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RESEARCH & KNOWLEDGE

Research Article

Development and identification of *Garra* Larvae in Thailand

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Abstract - This study provides new data on the developmental morphology for the identification of three species of fish found in Thailand, stone-lapping minnows (*Garra cambodgiensis*), sooty garra (*G. fuliginosa*), and Tenassarim garra (*G. notata*). Larval specimens were collected from the artificial breeding tanks and rearing ponds at the Mae Hong Son and Nan Fisheries Research Stations. The larvae were divided into four developmental stages, i.e., yolk sac, larval, post-larval, and juvenile. Their morphometric and meristic characteristics, including the number of myomere, dorsal-and anal-fin rays, and the chromatophore pigment patterns, were studied and used for the species identification. For the *Garra* larvae, the chromatophore pigmentation on the gut, midline, and dorsal and ventral parts of the body were the important characteristics. In addition, the juvenile stage can be distinguished by the chromatophore pigmentation on the gut, midline, and the dorsal and ventral parts of the body.

Keywords: Garras, yolk sac**,** fish larvae, myomeres, chromatophore pigment

1. Introduction

Family Cyprinidae (Cyprinid fishes) with 220 genera, or about 2,420 species, is the largest family of the freshwater fishes (Nelson, 2006). Genus *Garra* is a fresh water living fish belonging to this family that has a widely distribution range from China, Southeast Asia, India, and the Middle East as far as North and Central Africa. The majority of more than 140 species of *Garra* have been reported worldwide, but only seven species have been found in Thailand and Southeast Asia (Smith, 1945; Vidthayanon *et al*., 1997).

A recent survey study revealed details about the low water level in much of the Salween, Chao Phraya, and Mekong river basins, the exposition of traditional spawning grounds to dryness, insufficient natural reproduction, and also the rapid planning policy for artificial breeding culture and conservation of these species.

Fortunately, three species of *Garra*, *Garra cambodgiensis* (Tirant, 1884), *G. fuliginosa* (Fowler, 1937) and *G. notata* (Blyth, 1860), can be used as the artificial breeding specimens in the ponds at Mae Hong Son and Nan Fisheries Research Stations in the northern part of Thailand.

The habitat of *G. cambodgiensis* and *G. fuliginosa* is the Mekong River Basin in Thailand, Laos PDR, Cambodia and Vietnam. The Chao Phraya and Mae Klong river systems in the central and western parts Thailand and some small drainages in peninsular Thailand, especially Mae Tang Stream in Chiang Mai Province and Meuy River in Mae Hong Son Province, are reportedly also to be the habitats of these species (Rainboth, 1996; Kottelat, 2001; Vidthayanon *et al*., 1997). Whilst, *G. notata* (Blyth, 1860) has been found at the high altitudes between 120-1,500 m in rocky mountain streams, rapid zones and waterfalls of the Salaween and Great Tenassarim (Tarinntharyi) Basin in Myanmar, Yunnan (Southern China) and water courses adjacent to these region in the western part of Thailand, such as Meuy River in Mae Hong Son Province (Yang *et al*., 2012; Vidthayanon *et al*., 1997).

The typical characteristics of the *Garra* species differ from Garrina members by the first two pectoral-fin rays being thickened, fleshy and unbranched as well as a possession of 10-11 dorsal-fin rays. The numbers of lateral line scales in *G. cambodgiensis*, *G. fuliginosa* and *G. notate* were 32-35, 32-34 and 33-34, respectively (Kottelat, 2001; Rainboth, 1996).

Larval development in cyprinid fishes is well documented (Soda and Kimura, 2006). However, the morphological information of *G. cambodgiensis*, *G. fuliginosa* and *G. notata* larvae and juveniles in Thailand has not been reported yet. Here, we describe the comparative development of these three fishes.

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2. Materials and methods

Garras fish specimens at different ages from hatching to juvenile stage were collected from the breeding tanks. The larvae were divided into various stages, i.e., just hatching (yolk sac larval stage), larval stage at which the flexion of the urostyle was found, post larval stage at which the pelvic fins just began to develop and juvenile stage at which all the meristic characteristics were completely developed and also the scales began to develop. The specimens at different ages from just hatching, 6-hours, 12-hours, 1, 2, 5, 7, 8, 12, 15, 19, 23, 27, 31, 35, 40, 45 and 50-days old were collected and preserved in 10% formalin solution. After two weeks, an intensive study on the developmental characteristics and the drawing of the specimens were conducted. Thereafter, the specimens were transferred to 70% ethanol, preserved, and deposited at the National Inland Fisheries Institute Collection, Department of Fisheries, Ministry of Agriculture and Cooperation, Thailand.

The identification and determination of each developmental stage were carried out in the laboratory under a stereomicroscope and the drawing was performed with a camera lucida. The differences among the meristic characteristics and chromatophore pigment patterns were used for the identification of the fish species (Termvidchakorn and Hortle, 2013). Figure 1 shows the different larval stages of the *Garra*.

