korean rose bitterling, *Rhodeus uyekii*, and determined the changes in its morphometric characteristics. The *R. uyekii* from 1 days post hatching (DPH) to 21 DPH, the eye diameter (ED) was 5.4 at 5 DPH. Thereafter, the ED/total length (TL) ratio increased to 10.7 at 21 DPH (p<0.05). The yolk length (YL) decreased from 95.4 to 1.1 by 21 DPH, and this rate of decrease was greater than that for any other dimension (p<0.05). 12 morphometric dimensions/TL for the *R. uyekii* were measured at each sampling day from 21 DPH to 170 DPH. At just hatching, the average TL and BW were 6.1±0.09 mm and 4.9±0.07 mg, respectively. At 53 DPH, the average TL was 12.9±0.28 mm and the average BW was 14.7±0.72 mg; the total length growth equation was TL=5.507e^{-0.039t} (R²=0.916). Further, the body weight growth equation was BW=3.3647e^{-0.029t} (R²=0.9354). The dimensions with regard to body depth showed the greatest growth rates in the external characteristics of the fish (p<0.05). The patterns of the morphometric characteristics measured in this study can be classified in three ways. The patterns were shown to be increased (y=0.0992x+12.07, R²=0.8333), decreased (y=−0.0569x+42.029, R²=0.8395) or maintained (y=0.005x+18.263, R²=0.3678) from 21 DPH to 170. These results will provide useful indices for the successful rearing of the *R. uyekii*.

Key words: Classical dimension, Early growth, Morphometrical change, *Rhodeus uyekii*, Truss dimension

INTRODUCTION

Morphological differences in groups and species are classified in terms of the overall body shape or specific morphoanatomical characteristics (Sträuss & Bond, 1990). Among the body shape characteristics of fish, morphometric traits, unlike meristic traits, can be measured as mensural characteristics. Although the body shape of fish is largely determined by genetic factors (Riddell et al., 1981; Taylor & McPhail, 1985), the use of morphometric analyses to distinguish the genetically discrete groups within a fish species is limited by the difficulty of measuring the environmentally induced variations in the body shape (Ihssen et al., 1981; Currens et al., 1989; Park et al., 2001).

Both truss and classical dimensions are used to describe the body shapes of fish (Hubbs & Lagler, 1947; Sträuss & Bookstein, 1982). Truss dimensions consist of a systematically arranged set of distances that are measured between a set...
of preselected anatomical landmarks (Sträuss & Bookstein, 1982; Sträuss & Bond, 1990). These landmarks are identified based on local morphological features and are chosen to divide the body into functional units (Sträuss & Bond, 1990). Truss dimensions, which include components of body depth and length along the longitudinal axis, have theoretical advantages over classical morphometric characteristics in discriminating the different groups (Park et al., 2007).

Korean rose bitterling, *Rhodeus uyekii*, belongs to the family Cyprinidae. It is an endemic species that is found in the water system of Nakdong river, Korea; furthermore, it likes flow slow, lush aquatic plants and mud at the edge of a river or reservoir pond (Jeon, 1982). The spawning season of *R. uyekii* is from April to June. During this period, females extend their ovipositor and spawning to the gill chamber of Unionidae fresh water bivalves, and male dense coloration (Uchida, 1939; Nakamura, 1969). The body shape of the *R. uyekii* is compressiform that has high body depth; usually, males have higher body depth than females. Further, males have a light brown body color at the dorsal region, and the caudal peduncle side has a dense color of silverlight. Males have black color in anal fin out side, and inside small light white, meanwhile female has not pattern. Therefore, males are particularly treated as aquarium fish because they have a visual effect. Recently, *R. uyekii* demand urgent conservation because easily exposure to biological and chemical pollution (Kang et al., 2006).

