

Development and Identification of Three Species of Thai Ricefish, *Oryzias*, in the Mekong Basin

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ABSTRACT.– The development and species identification of three species of *Oryzias*, *O. curvinotus*, *O. pectoralis* and *O. songkharmensis*, were observed from captive bred samples. Ricefish larvae were collected from breeding tanks and rearing ponds and divided into three developmental stages; the larval, post-larval and juvenile stages. The development of morphometric, meristic and chromatophore pigment patterns were studied in each developmental stage. The number of dorsal and anal fin rays, and the chromatophore pigment pattern were found to be useful as characters for species discrimination in each of the three larval developmental stages. The chromatophore pigment pattern on the gut, dorsal ventral and midline of the body were found to be important characters for discrimination between these three species of *Oryzias* larvae. The chromatophore pigment pattern on other parts of body, such as the caudal, dorsal and anal fin rays, and the number of myomere, dorsal and anal fin rays were also used for discrimination between these three species in both the larvae and juvenile stages. The small strip along the dorsal and ventral parts of the caudal fin was diagnostic for *O. songkharmensis*, whilst the strip at the midline of the caudal fin and the chromatophore band at the anterior part of caudal were diagnostic for *O. pectoralis* and *O. curvinotus*, respectively.

KEY WORDS: *Oryzias*, larvae, identification, development, chromatophore pigment

INTRODUCTION

Ricefish in the genus *Oryzias* Jordan and Snyder 1906, are small fish distributed in shallow freshwater and brackish habitats. They are commonly found in ponds, ditches, and paddy fields. To date, 28 species have been described in the South, Southeast and East Asia (Asai et al., 2011). In Thailand six species of *Oryzias* have been currently recorded, there are three species in the lower Mekong river basin for which there are no available studies about their larval development. They are (i) the Hainan medaka, *O. curvinotus* (Nichols and Pope, 1927), which is found in North Vietnam and South China, including Hainan and Hong

Kong (Uwa and Parenti, 1988; Kottelat, 2001a, b). (ii) The pectoral-fin spot medaka, *O. pectoralis* Roberts, 1998, which is widely distributed from Laos to north Vietnam (Roberts, 1998; Kottelat, 2001a), and (iii) the recently described small ricefish, *O. songkharmensis* Magtoon, 2010, which is found in the Songkharm river basin, Thailand, and along the Mekong basin to Laos (Magtoon, 2010).

Oryzias ricefish have a general description of: a laterally compressed body, a depressed head with moderately large eyes, 28 -31 vertebrae, with the dorsal and anal fin bases situated from the 17th to 20th - 21st and the 10th - 11st to the 19th - 20th vertebrae, respectively. The number of

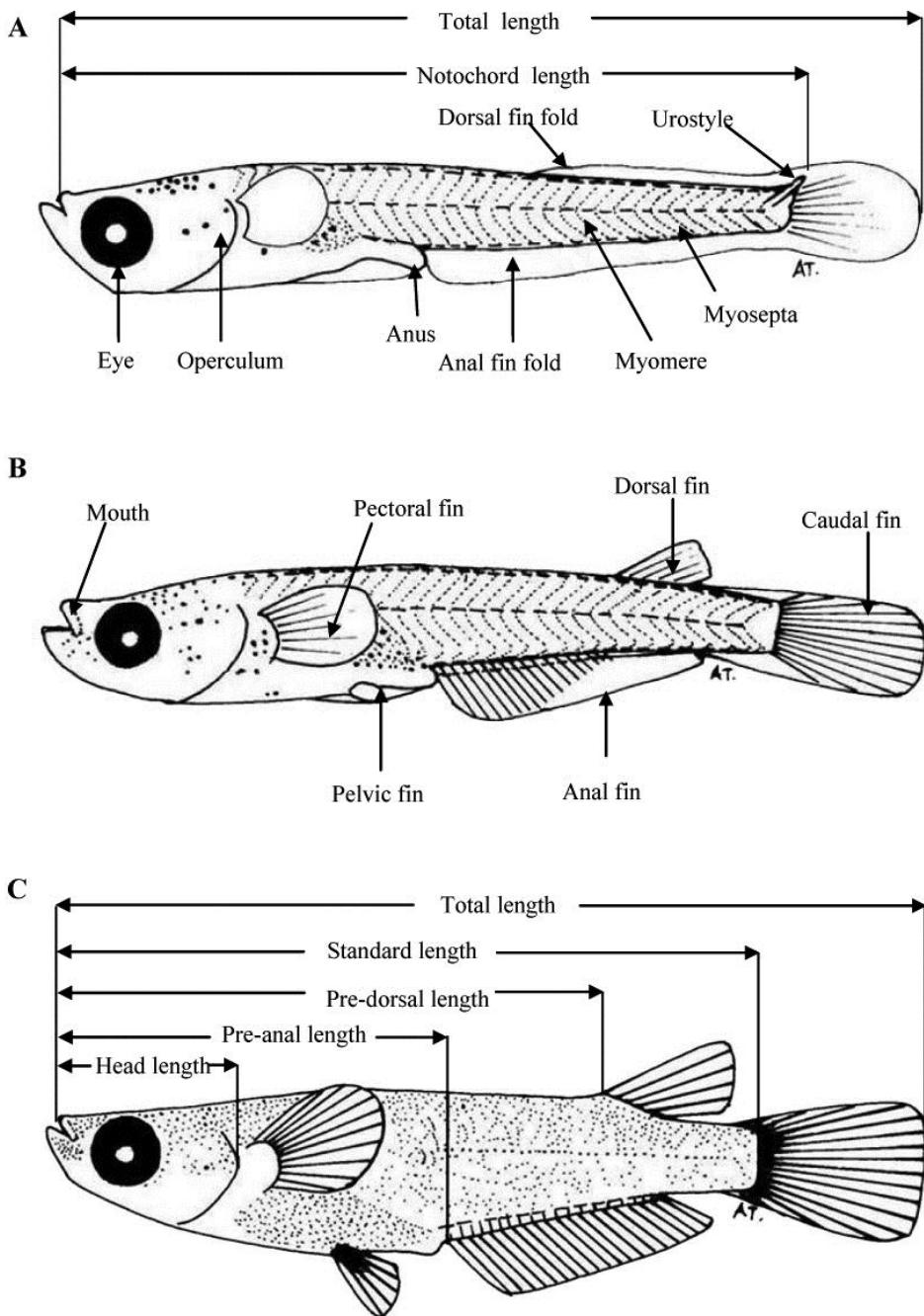


FIGURE 1. Meristic characters of ricefish larvae. A, larval stage; B, post-larval stage and C, juvenile.