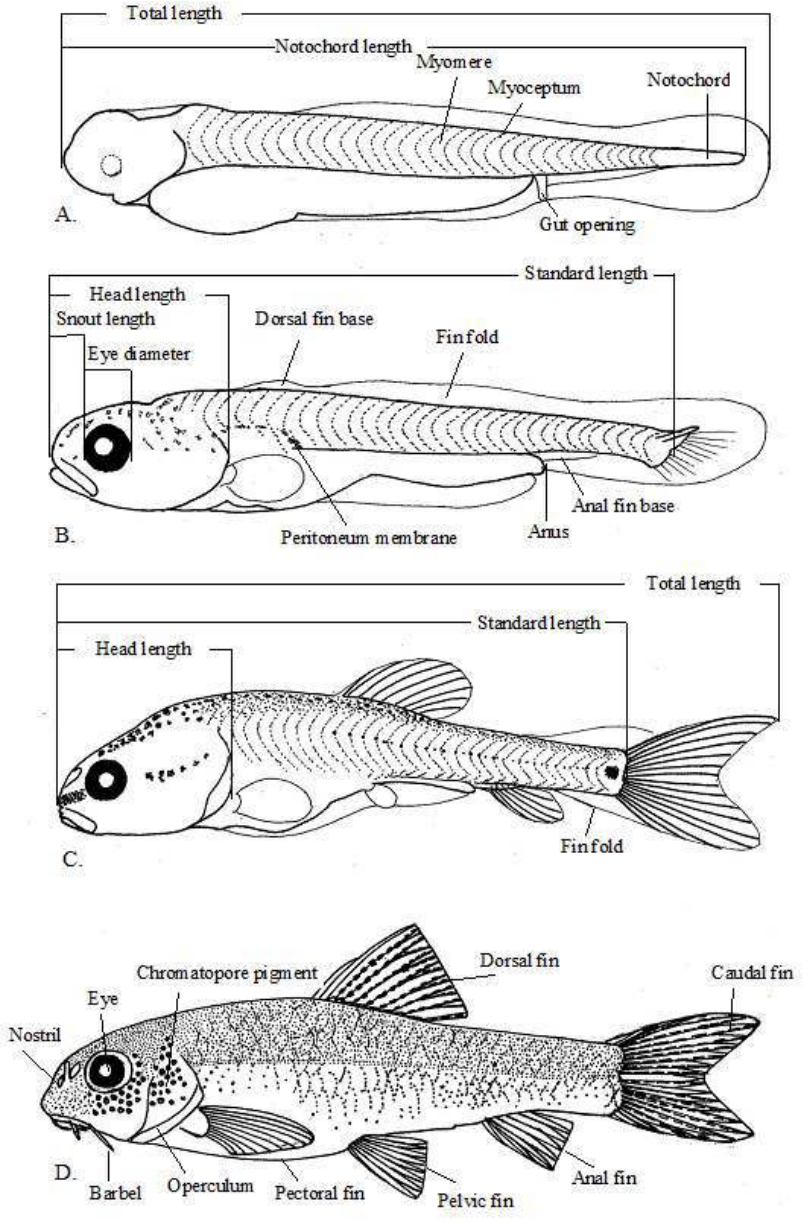

Figure 1. Morphological characteristics of *Garra* fish larvae and juvenile.

A. Yolksac larval stage B. Larval stage

C. Post larval stage D. Juvenile

3. Results

The developmental larval stages of the *Garra* (yolk sac stage, larval stage, post larval stage, and juvenile stage) showed the different characteristics and chromatophore pigment patterns.

3.1 Development of stone-lapping minnows (*Garra cambodgiensis*)

Six-hour old larva, 4.1 mm TL (Fig. 2A): The larva showed a long and slender body. The round body head was separated from the yolk and the body. The eyes and mouth had not developed yet. The yolk sac presented on the ventral of the body. The gut opening was about two thirds of the body. The number of myomere was 34 (24+10). The anterior part of the yolk was bigger than the posterior part. The fin fold covered both the dorsal and ventral parts of the body. Chromatophore pigments did not occur on the body.

One-day old larva, 4.6 mm TL (Fig. 2B): The characteristic features of the mouth part and eyes were developed. The yolk sac was smaller than that found in the previous stage because it was used for the development of the larva. The number of myomere was 34 (24+10). The gut opening was about two thirds of the body. The urostyle was straight. The pectoral fins were developed. The fin fold covered both the dorsal and ventral parts of the body. The chromatophore pigments occurred on the eyes, cheeks, head, peritoneum membrane, and the dorsal, ventral, and mid line of the body.

Seven-day old larva, 6.5 mm TL larvae (Fig. 2C): The mouth part comprising maxilla and mandible were developed. The yolk sac dispersing the gut opening was about two thirds of the body. The number of the myomere was 34 (23+11). The development of the fin fold at the caudal ray to the caudal fin was observed. The urostyle had a flexion. The supporting bone and caudal ray were also developed.

Fifteen-day old larva, 8.0 mm. TL (Fig. 2D): The larva showed the nearly complete development of the caudal fin and the development of the dorsal and anal fins from the fin fold. There were the dorsal and anal fins, and the anal fin base and rays. Whereas, the pectoral fins were not developed completely. There were 34 (23+11) myomeres on the body. Increasing melanophore pigments were found on the body, cheeks, and the dorsal, ventral, and mid line of the body. Big dots of melanophore pigments were found on the lateral and dorsal parts of the caudal peduncle, gut, and dorsal part of the body.

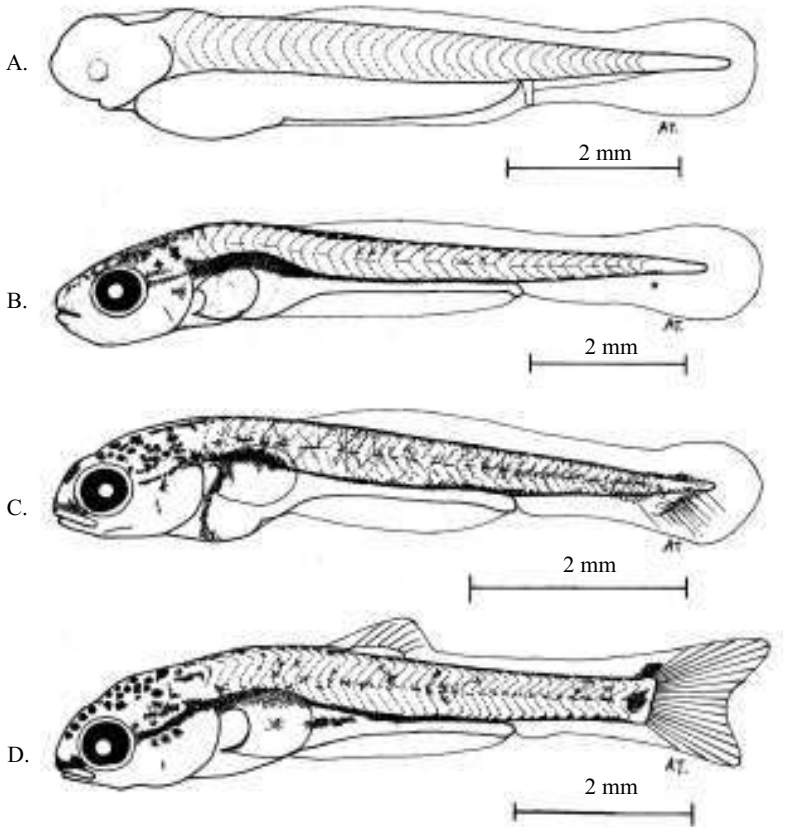

Figure 2. Developmental stages of stone-lapping minnows (*G. cambodgiensis*) from artificial breeding.