Early life history of the *R. uyekii* is as follows: reared in the laboratory, egg and larval development and seasonal sex ratio of Korean endemic fresh water fish *R. uyekii*, reproductive cycle of the spring-spawning bitterling, osteological study of *R. uyekii*, elongation of the ovipositor in *R. uyekii*, temperature-dependent index of mitotic interval (\(t_0\)) for chromosome manipulation in *R. uyekii* and morphometric traits and cytogenetic analysis in induced cross and reciprocal hybrid between *R. uyekii* and *R. notatus*; all of these factors were studied with regard to the *R. uyekii* (Kim and Han, 1990; Park and Kim, 1990; An, 1995; Kim, 1997; Chae, 2001; Kim et al., 2011, 2012).

More information about the developmental stages of the *R. uyekii* is required because high levels of hatchery mortality are common during the early life stages. However, there have been no detailed reports. Because research into the early larval development of the *R. uyekii* is currently insufficient, in this study, we investigated the process of yolk absorption in *R. uyekii*, and also determined the changes in its morphometric characteristics.

**MATERIALS AND METHODS**

1. Experimental fish

*R. uyekii* broodstock was transported to a mariculture facility at the Fishery Genetics and Breeding Sciences Laboratory of the Korea Maritime and Ocean University in Busan, Republic of Korea, from Inland Aquaculture Research Center, National Fisheries Research & Development Institute (NFRDI), Jinhae, Republic of Korea. The fish were raised in a glass tank (W 120 cm×L 30 cm×H 50 cm) until the start of the experiment. The water temperature was maintained at 20±1 °C.

2. Artificial spawning and hatching

Since the spawning season of the *R. uyekii* from late April to the middle of June 2013, dense colored males and ovipositor extended females were selected and used as broodstock. After the egg was from extended ovipositor female using the abdominal compression method, were inseminated using a wet method semen that through pressure on the abdomen of male were sorted within 2 minutes. A total of 2,000 eggs were reared in the plastic tank (Fresh Plus, Mirae Chemical, Republic of Korea)(W 30 cm×L 20 cm×H 15 cm) until hatching. The water depth and L:D ratio were maintained at 10 cm and 13:11, res-
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pectively. The water in the hatch tank was replaced two times per day using aerated bottled water; further, the water temperature was maintained at 22±0.5°C.

3. Rearing conditions

The hatched larvae each divided into 10 fish in the transparent plastic containers (Coolrara, Easyfilm, Republic of Korea) (W 10 cm×L 5 cm×H 3 cm), and aeration bottled water (Natural mineral water, Pulmuone, Republic of Korea) was used for rearing, being replaced two times per day. Other rearing conditions were maintain under the same conditions of “Artificial spawning and hatching in this section,” as previous stated. Since larvae feed at completely yolk absorption and the first feeding, 21 days post hatching (DPH), *Artemia* sp. was feeding. The water temperature was maintained at 22±0.5°C; further, an analysis was made each day with regard to the average water temperature and integral water temperature during the rearing period.

4. Specimens and morphometrical observation

Newly hatched larvae (*n* = 50) were daily sampled from hatching to the DPH at 2 days interval from 7 to 21 DPH, at 4 days interval from 25 to 41 DPH, at 6 days interval from 47 to 83 DPH, and at 30 days interval from 110 to 170. Body outline measurements were made for 13 distances between landmarks for both truss and classical dimensions (Table 1, Fig. 1). Total length and body weight for the sampled larvae at different stages were measured to the nearest 0.01 g and 0.01 cm using an electric balance (AX 200, Shimadzu Corp., Japan) and a digital Vernier calipers.

### Table 1. Morphometric dimensions for Korean rose bitterling, *Rhodeus uyekii*, used in this experiment*

| Morphometric dimensions | TL  | HHAV | HHAP | HHAC | ED |
|-------------------------|-----|------|------|------|----|
| Total length            |     |      |      |      |    |
| Horizontal distance     |     |      |      |      |    |
| Most anterior extension of the head and the anterior insertion of the ventral fin |     |      |      |      |    |
| Most anterior extension of the head × longest point of gill cover from maxilla | 1 × 8 |      |      |      |    |
| Anterior insertion of primary dorsal fin × anterior insertion of the pectoral fin | 2 × 7 |      |      |      |    |
| Anterior insertion of primary dorsal fin × verticality position of the primary dorsal fin ray’s anterior insertion | 2 × 5 |      |      |      |    |
| Anterior insertion of primary dorsal fin × anterior insertion of the ventral fin | 2 × 6 |      |      |      |    |
| Anterior insertion of primary dorsal fin × posterior insertion of the anal fin | 2 × 4 |      |      |      |    |