dorsal- and anal-fin rays differs among the species. *O. curvinotus* has 5 - 6 dorsal-fin rays and 17 - 20 anal-fin rays (Parenti, 2008). *O. pectoralis* has 6 - 7 dorsal-fin rays and 19 - 20 anal-fin rays (Roberts, 1998; Parenti, 2008), whilst *O. songkharmensis* has 6 - 7 dorsal-fin rays and 15 - 16 anal-fin rays (Magtoon, 2010).

Iwamatsu (1994) studied the developmental stages of the medaka (*O. latipes*) from unfertilized eggs, fertilized eggs to hatching stages of embryo, and from the hatching larvae to the young stage of medaka. The just hatched larvae of medaka are not fully developed but do so after hatching, including the development of the eye, mouthparts and caudal fin. The number of fin rays and chromatophore pigment pattern were found to be useful for species identification of larvae. In addition, the four species, *O. javanicus*, *O. dancena*, *O. mekongensis* and *O. minutillus*, were studied in terms of their development and species identification (Termvidchakorn and Magtoon, 2008).

In this article, the development of morphometric, meristic and chromatophore pigment patterns among three more species of ricefish in the genus *Oryzias*, *O. curvinotus*, *O. pectoralis* and *O. songkharmensis*, were identified.

MATERIALS AND METHODS

The specimens were collected from breeding tanks at different ages from just hatched to the juvenile stage. The larvae were then divided into three stages (Fig. 1); (i) the larval stage, from larvae that had just hatched out of the egg shell up to when the pelvic fin had just started to develop, (ii) the post-larval stage, from when the pelvic fin had just started to develop (visibly form) up to when the scales were started to visibly

develop and (iii) the juvenile stage, from when the scales had just started to visibly develop up to when the scales were completely developed.

For each species, five specimens of each of the three developmental stages were collected and preserved with 10% (v/v) formalin solution and changed to 4% (v/v) formalin solution after 2 weeks. The developmental stages and identification were then studied under a stereo light microscope with drawing aided by the use of a camera lucida for each stage. The different meristic characters and chromatophore pigment patterns were studied for each species and developmental stage so as to be able to identify species-specific characters for each developmental stage.

All materials specimens deposited on National Inland Fisheries Institute (NIFI), Department of Fisheries, Ministry of Agriculture and Co-operatives, Thailand.

RESULTS

The development of the ricefish larva.– Ricefish of the genus *Oryzias* show a long embryonic development in their incubation period. When the larvae hatch out from the eggshell some parts of their body have developed beyond the yolk sac stage and show larval period characters, whilst the rest develop after hatching.

The development of the Hainan medaka (*Oryzias curvinotus*) larvae.– All data relates to larvae collected from hatching ponds and not wild fish.

At hatching the larvae (Fig. 2A) were 4.1 ± 0.1 mm long with a laterally compressed body. The head is slightly depressed, the mouth open, and the maxilla and mandibles are already developed. The large eyes are about one third the size of the

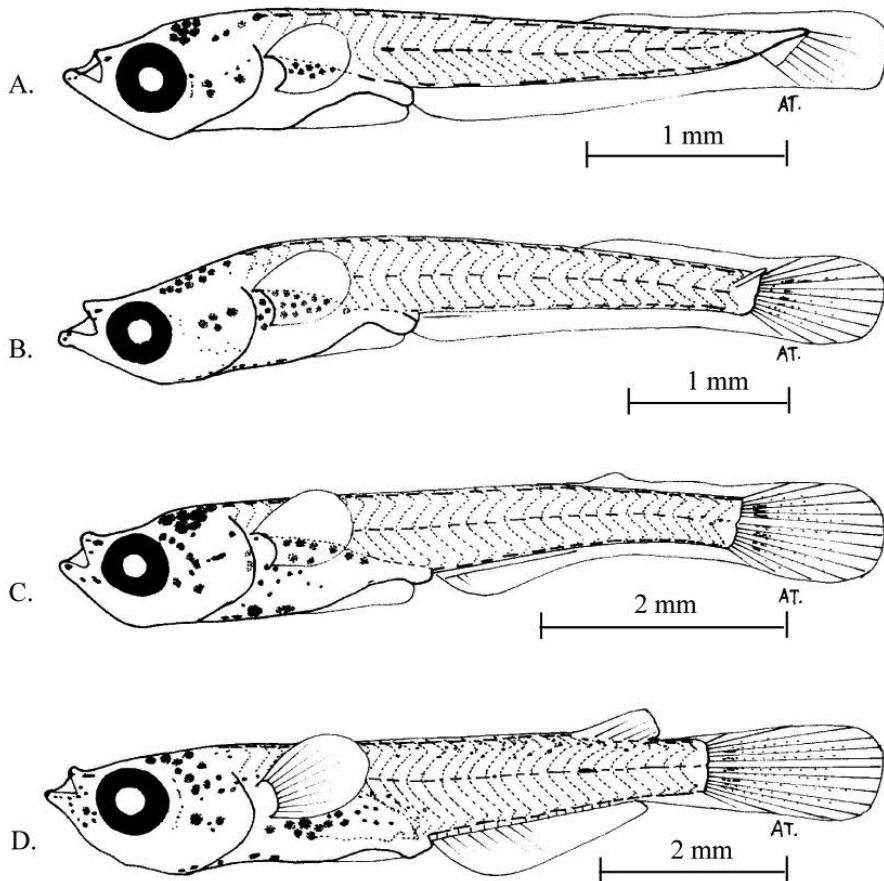


FIGURE 2. The developmental stages of the Hainan medaka ricefish (*Oryzias latipes*) from artificial breeding. **A**, 4.1 mm long just hatched larvae; **B**, 5.3 mm long 1-day old larvae; **C**, 6.6 mm long 3-day old larvae and **D**, 8.9 mm long 5-day old larvae.