A. 6 hours larva about 4.1 millimeters in length.

B. 1 day old larva about 4.6 millimeters in length.

C. 7 days old larva about 6.5 millimeters in length.

D. 15 days old larva about 8.0 millimeters in length.

Nineteen-day old larva, 8.7 mm TL (Fig. 3A): The larva had a complete development of the dorsal, anal, and caudal fin rays, but they were small. The pelvic fins started to develop as a thin membrane at the ventral part of the body. After the origin of the dorsal fin, the presence of the fin fold was still observed. The nostrils were developing. The chromatophore pigments were increased on the snout, cheeks, and peritoneum membrane at the origin of the pectoral fins as a big hole on the head. The caudal fin developed from round to fork shaped.

Twenty seven-day old larva, 12.6 mm TL (Fig. 3B): The larva showed an increase in size of the pelvic fins and the development of the pelvic fin rays. The fin fold was still present between the pelvic fins. The anal fin was developed completely in both size and number of rays. The caudal fin showed a forked shape. The nostrils were developed. The chromatophore pigments were increased at the end of the mid line of the body, from the anterior part of the mid line and the dorsal part of the body.

Thirty five-day old larva, TL (Fig. 3C): The larva showed the development of the maxilla, barbells, and inferior mouth. The shape and size of the dorsal, anal, pelvic, and caudal fins were developed completely. The pectoral fins were developing their fin rays. The chromatophore pigments were increased on the dorsal part of the body and a band on the body mid line.

Forty five- day old juvenile, 27.5 mm TL (Fig. 3D) : The juvenile showed a complete development of the head, mouth, barbells, fins, and chromatophore pigment patterns. The scales were the last developmental part. It was similar to an adult fish.

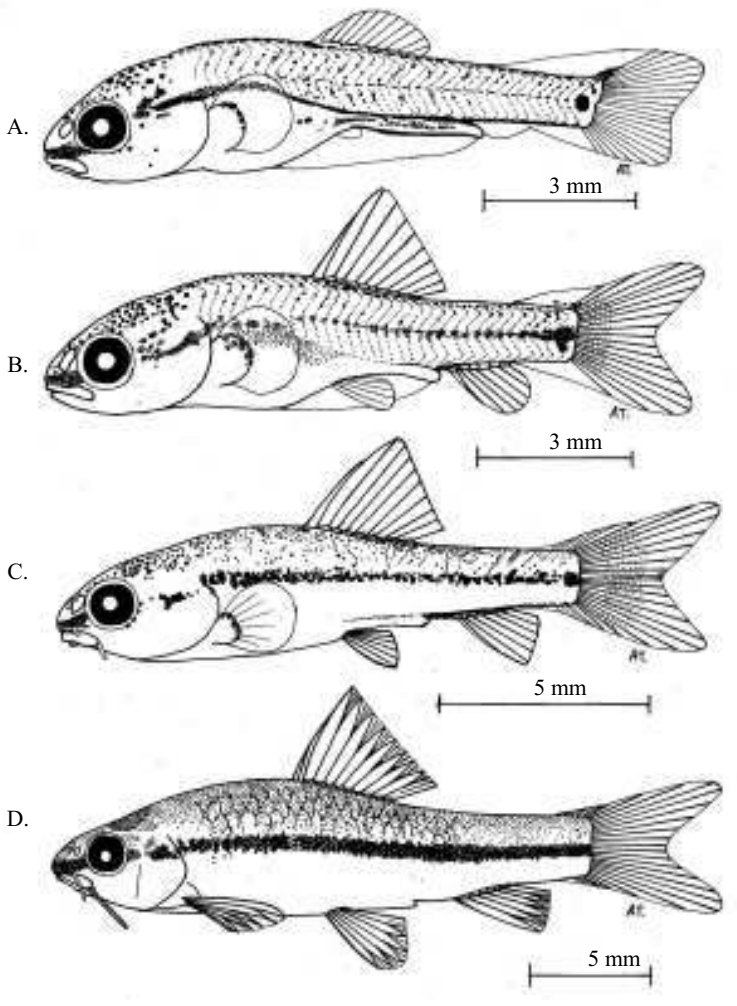

Figure 3. Developmental stages of stone-lapping minnows (*G. cambodgiensis*) from artificial breeding.

A. 19 days old larva about 8.7 millimeters in length.

B. 27 days old larva about 12.6 millimeters in length.

C. 35 days old larva about 16.1 millimeters in length.

D. 45 days old juvenile about 27.5 millimeters in length.

3.2 Development of sooty garra (*Garra fuliginosa*)

Six-hour old yolk sac larva, 3.7 mm TL (Fig. 4A): The yolk sac larva showed a long and slender body. The head was round and separated from the body. The mouth and eyes had not developed yet. The yolk sac occurred at the ventral part of the body, next to the head near the body. There were 33 (26+7) myomeres. The gut opening occurred about two thirds of the body. The urostyle was straight. The fin fold covered both the dorsal and ventral parts of the body. At this stage, an occurrence of chromatophore pigments on the body was observed.

One-day old larva, 3.9 mm TL (Fig. 4B): The larva showed the formation of the mouth and development of black eyes. The size of the yolk sac was decreased and the yolk was absorbed for the organ development. The urostyle was straight. The number of myomere was 32 (25+7). The pectoral fins and fin fold covered both the dorsal and ventral parts of the body.

Five-day old larva, 5.1 mm TL (Fig. 4C): The mouth part, maxilla, and mandible were developed. The flexile urostyle could be seen. The supporting bones (plural and hypural bone) and caudal fin rays were developed. The dorsal and anal fin bases were also developed. The gut opening was about two thirds of the body length. The chromatophore pigments occurred on the dorsal part of the head and peritoneum membrane.

Seven-day old larva, 7.7 mm TL (Fig. 4D): The caudal, dorsal, and anal fin rays of the larva were developed completely. The chromatophore pigments were increased on the dorsal part of the head and the body. A large black round chromatophore pigment patch covered the snout and the caudal part of the body, and was also on the mid line of the body next to the end of the dorsal fin.