* Each dimension is demonstrated in Fig. 1.
(CD-20 CP; Mitutoyo, Japan), respectively. Prior to the measurements, the larvae were anaesthetized with 300 ppm lidocaine-HCl/NaHCO₃ at 25 °C, according to the method of Park et al. (1988). Furthermore, a mimetic diagram was produced according to the growth for indicating the morphological changes.

The following horizontal distances were measured: total length (TL), the horizontal distance between the anteriormost head and the anterior insertion of the ventral fin (HHAV), the horizontal distance between the anteriormost extension of the head and the anterior insertion of the pectoral fin (HHAP), the horizontal distance between the anteriormost extension of the anal fin and the anterior insertion of the caudal fin (HHAC), and ED. Other distances (truss morphometric dimensions and classical morphometric dimensions) were direct distance measurements.

The external truss morphometric dimensions measured were as follows: 1 × 8, the anteriormost extension of the head × the longest point of the gill cover from the maxilla; 2 × 7, the anterior insertion of the primary dorsal fin × the anterior insertion of the pectoral fin; 2 × 5, the anterior insertion of the primary dorsal fin × the vertical position of the anterior insertion of the primary dorsal fin ray; 2 × 6, the anterior insertion of the primary dorsal fin × the anterior insertion of the ventral fin; 2 × 4, and the anterior insertion of the primary dorsal fin × the posterior insertion of the anal fin (Fig. 1A). Each external morphometric dimension was analyzed relative to the TL.

The external classical morphometric dimensions measured were as follows: 1 × 2, the anteriormost extension of the head × the anterior insertion of the primary dorsal fin; 1 × 5, the anteriormost extension of the head × the vertical position of the anterior insertion of the primary dorsal fin ray; and 2 × 3, the anterior insertion of the primary dorsal fin × the anterior insertion of the caudal fin (Fig. 1B). Each external morphometric dimensions was analyzed relative to the TL. The TL and the body weight of each larva were measured, and their relationship, which indicated an exponential growth curve, was analyzed. The values given are morphometric dimensions/TL. In addition, the curves were drawn depending on the growth with the days post hatching; in particular, the main trendy dimensions were indicated as a linear function.

5. Statistical analysis

The study was performed in triplicate and the results are reported as means ± SD (n = 50), unless otherwise stated. The data were analyzed with a one-way analysis of variance (ANOVA) using the SPSS statistical package (SPSS 9.0, SPSS Inc., Chicago, IL, USA). The means were compared with Duncan’s multiple range test and were considered significantly different at p<0.05.
RESULTS

*R. uyekii*, hatched at two days (48 h) post fertilization with integral water temperature (IWT) after fertilization 43°C. From just hatching (IWT after hatching 0°C) to 170 days post hatching (DPH)(IWT after hatching 3.740°C), the total length (TL) and body weight (BW) growth, showed in Fig. 2. At just hatching, the average TL and BW were each 6.1±0.09 (SD) mm and 4.9±0.07 (SD) mg, respectively; at 170 DPH, the average TL was 12.9±0.28 (SD) mm and the average BW was 14.7±0.72 (SD) mg. Total length showed continuous growth from just hatching to 53 DPH; the total length growth equation was TL= 5.507(e^{0.0330t}) (R²=0.916)(TL, total length; t, time)(Fig. 1A). Body weight was slightly reduced from just hatching to completely yolk absorption time, but after feeding began, it rapidly increased. The body weight growth equation was BW=3.3647e^{0.0296t} (R²=0.9354)(BW, body weight; t, time) (Fig. 1A). Yolk absorption is complete at 21 DPH (462°C IWT), and showed a larva form. The increases in TL and BW showed a positive relationship. TL increased by 10.6 mm from 21 DPH to 170 DPH. Thereafter, the Korean rose bitterling ultimately grew to 23.9 mm (Fig. 2). The BW of the Korean rose bitterling had increased to 253.2 mg at a steady rate by 170 DPH (Fig. 2). Further, the rate of the increase in TL was more regular than the increase in BW (Fig. 2).