head, and the gut opening is at 40% of the total length. The urostyle is flexion and the pleural and hyplural support bones are developed. Dorsal and anal finfolds were present, whilst the caudal fin rays had started to develop. Chromatophore pigment was present on the head, snout, operculum, and the dorsal part of the stomach, dorsal, ventral and mid line of the body.

At 1-day old (Fig. 2B) the larvae are 5.3 ± 0.1 mm long, have developed caudal fin rays and an increased level of chromate-

phore pigment on the operculum, dorsal and ventral parts of stomach, and the caudal fin.

By 3 days after hatching the larvae (Fig. 2C) are 6.6 ± 0.1 mm long and the mouth and caudal fin rays are completely developed. The dorsal anal finfold and finbase were starting to develop, and the amount of pigment on the head, mouth and ventral part of stomach had increased.

Five days after hatching the larvae (Fig. 2D) were 8.2 ± 0.2 mm long and the dorsal and anal fin rays had started to develop. The

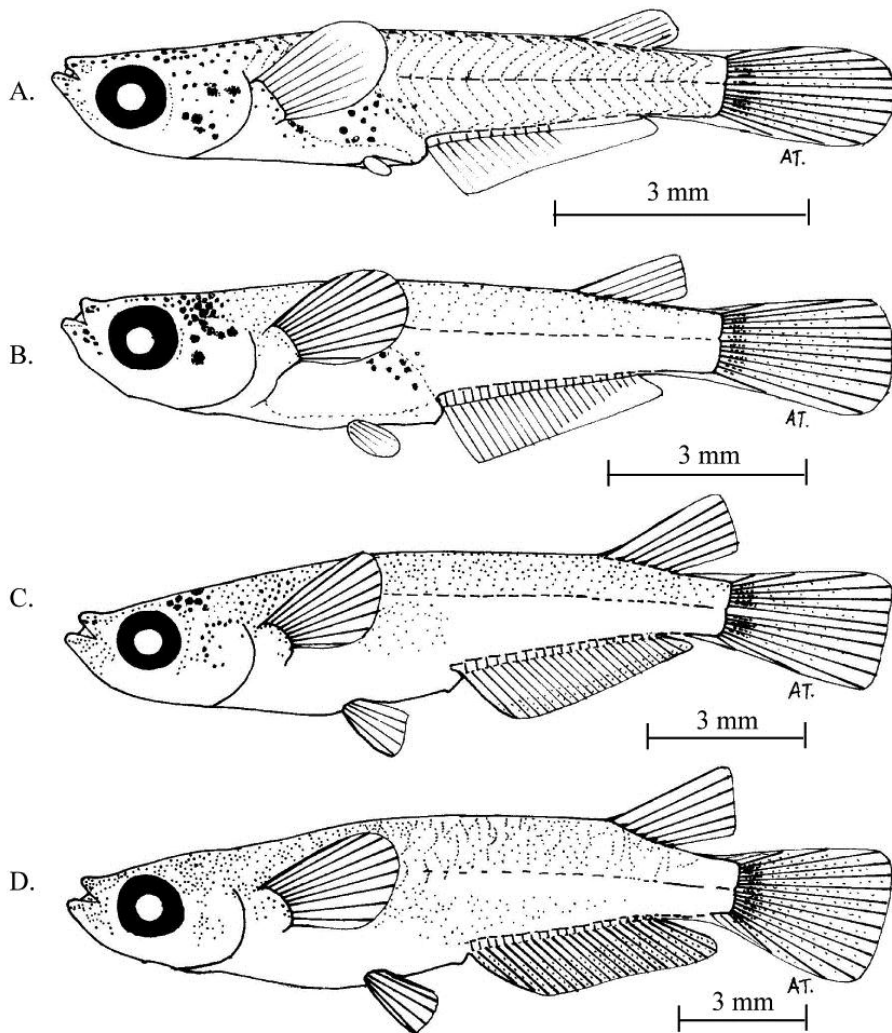


FIGURE 3. The developmental stages of the Hainan medaka ricefish (*Oryzias curvinotus*) from artificial breeding. **A**, 9.9 mm long 7-day old post larvae; **B**, 12.4 mm long 9-day old post larvae; **C**, 15.6 mm long 12-day old post larvae and **D**, 19.4 mm long 15-day old juvenile.

pigment level on the mouthparts and operculum had increased further.

At 7-days old the larvae (Fig. 3A) were 9.9 ± 0.2 mm long and the pectoral, dorsal and anal fin rays were clearly developing. The pectoral fin was starting to form from the finfold at the ventral part of the body in

front of the anus. The chromatophore pigment level was increasing on the mouth, operculum, pectoral finbase, stomach over the pelvic fin and the anterior dorsal parts of the body.

By nine days the larvae (Fig. 3B) were 12.4 ± 0.2 mm long. The pectoral and dorsal

fin rays were completely developed while the anal and pelvic fin rays were still developing. The chromatophore pigment

chromatophore pigment levels had increased further on the mouth, operculum, pectoral finbase and anal fin.