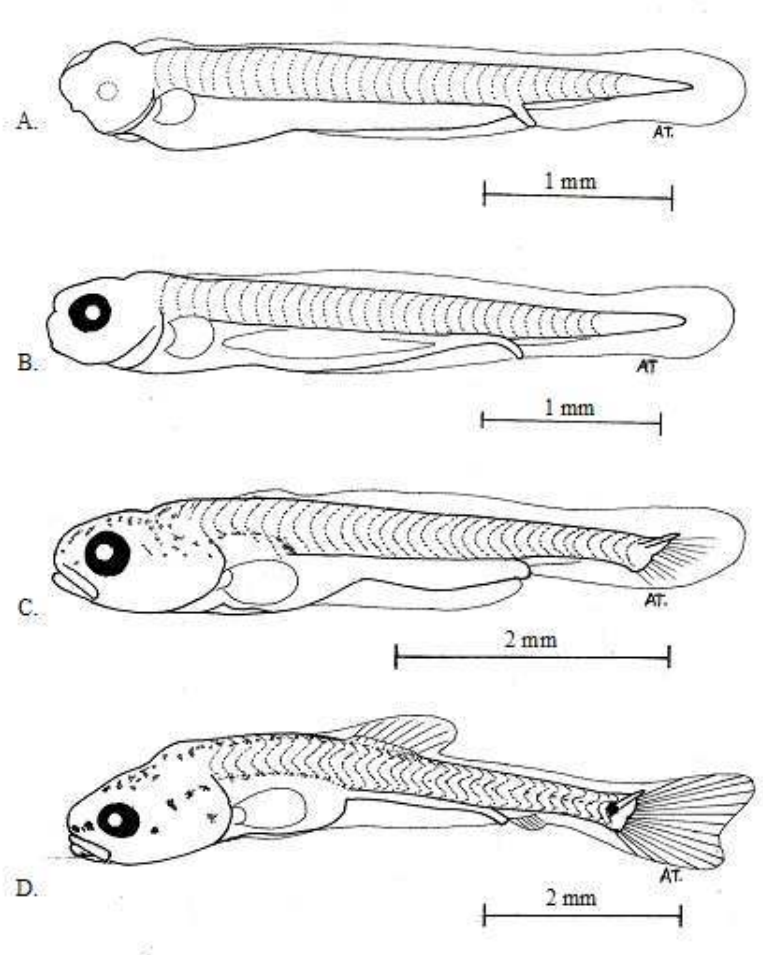

Figure 4. Developmental stage of sooty garra (*Garra fuliginosa*) from artificial breeding.
A. 6 hours larva about 3.7 millimeters in length.
B. 1 day old larva about 3.9 millimeters in length.
C. 5 days old larva about 5.1 millimeters in length.
D. 7 days old larva about 7.7 millimeters in length.

Eleven-day old larva, 9.4 mm TL (Fig. 5A): The larva showed the complete development of the dorsal, anal, and caudal fin ray numbers. The pelvic fins started to develop. The chromatophore pigments were increased on the dorsal part of the head, cheeks, along the dorsal part of the body, snout, and along the mid line of the body next to the origin of the dorsal fin.

Nineteen-day old larva, 13.7 mm TL (Fig. 5B) : The larva showed the development of the pectoral and pelvic fin rays while the maxillary barbells occurred at the angle of the maxilla. The mouth started to move downward. The chromatophore pigments were increased on the head, along the dorsal part of the body, a dark patch from the snout through the cheeks to the mid line of the body, and ending prior to the commencement of the caudal fin.

Thirty one-day old larva, 19.5 mm TL (Fig. 5C): The larva showed the complete development of the fins and barbells. The mouth moved more downward and became the inferior mouth. The increasing chromatophore pigments were found to be similar to those in the juvenile stage.

Fifty-day old juvenile, 29.2 mm TL (Fig. 5D): The larva had a complete development of the fins, mouth, barbells, and chromatophore pigment patterns. The scales started to develop as a juvenile fish.

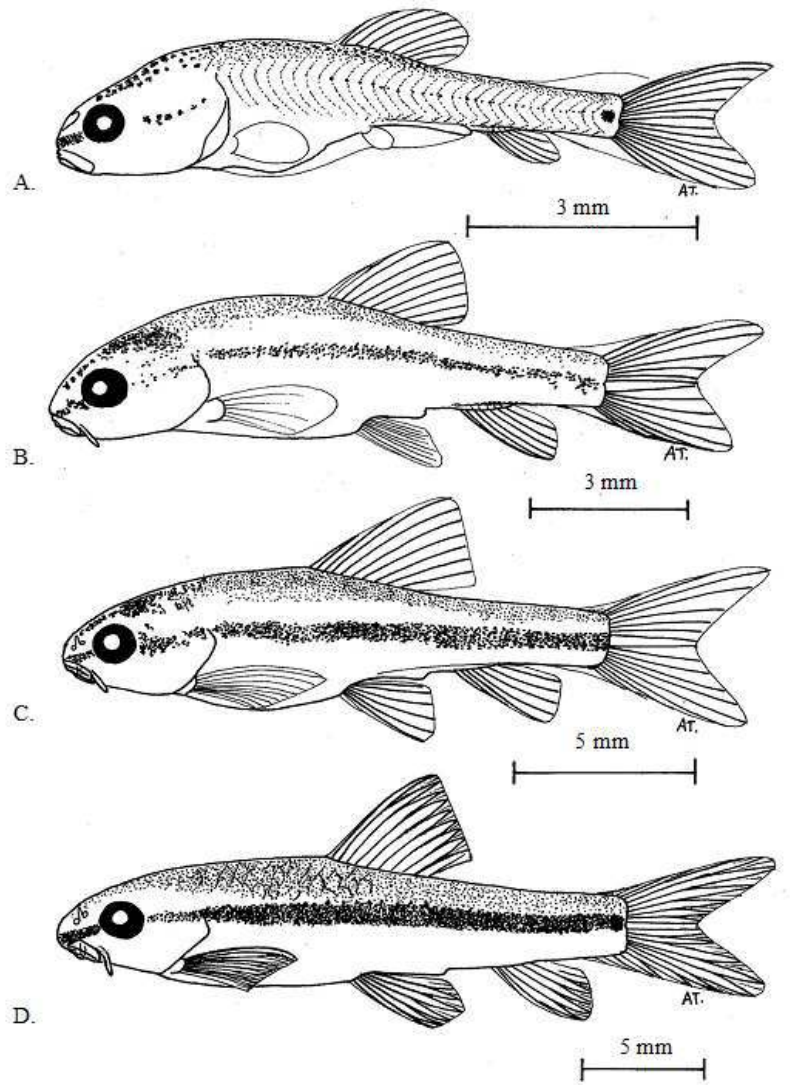

Figure 5. Developmental stage of sooty garra (*Garra fuliginosa*) from artificial breeding.
A. 19 days old larva about 8.7 millimeters in length.
B. 27 days old larva about 12.6 millimeters in length.
C. 35 days old larva about 16.1 millimeters in length.
D. 45 days old juvenile about 27.5 millimeters in length.