Table 2 lists two morphometric dimensions/tl for the Korean rose bitterling from 1 DPH to 21 DPH. In this experiment, the eye diameter (ED) was 5.4 at 5 DPH. Thereafter, the ED/TL ratio increased to 10.7 at 21 DPH (p<0.05). Yolk length (YL) decreased from 95.4 to 1.1 by 21 DPH, and this rate of decrease was greater than that for any other dimension (p<0.05). Table 3 lists 12 morphometric dimensions/TL for the Korean rose bitterling, which were measured at each sampling day from 21 DPH to 170 DPH.

Table 2. Results of 2 characteristics in Korean rose bitterling, *Rhodeus uyekii*, from 1 day to 21 days post hatching*

| Day post hatching | Morphometric dimension/TL (%) |
|-------------------|-------------------------------|
| 1                 | ED A                          |
| 2                 | 95.4 ± 3.12                  |
| 3                 | 94.7 ± 2.50                  |
| 4                 | 91.9 ± 2.92                  |
| 5                 | 90.1 ± 1.10                  |
| 6                 | 5.4 ± 0.70                   |
| 7                 | 6.4 ± 0.74                   |
| 8                 | 8.0 ± 1.5                    |
| 9                 | 7.0 ± 0.80                   |
| 10                | 7.4 ± 0.85                   |
| 11                | 7.6 ± 0.86                   |
| 12                | 7.4 ± 0.73                   |
| 13                | 7.7 ± 0.76                   |
| 14                | 7.5 ± 0.98                   |
| 15                | 7.9 ± 0.61                   |

*The values are means ± SD (n=50) of triplicate groups. Different capital letters on the values indicate statistical significance among days post hatching (p<0.05). Each dimension is demonstrated in Fig. 1. ED: eye diameter, TL: total length, YL: yolk length.
Table 3. Results of 11 characteristics in Korean rose biterling, *Rhodeus uyekii*, by days post hatching*  

| Day post hatching | Morphometric dimension/TL (%) |
|-------------------|--------------------------------|
|                   | HHAP                          | HHAV                          | HHAC                          |
|                   | 1 × 2                          | 1 × 8                          | 1 × 5                          |
| 21                | 14.7 ± 2.26**                  | 42.9 ± 2.016**                 | 36.9 ± 1.04**                  |
|                   | 18.6 ± 1.36**                  | 41.4 ± 2.16**                  | 41.4 ± 2.16**                  |
| 25                | 15.9 ± 3.82**                  | 41.0 ± 2.32**                  | 41.0 ± 1.68**                  |
|                   | 18.0 ± 1.72**                  | 41.0 ± 2.76**                  |                                |
| 29                | 16.7 ± 3.17**                  | 41.1 ± 2.35**                  | 41.8 ± 1.45**                  |
|                   | 18.1 ± 1.23**                  | 41.1 ± 1.45**                  |                                |
| 33                | 17.7 ± 2.56**                  | 40.3 ± 2.90**                  | 35.3 ± 1.10**                  |
|                   | 40.4 ± 2.66**                  | 27.3 ± 1.90**                  |                                |
| 37                | 17.3 ± 2.01**                  | 40.9 ± 3.06**                  | 34.7 ± 1.43**                  |
|                   | 41.4 ± 1.12**                  |                                |                                |
| 41                | 17.7 ± 3.18**                  | 39.3 ± 2.41**                  | 33.1 ± 1.59**                  |
|                   | 41.5 ± 0.84**                  | 18.3 ± 2.44**                  |                                |
| 45                |                                |                                |                                |
| 49                |                                |                                |                                |
| 53                |                                |                                |                                |
| 57                |                                |                                |                                |
| 61                |                                |                                |                                |
| 65                |                                |                                |                                |
| 69                |                                |                                |                                |
| 73                |                                |                                |                                |
| 77                |                                |                                |                                |
| 81                |                                |                                |                                |
| 85                |                                |                                |                                |
| 93                |                                |                                |                                |
| 101               |                                |                                |                                |
| 105               |                                |                                |                                |
| 113               |                                |                                |                                |
| 117               |                                |                                |                                |
| 121               |                                |                                |                                |
| 125               |                                |                                |                                |
| 133               |                                |                                |                                |
| 137               |                                |                                |                                |
| 141               |                                |                                |                                |
| 145               |                                |                                |                                |
| 153               |                                |                                |                                |