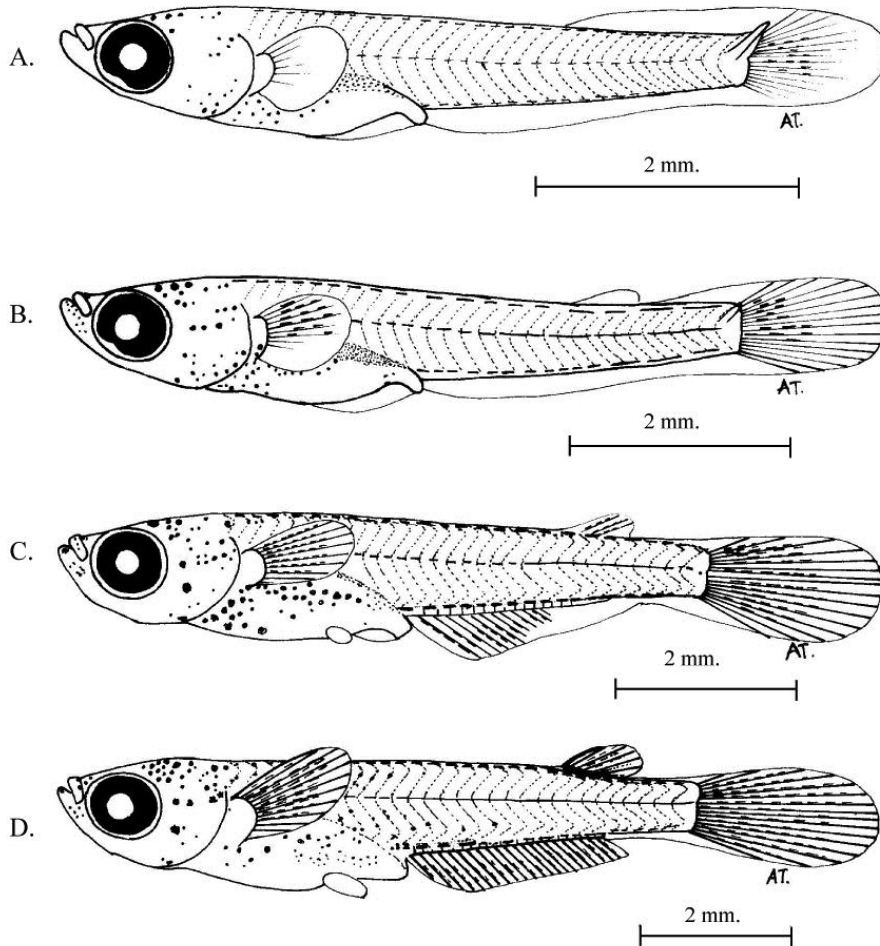


FIGURE 4. The developmental stages of the pectoral-fin spot medaka (*Oryzias pectoralis*) from artificial breeding. **A**, 6.2 mm long just hatched larvae; **B**, 7.4 mm long 4-day old larvae; **C**, 9.2 mm long 7-day old post larvae and **D**, 10.9 mm long 9-day old post larvae.

level was increasing on the dorsal part of body.

At 12-days old the larvae (Fig. 3C) were 15.6 ± 0.1 mm long with completely developed fin rays. The number of dorsal and anal fins was 17 and 23, respectively, and they had 5 - 6 pectoral fin rays. The

Finally, at 15-days old (Fig. 3D) the larvae were 19.9 ± 0.2 mm long, the scales were started to form and the chromatophore pigment levels were increasing on the ventral part of the body on the stomach to the caudal peduncle.

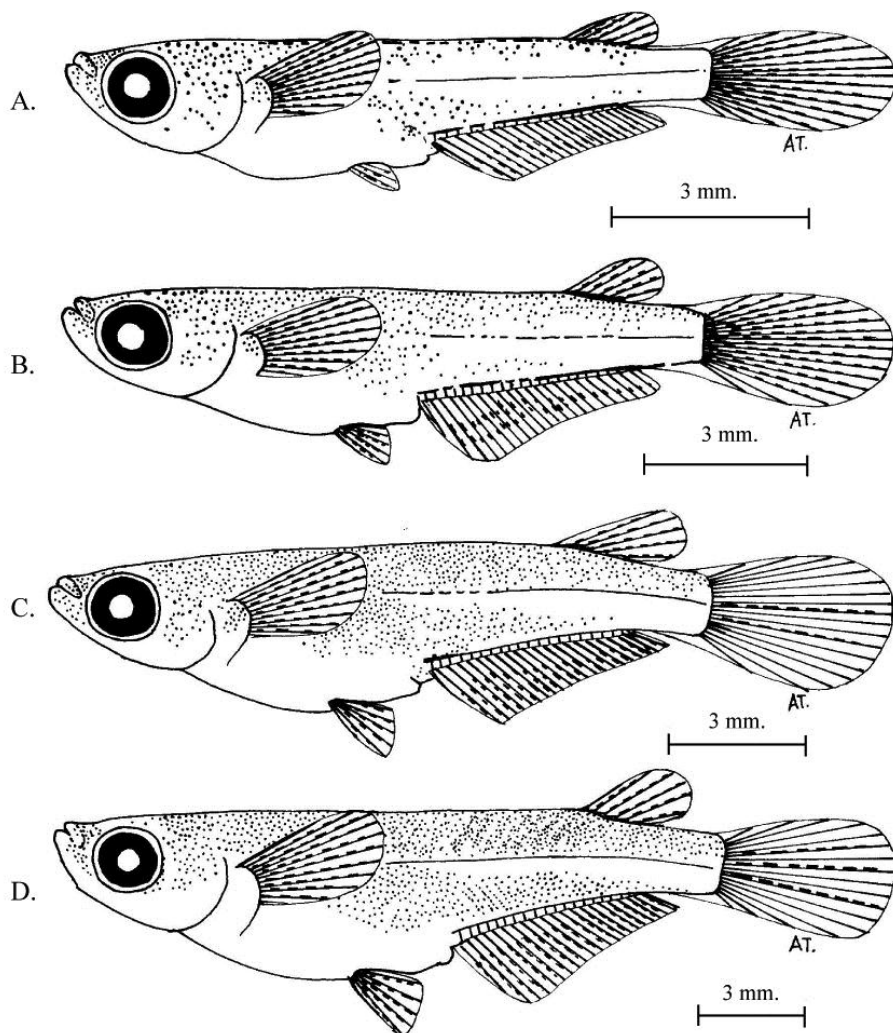


FIGURE 5. The developmental stages of the pectoral-fin spot medaka (*Oryzias pectoralis*) from artificial breeding. **A**, 12.7 mm long 12-day old post-larvae; **B**, 15.3 mm long 15-day old post-larvae; **C**, 18.3 mm long 19-day old post-larvae and **D**, 22.7 mm long 23-day old juvenile.