3.3 Development of Tenassarim garra (*Garra notata*)

Six-hour old yolk sac larva, 5.0 mm TL (Fig. 6A): The larva showed a long and slender body. The head was round and separated from the body. The mouth and eyes had not developed yet. The urostyle was straight. The yolk sac presented at the ventral part of the body at the anterior end next to the margin of the dorsal fin fold. It was small and parallel to the ventral part of the body. The gut opening was about two thirds of the body from the head. Thirty-four (34; 24+10) myomeres occurred on the body. The fin fold covered both the dorsal and ventral parts of the body. The pectoral fins presented just behind the head and on the dorsal part of the yolk. No chromatophore pigments occurred on any part of the body.

One-day old larva, 6.0 mm TL (Fig. 6B) : The larva showed the forming of the mouth. The yolk sac was smaller than that in the previous stage. There were 34 (22+12) myomeres occurring on their body. The fin fold covered both the dorsal and ventral parts of the body. The caudal fin was developed at the posterior end of the fin fold as the caudal area. The eyes were dark. The chromatophore pigment dots occurred on the head, mid line of the body, and peritoneum membrane.

Three-day old larva, 6.4 mm TL (Fig. 6C): The larva at this stage showed the flexion of the urostyle and the development of the supporting bones. The yolk sac was small and had nearly disappeared. The fin fold was divided into the caudal fin, but the dorsal and anal fins had not divided yet. The caudal rays were developing. The chromatophore pigments were increased on the head, cheeks, and along the body.

Seven-day old larva, 8.5 mm TL (Fig. 6D): Alterations found in the larva were the complete development of the caudal fin, while the dorsal and anal fins were developing from the fin fold at the anterior, dorsal and anal parts to be the fin bases and fin rays. However, the fin fold still covered the body. The maxilla and mandible were developed on a terminal shape. The nostrils were formed. The chromatophore pigments were increased on the head, snout, under the eyes, and on the anterior part of the caudal fin rays.

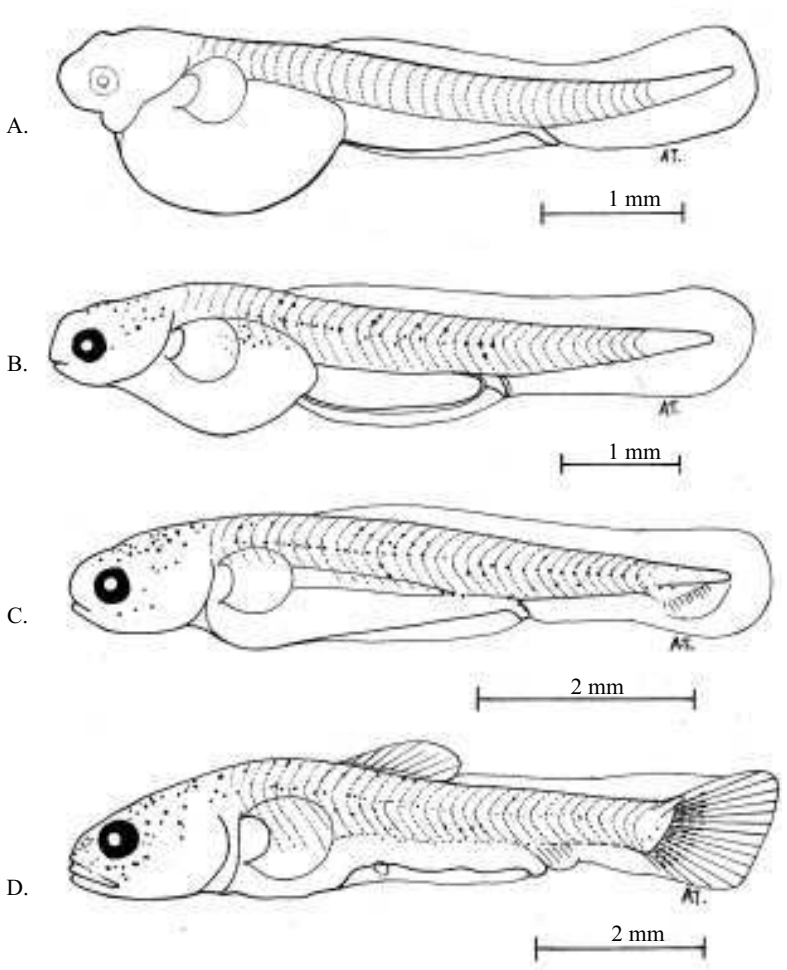

Figure 6. Developmental stage of Tenassarim garra (*Garra notata*) from artificial breeding.
A. 6 hours larva about 5.0 millimeters in length.
B. 1 day old larva about 6.0 millimeters in length.
C. 3 days old larva about 6.4 millimeters in length.
D. 7 days old larva about 8.5 millimeters in length.

Nine-day old larva, 10.7 mm TL (Fig. 7A): The larval head was developed prominently. The larva showed the development of the pelvic fins at the ventral part of the body and under the dorsal fin as a pair of sheets between the fin fold. The caudal fin became fork shaped. The chromatophore pigments were increased on the head, snout, cheeks, dorsal part of the body, and anterior part of the dorsal fin rays and caudal fin rays.

Twelve-day old larva, 12.5 mm TL (Fig. 7B): The larva showed the development of the barbells at the angle of the maxilla. The pelvic fins started to develop the rays. The nose was divided into two lobes. The chromatophore pigments were increased on the head, cheeks, and all over the body.