*For abbreviations and each dimension, see Table 1 and Fig. 1. The values are means ± SD (n=50) of triplicate groups. Data were analyzed using one- and two-way ANOVA on data transformed to the arcsine of the square root. Different capital letters on the values indicate statistical significance among days post hatching (p<0.05). Different small letters on the values indicate statistical significance among ratios of morphometric dimension/total length (TL) (p<0.05).

The horizontal distance between the most anterior extension of the head and the anterior insertion of the pectoral fin (HHAP), most anterior extension of the head × anterior insertion of the primary dorsal fin (1 × 2), most anterior extension of the head × longest point of gill cover from maxilla (1 × 8), most anterior extension of the head × verticality position of the primary dorsal fin ray’s anterior insertion (1 × 5), and ED did not differ significantly from 31 DPH to 170 DPH (p>0.05). During 170 DPH, the horizontal distance between the most anterior extension of the head and the anterior insertion of the ventral fin (HHAV), horizontal distance between the most anterior extension of the anal fin and the anterior insertion of the caudal fin (HHAC) and anterior insertion of primary dorsal fin × anterior insertion of the caudal fin (2 × 3) decreased gradually, whereas the anterior insertion of primary dorsal fin × anterior insertion of the pectoral fin (2 × 7), anterior insertion of primary dorsal fin × verticality position of the primary dorsal fin ray’s anterior insertion (2 × 5), anterior insertion of primary dorsal fin × anterior insertion of the ventral fin (2 × 6) and anterior insertion of primary dorsal fin × posterior insertion of the anal fin (2 × 4) gradually increased significantly (p<0.05). The dimension 2 × 7 increased to 130.6%, 2 × 5 increased to 240.2%, 2 × 6 increased to 231.2% and 2 × 4 increased to 229.3%; these dimensions conveyed the greatest growth rates in the external characteristics of fish, respectively (p<0.05).

The relationship between YL/TL and DPH was y=−4.375x+111.0, R²=0.894 (y is YL/TL, x is days post hatching) (Fig. 3).
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Fig. 4. Relationship between days post hatching and horizontal distance between the most anterior extension of the head and the anterior insertion of the ventral fin (HHAV) \( y = 0.0992x + 12.07, R^2 = 0.8333 \), anterior insertion of primary dorsal fin × verticality position of the primary dorsal fin ray’s anterior insertion (2×5) \( y = -0.0569x + 42.029, R^2 = 0.8395 \), and most anterior extension of the head × longest point of gill cover from maxilla (1×8) \( y = 0.005x + 18.263, R^2 = 0.3678 \) in the Korean rose bitterling, *Rhodeus uyekii*, from 21 days post hatching (DPH) to 170 DPH (Fig. 4).