The developmental stages of the pectoral-fin spot medaka (*Oryzias pectoralis*) larvae.— As above, all data relates to artificially reared fish and not wild fish.

Two-day old larvae (Fig. 4A) were 6.2 ± 0.2 mm long with a laterally compressed body, a slightly depressed head, large eye (20% size of the head), open

mouth, the urostyle was flexion and the support bones were developed. The caudal fin rays were developed while the pectoral fin rays were developing. The dorsal and anal fin folds were not present. Chromatophore pigment was present on the dorsal, lateral and mid line of the body and also on the caudal fin.

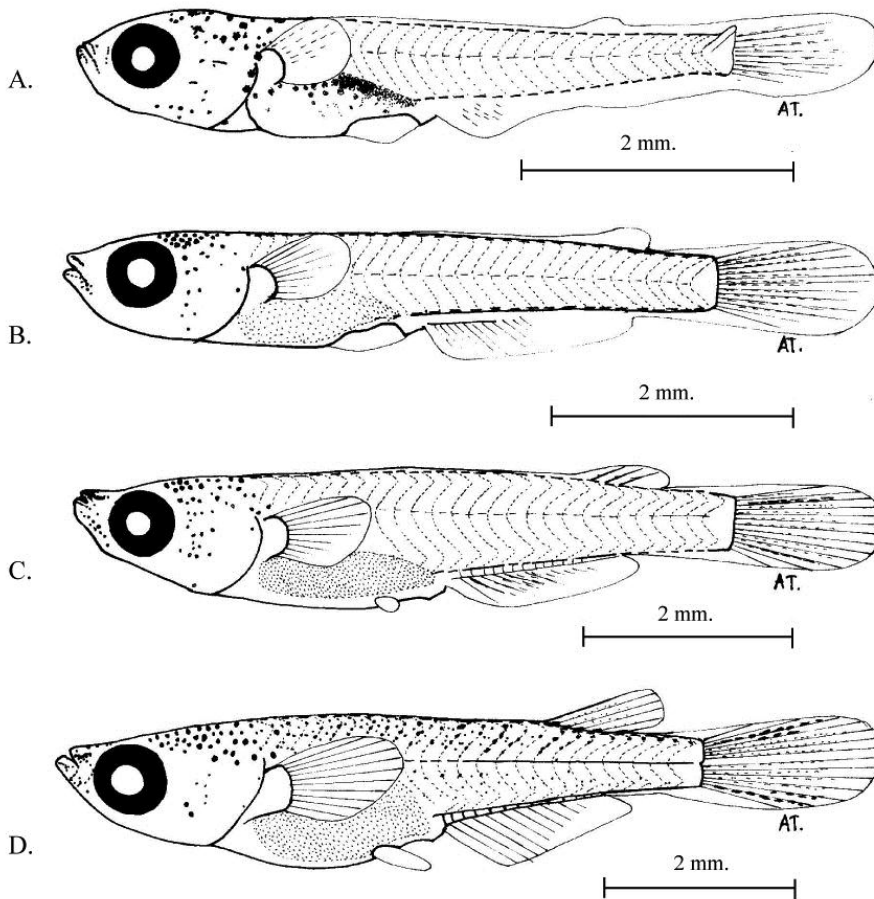


FIGURE 6. The developmental stages of the Songkharm river ricefish (*Oryzias songkharmensis*) from artificial breeding. **A**, 5.9 mm long 3-day old larvae; **B**, 6.4 mm long 5-day old larvae; **C**, 7.6 mm long 7-day old larvae and **D**, 8.6 mm long 9-day old larvae.

By five days after hatching the larvae (Fig. 4B) were 7.4 ± 0.2 mm long with developing dorsal and anal fins from the fin folds. The chromatophore pigments had increased on the head, caudal fin, and the anterior and dorsal parts of the stomach, and were also found on the pectoral fin and mandible part of the mouth.

The 7-days old larvae (Fig. 4C) were 9.2 ± 0.2 mm long and showed development of the pectoral, dorsal and anal fin rays with the start of the formation of the pelvic fin from the fold at the ventral part of the body

before the anus opening, and also the dorsal and anal fin rays. The chromatophore pigments had increased on the stomach, pectoral and caudal fins and were also found on the dorsal and anal fins.

At 9-days old the larvae (Fig. 4D) were 10.9 ± 0.2 mm long and had complete development of the pectoral, dorsal, anal and caudal fin rays, while the pelvic fin was developing. The chromatophore pigment had increased further on the head, dorsal and ventral parts of the body and appeared on the completely developed fin rays.