Fifteen-day old larva, 13.9 mm TL (Fig. 7C): The nose of the larva was divided into two lobes. The barbells on the maxilla were also formed with an anterior one on the maxilla. The mouth became more downward. The pectoral fins formed the rays, and the other rays were developed completely. The caudal fin showed the forked shape much better than that found in the previous stage. The chromatophore pigments were increased on the body and the dorsal and anal fins.

Forty-day old post-larva, 26.8 mm TL (Fig. 7D): The post-larva showed the characteristics the same as a juvenile for the inferior mouth with two maxillary barbells, the pectoral fins moved downward to near the belly, the caudal fin became a forked caudal fin. In addition, the scales were formed as the complete development of the larva.

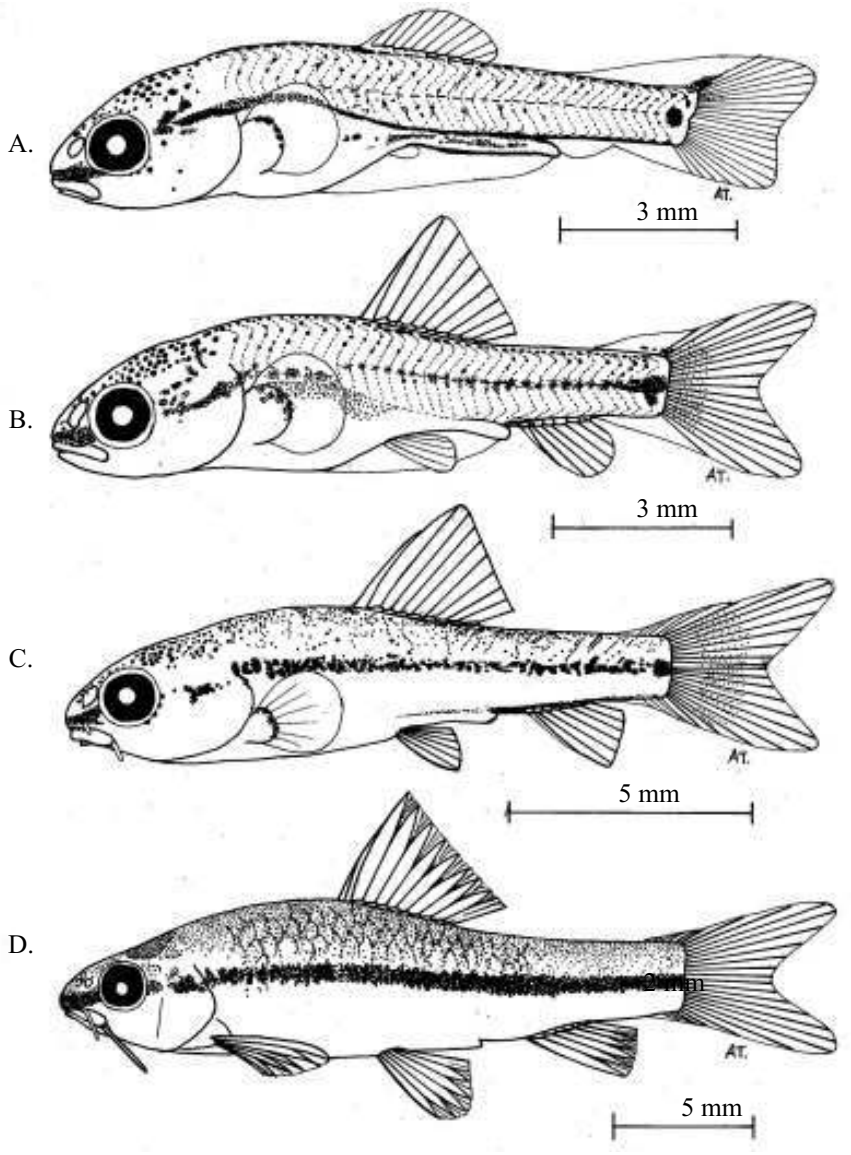

Figure 7. Developmental stage of Tenassarim garra (*Garra notata*) from artificial breeding.
A. 9 days old larva about 10.7 millimeters in length.
B. 12 days old larva about 12.5 millimeters in length.
C. 15 days old larva about 13.9 millimeters in length.
D. 40 days old post-larva about 26.8 millimeters in length.

3.4 Identification keys for 3 *Garra* species

Key to identifying yolk sac stage (Fig. 8)

1 a. The yolk sac looks like a rectangular shape…….. ……..…..……..…… 2
b. The yolk sac looks like an oval shape…….. ……..…..……..…*G. notata*
2 a. The anterior part of the yolk sac looks like an oval shape……..……..… *G. cambodgiensis*
b. The yolk sac looks like a rectangular shape…… ……..……..……..… *G. fuliginosa*

Key to identifying larval stage (Fig. 9)

1 a. The larvae have some pigments on head……..2
b. The larvae have many pigments on head……...… ……..… *G. cambodgiensis*
2 a. The larvae have a dark spot line at the mid line of the body…….…… ……. …….. *G. notata*
b. The larvae do not have a dark spot line at the mid line of the body…..…...…….. *G. fuliginosa*

Key to identifying post larval stage (Fig. 10)

1 a. The larvae have a dark band at the snout……..……...……..……2
b. The larvae do not have a dark band at the snout……..…… *G. notata*
2 a. The larvae have a dark band at the dorsal part of the body…..……...… *G. fuliginosa*
b. The larvae have a dark band at both the dorsal and ventral parts of the body …….. *G. cambodgiensis*

Key to identifying juvenile stage (Fig. 11)

1 a. The large band occurs on the mid line of the body ……..……...……..……2
b. The large band does not occur on the mid line of the body……..…… *G. notata*
2 a. The dark band occurs from the head to the dorsal part of the body……..…… *G. cambodgiensis*
b. The small dark band occurs on the dorsal part of the body…… ……..…….. *G. fuliginosa*

The larvae of the genus *Garra* were identified to species level using the differences in the morphometrics and chromatophore pigment patterns in each stage (Figure 8-11).