As shown in Fig. 4, the patterns of the morphometric characteristics measured in this study can be classified in three ways. HHAV, 1×8 and 2×5 are the main traits of each curve, and patterns were shown increased (\( y = 0.0992x + 12.07, R^2 = 0.8333 \)), decreased (\( y = -0.0569x + 42.029, R^2 = 0.8395 \)) or maintained (\( y = 0.005x + 18.263, R^2 = 0.3678 \)) from 21 DPH to 170 DPH (Table 3). Although limited, research related to the early growth of the *R. uyekii* has shown that when they hatch, they absorb the whole yolk sac and then begin to emerge (Dill, 1969; Brannon, 1972; Zhang et al., 1995). However, it is unclear how the yolk sac changes in shape from the start of its absorption. In this study, yolk length (YL) decreased with time. After hatching, YH also decreased, but the reduction was smaller than that in YL. This decrease in yolk means that, as in the larvae, the juvenile reverts from endogenous feeding to exogenous feeding after the yolk sac declines (Dill, 1969; Brannon, 1972; Zhang et al., 1995). It is generally reported that the absorptive function of the alimentary canal in fish is initiated either before or after the absorption of the yolk sac. In juvenile *Coregonus fera*, the yolk sac is completely

**DISCUSSION**

The time taken for the hatching of the *R. uyekii*, was 48 h in 22±1°C, which was a similar result to Kim & Han (1990), which reported 50 h in 17.5±1°C, and Suzuki et al., (1985) 46 h in 22±1°C. This result is relative more longer or shorter than that of previous research on other species of rose bitterling, *R. ocellatus*, 39 h in 17.8–18.0; slender bitterling, *Acheilognathus lanceolatus*, 84 h in 17–21°C; flat bitterling, *Paracheilognathus rhombea*, 96 h in 16–19°C. It seems that the time taken for hatching was affected by the water temperature and different inter species. Makeyeve (1976) reported that the yolk sac larvae yolk bump was to prevent the drop out from the shellfish gill chamber as an anchor. In this experiment, even though taken the larvae what through artificial spawning and rearing, appeared yolk bump. In particular, a yolk bump will be important to distinguish the trait of *Rhodeus* compared with other Genus.

The truss dimensions are the primary units of classification for the external anatomical markers that are normally assessed in fish (Strauss & Bond, 1990). The truss dimensions are the length measurements that refer to the ratios between the width of the fish’s body and the axis of the fish’s length. Theoretically, the truss dimensions are a better measurement of the shapes of fish than the classical dimensions (Strauss & Bookstein, 1982; Currens et al., 1989).

The combined morphometric traits of the *R. uyekii* showed a tendency to increase from 21 days post hatching (DPH) to 170 DPH (Table 3). Although limited, research related to the early growth of the *R. uyekii* has shown that when they hatch, they absorb the whole yolk sac and then begin to emerge (Dill, 1969; Brannon, 1972; Zhang et al., 1995). However, it is unclear how the yolk sac changes in shape from the start of its absorption.
absorbed after 11 DPH, and during this period, the absorptive function of the midgut epithelium begins to function (Loewe & Eckmann, 1988).

A morphometrical study of the early growth period was reported on cyprinid loach, *Misgurnus anguillicaudatus*, Ussurian bullhead, *Leiocassis ussuriensis* and Korean bullhead, *Pseudobagrus fulvidraco* (Han et al., 2013; Lim et al., 2013). As mentioned earlier with Han et al. (2013) and Lim et al. (2013), in cyprinid loach, dimensions with regard to body length were significantly increased during early growth 60 DPH (*p*<0.05), and Ussurian bullhead and Korean bullhead were growth to constant ratio in early growth period after maintain, on the other hand *R. uyekii* shown dimensions about body depth gradually increasing, and relatively dimensions about body length decreasing trend in early growth period.

As aforementioned, there has been insufficient research into the early growth of *R. uyekii* In this study, morphometric characteristics as well as the absorption of the yolk sac were investigated from 1 DPH to 170. These results will provide useful indices for the successful rearing of the *R. uyekii*.

ACKNOWLEDGMENTS

This research was supported by a research grant (RP-2014-AQ-022) from the Inland Aquaculture Research Center, National Fisheries Research & Development Institute (*NFRDI*), Republic of Korea. The comments of the anonymous reviewers greatly improved the quality of the manuscript. We declare that all experiments in this study comply with the current laws of the Republic of Korea (Ordinance of Agriculture, Food and Fisheries, No. 1, Regarding Experimental Animals, No. 9,932).

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