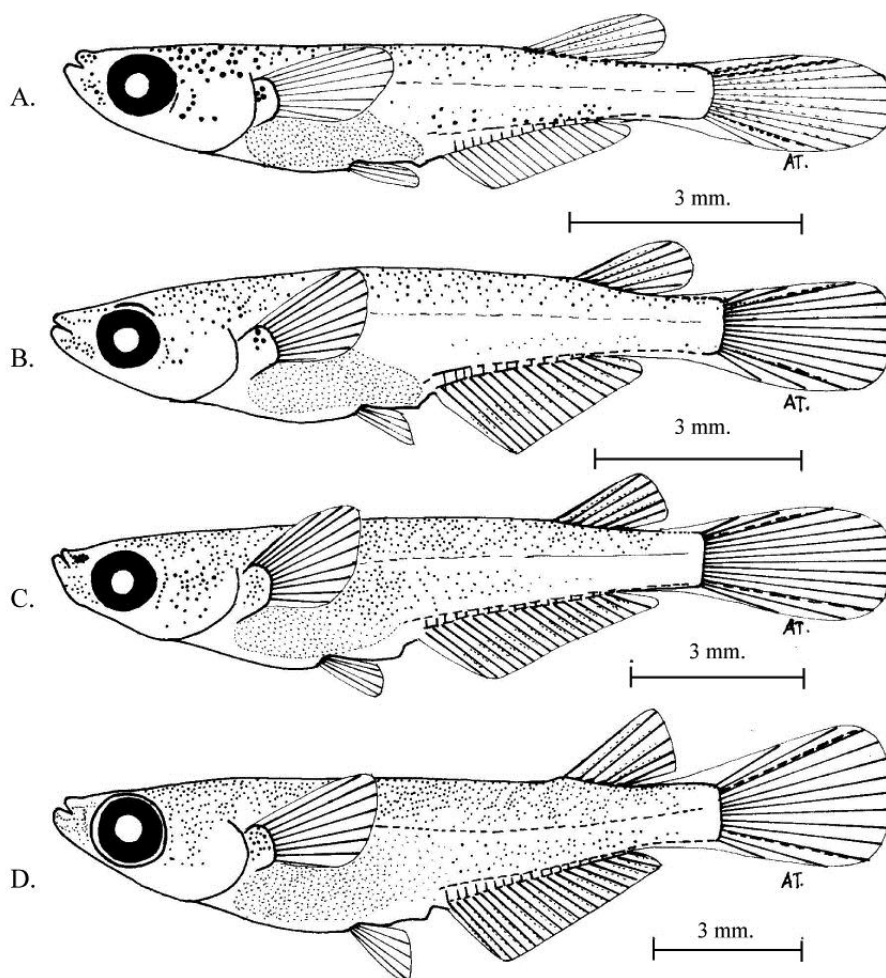


FIGURE 7. The developmental stages of the Songkharm river ricefish () from artificial breeding. A, 10.6 mm long 12-day old larvae; B, 11.9 mm long 15-day old larvae; C, 14.6 mm long 19-day old post larvae and D, 16.8 mm long 23-day old post larvae.

The 12-day old larvae (Fig. 5A) were 12.7 ± 0.2 mm long and visual evidence of muscle and the myomere systems had disappeared. The pelvic fin rays were developed and the chromatophore pigment had increased on the head, nape, and the dorsal and ventral parts of the body, and also appeared on the base of the pectoral and pelvic fins.

At 15-days old (Fig. 5B) the larvae had reached 15.3 ± 0.2 mm long and showed some further changes in the level of the chromatophore pigment, being increased on the head, dorsal and ventral parts of body.

At 19-days old (Fig. 5C) the larvae were 18.3 mm long and showed an increasing amount of chromatophore pigment on their operculum, the base of pectoral fin, and the dorsal and ventral parts of body, whilst that

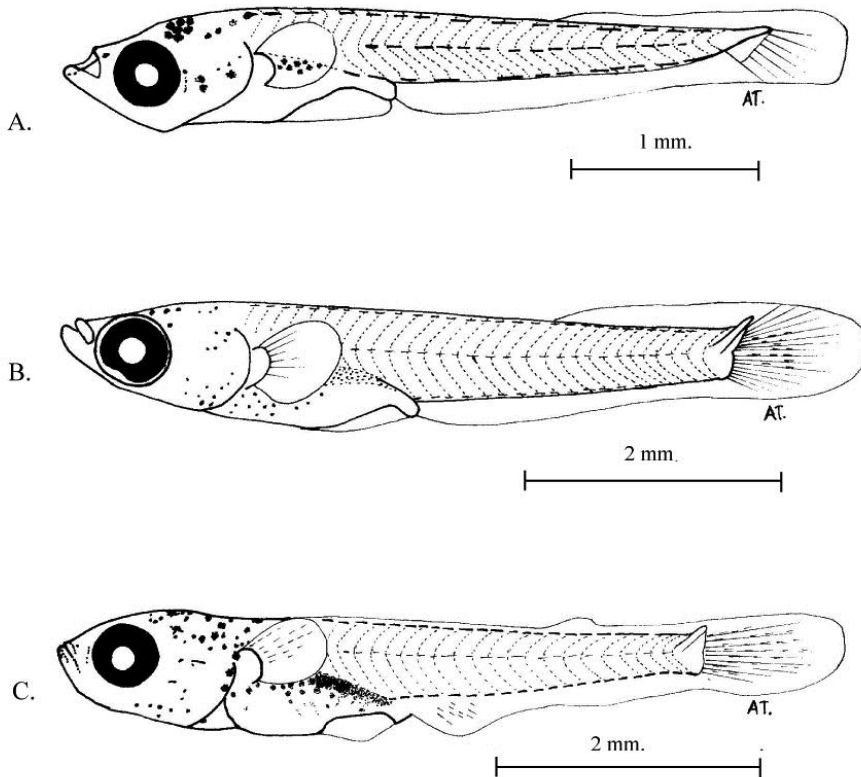


FIGURE 8. The differences in the meristic characters and chromatophore pigment levels in the larval stage of the three ricefish species. **A**, 1-day old (5.3 mm long) *O. curvinotus*; **B**, just hatched (6.2 mm long) *O. pectoralis*; and **C**, 3-day old (5.9 mm long) *O. songkharmensis*.

on the caudal fin had changed into a 2 dot line.

Finally, the 23-day old larvae (Fig. 5D) were 22.7 ± 0.2 mm long with clear signs of the scales starting to form at the dorsal part of the body. The chromatophore pigment on the caudal fin showed 3 dot lines at the center of the fin.

The developmental stages of the Songkharm river ricefish (*O. songkharmensis*).—As above all data relates to artificially reared fish and not wild fish.