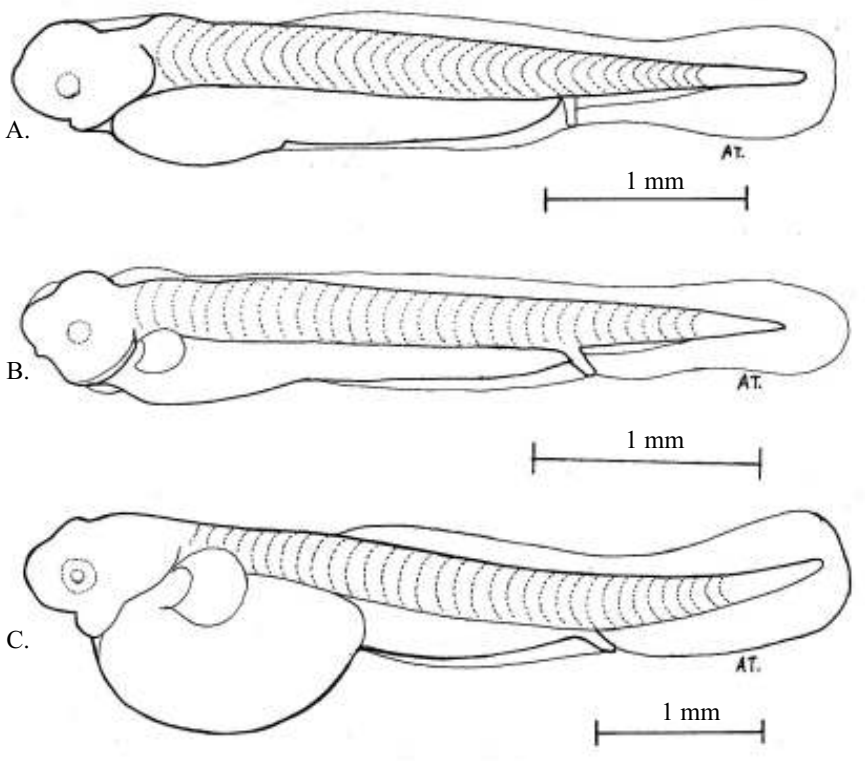

Figure 8. Species identification of yolk sac larval stage of genus *Garra* by comparison of yolk sac shape.
A. Stone lapping minnow (*Garra cambodgiensis*)
B. Sooty garra (*Garra fuliginosa*)
C. Tenassarim garra (*Garra notata*)

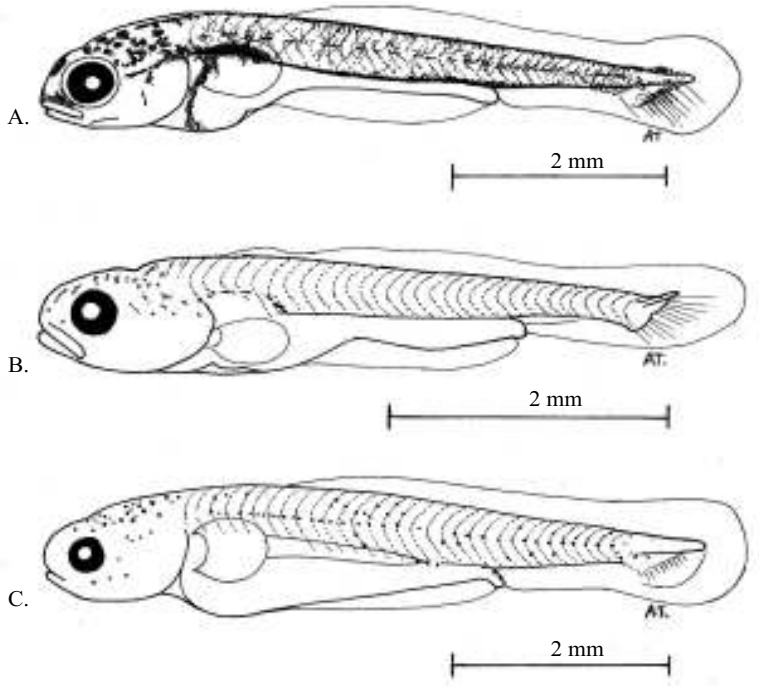

Figure 9. Species identification of larval stage of genus *Garra* by comparison of body shape and chromatophore pigment pattern.

A. Stone lapping minnow (*Garra cambodgiensis*)

B. Sooty garra (*Garra fuliginosa*)

C. Tenassarim garra (*Garra notata*)

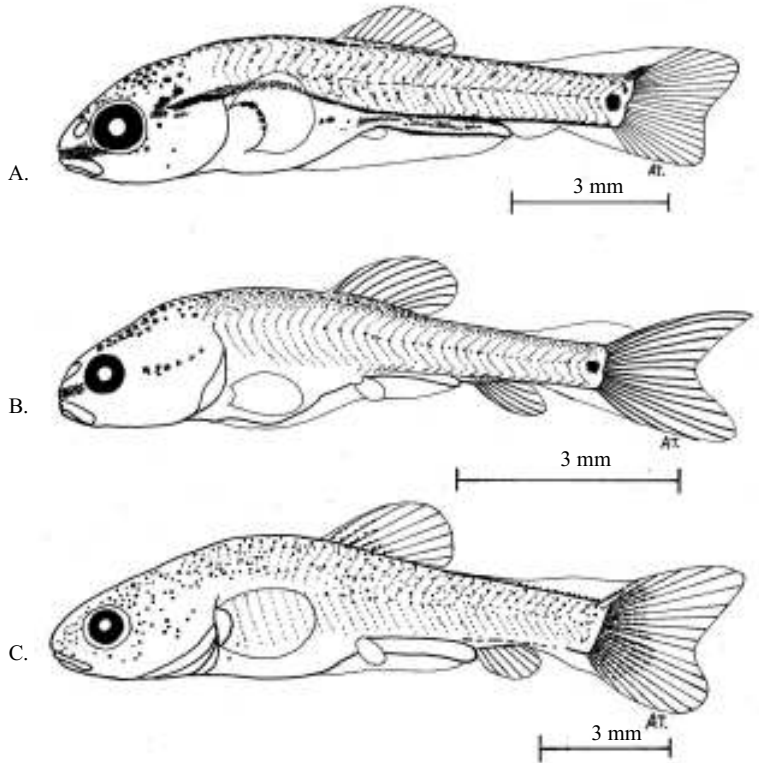

Figure 10. Species identification of post larval stage of genus *Garra* by comparison of body shape and chromatophore pigment pattern.