The 1-day old larvae (Fig. 6A) were 5.9 ± 0.2 mm long with a long, slender and

laterally compressed body. The head was slightly depressed, the mouth open and the large eyes had a diameter of about one third of the head length. The urostyle is flexion and the support bones were developed. The pectoral and dorsal fin rays are developing, whilst the dorsal and anal fins were formed from the fin fold at that area. Chromatophore pigment is present on the dorsal and ventral parts of head, mouthparts, rim of the operculum and peritoneum membrane. There is also a chromatophore pigment ditch line along the dorsal, ventral and mid line of the body.

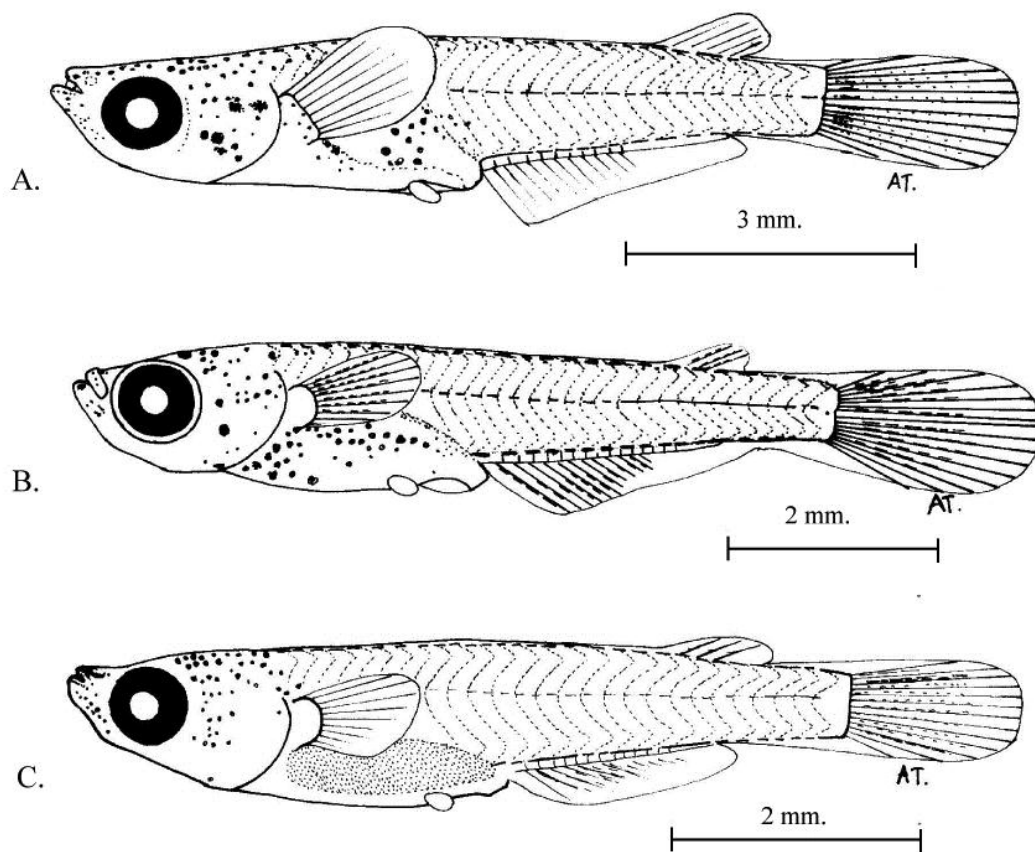


FIGURE 9. The differences in of the meristic characters and chromatophore pigment levels in the post larval stage among the three ricefish species (all are 7-days old). **A**, 9.9 mm long *O. curvinotus*; **B**, 9.2 mm long *O. pectoralis*; **C**, 7.6 mm long *O. songkharnensis*.

The 3-day old larvae (Fig. 6B) were 6.6 ± 0.13 mm long with the anterior part of the dorsal myomeres becoming flexion, and the pectoral, caudal, dorsal and anal fin rays are developing. The chromatophore pigment levels had increased on the mouth and the dorsal parts of the head, operculum and stomach.

At 7-days old (Fig. 6C) the larvae are 7.6 ± 0.14 mm long and show the flexion of myomeres as the dorsal and ventral parts of the body. This was the last fin that developed on their body. The caudal fin rays were completely developed, whilst the pectoral, dorsal and anal fin rays were

developing. Chromatophore pigment ditch lines are present on the dorsal and ventral parts of the caudal fin. The chromatophore pigment level had increased on the mouth, the dorsal parts of the head, and the opercula and stomach.

The 9-day old larvae (Fig. 6D) were 8.6 ± 0.15 mm long and had completely developed pectoral, dorsal and caudal fin rays whilst the anal fin rays were developing and the pelvic fin was forming. The chromatophore pigment level had increased on the mouth, and the dorsal parts of body from the head and nape to caudal part of body.

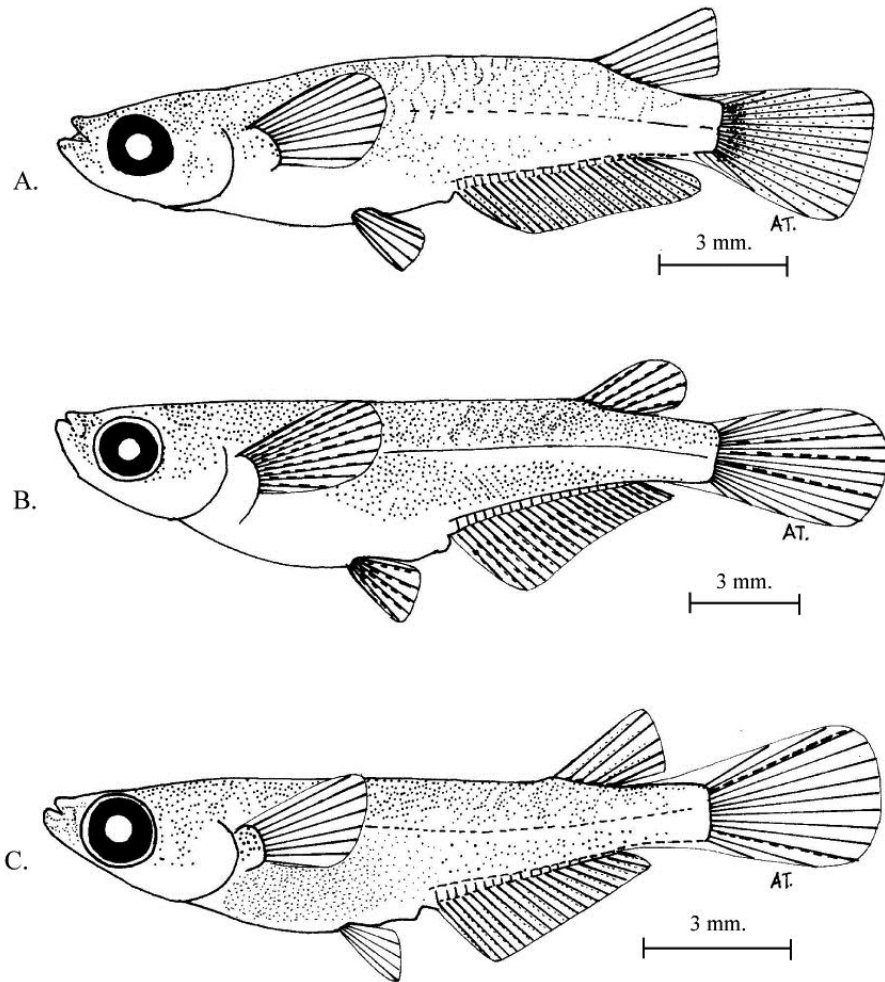


FIGURE 10. The differences in the meristic characters and chromatophore pigment levels in the juvenile stage among the three ricefish species. **A.** 5.3 mm long *Oryzias curvinotus*; **B.** 6.3 mm long *O. pectoralis*; **C.** 5.9 mm long *O. songkharnensis*. This should show the juvenile stage but here the legend is for larval, and indeed bar a size difference (6.3 vs 6.2 mm) is the same as figure 8 legend.

The 12-day old larvae (Fig. 7A) were 10.6 ± 0.21 mm long and had a thick myomere on their body obscuring the myoseptum. The pectoral fin rays were developing. The chromatophore pigment levels were increasing on the ventral parts of the body after the gut and on the base of pectoral fin and also were now present on the dorsal fin.

At 15-days old the larvae (Fig. 7B) were 11.9 ± 0.27 mm long with completely developed pelvic fin rays. The chromatophore pigment was present on the anal fin and showed increasing levels on the mouth, on dorsal parts of the body from the head to the caudal and ventral parts of the body.

The 19-day old larvae (Fig. 7C) were 14.6 ± 0.24 mm long and showed increased chromatophore pigment levels on their head,

mouth, pectoral fin base and both dorsal and ventral parts of the body, as well as on the dorsal and anal fins.

Finally the 23-day old larvae (Fig. 7D) were 16.8 ± 0.16 mm long and showed complete development, whilst the chromatophore pigment levels had increased on their whole body, the pectoral fin base, and the dorsal, anal and caudal fins. The scales were starting to form at the anterior dorsal part of the body.

Identification.— The larvae of the three species from the genus *Oryzias* described above (*O. curvinotus*, *O. pectoralis* and *O. songkharmensis*) can be identified to species from each other by using the different morphometric and chromatophore pigment patterns of each developmental stage.

Key to identified the larval stage of *Oryzias* larvae (Fig. 8A-C)

- 1a. Chromatophore pigment present on caudal fin.2
- 1b. Chromatophore pigment absent from caudal fin *O. curvinotus*
- 2a. Chromatophore pigment present on pectoral fin *O. pectoralis*
- 2b. Chromatophore pigment absent from pectoral fin. *O. songkharmensis*

Key to identified the post larval stage of *Oryzias* larvae (Fig. 9A-C)

- 1a. Chromatophore pigment present on dorsal fin2
- 1b. Chromatophore pigment absent from dorsal fin *O. curvinotus*
- 2a. Chromatophore pigment present on pectoral fin *O. pectoralis*
- 2b. Chromatophore pigment absent from pectoral fin *O. songkharmensis*

Key to identified the juvenile stage of *Oryzias* larvae (Fig. 10A-C)

- 1a. Chromatophore pigment absent from pectoral fin2
- 1b. Chromatophore pigment present on pectoral fin *O. pectoralis*
- 2a. Chromatophore pigment present on caudal fin *O. curvinotus*
- 2b. Band of chromatophore pigment present at the dorsal fin and ventral of caudal fin *O. songkharmensis*

DISCUSSION

There are three known species of ricefish in the genus *Oryzias* that occur in the northeastern part of Thailand and the countries along the Mekong River Basin. These are *O. songkharmensis* from the songkharm river area (a tributary of the Mekong River), *O. curvinotus* from Western China, Hainan and Hong Kong to Vietnam and *O. pectoralis* from the Indo-China area that occurs from Laos and North Vietnam. *Oryzias* larvae are well developed in the embryonic stage at the time of hatching, the larvae having passed through the yolk sac (prelarval) stage with complete absorption of the yolk sac before hatching. The larvae have well developed eyes, mouth, head and digestive tract, the urostyle is flexion and the caudal fin is developed upon hatching. The chromatophore pigment is present on their head, dorsal ventral and mid line of the body, but increases with their post-hatching development. The identification of the three species and developmental stages can be attained by using their meristic character (the number of dorsal, anal, pectoral and pelvic rays coupled with the chromatophore pigment pattern. However, for these three Thai species, the development of their dorsal pectoral and caudal fins were the

main characters used for identification between the three species.

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