A. Stone lapping minnow (*Garra cambodgiensis*)

B. Sooty garra (*Garra fuliginosa*)

C. Tenassarim garra (*Garra notata*)

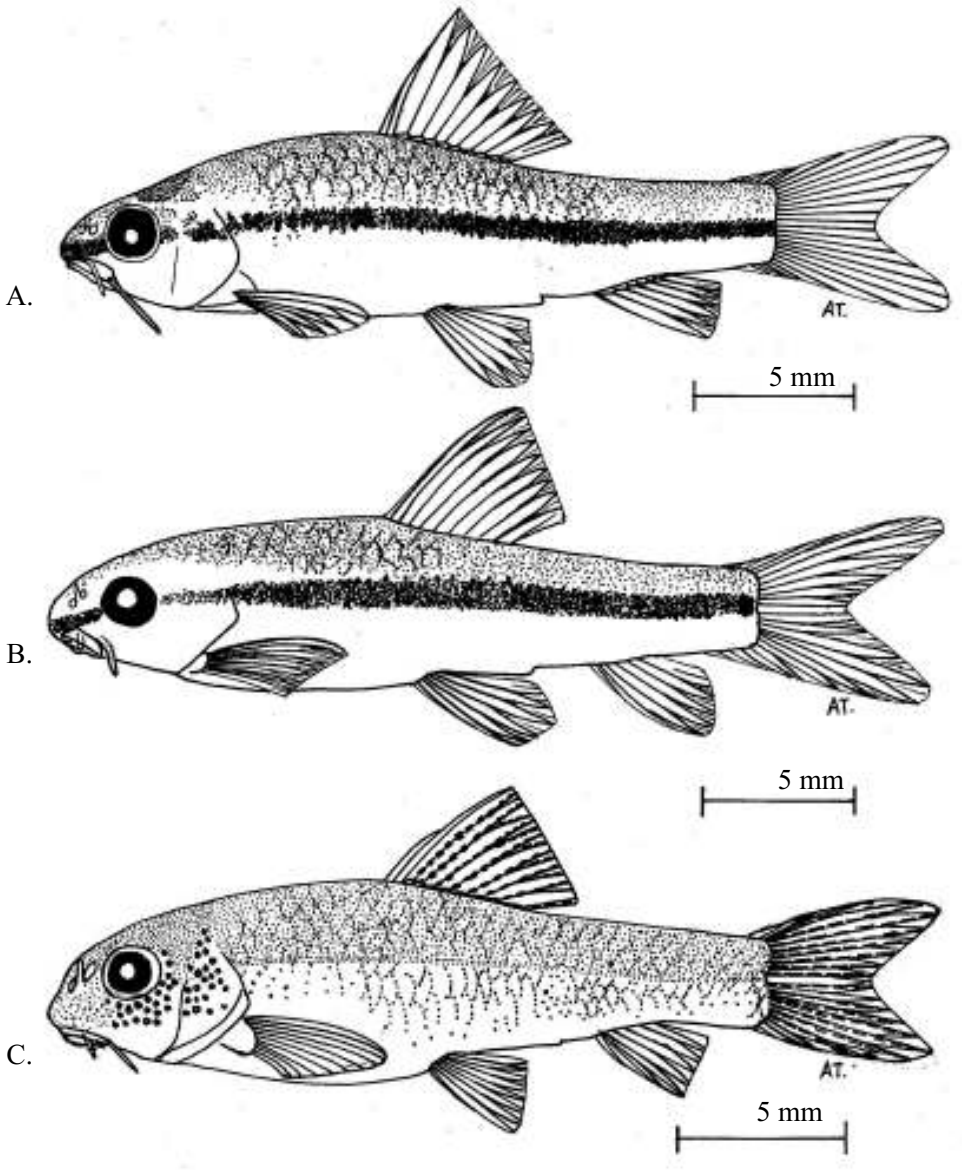

Figure 11. Species identification of juvenile stage of genus *Garra* by comparison of body shape and chromatophore pigment pattern.

A. Stone lapping minnow (*Garra cambodgiensis*)

B. Sooty garra (*Garra fuliginosa*)

C. Tenassarim garra (*Garra notata*)

4. Discussion and conclusion

The developmental identification of freshwater fish larvae in Thailand was carried out at the Department of Fisheries, Ministry of Agriculture and Cooperation, Thailand. The breeding specimens were used and examined accurately by comparing to natural specimens (Termvichakorn, 2003). According to the developmental larva of three garras, *G. cambodgiensis*, *G. fuliginosa,* and *G. notata,* at the six-hour-old point, no chromatophore pigment on any part of the body of the fishes was observed. In addition, *G. cambodgiensis* and *G. notata* had 34 (24 + 10) in the number of myomere and the shape of the yolk sac looked like an oval shape. Whilst, *G. fuliginosa* had 33 (26 + 7) myomeres and the shape of the yolk sac was similar to a rectangular shape.

In the larval stage of the three garras, the position of the chromatophore pigments on the fishes was used to identify the species. *G. cambodgiensis* had much more chromotophore pigments on the head, while *G. notata* had the chromotophore pigments looking like a dark spot line at the mid-line of the body. In contrast, a disappearance of the dark spot at the mid-line of the body was noted in *G. fuliginosa*.

In the post larval stage of these fishes, the different positions of the chromatophore pigments as a dark band on the bodies of the fishes were identified. *G. cambodgiensis* had a dark band occurring on both the dorsal and ventral parts of the body, while *G. fuliginosa* had a dark

band only at the dorsal part of the body. Whereas, the dark band at the snout disappeared in *G. notata*. The post larval stage can be recognized by the forming of scales. The scales were formed after the chromatophore pigment patterns showed the scale mark on the skin. Therefore, the chromatophore development is affected by the growing conditions. Further study should be conducted for the identification of the natural specimens to confirm whether the differences among the melanophore pigment patterns observed in larval and juvenile stages can be used to distinguish these fishes.

In the juvenile stage of the fishes, the dark band of the chromatophore pigments or melanophores was also used for the identification of juveniles. *G. cambodgiensis* had chromatophore pigments occurring from the head region to the dorsal part of the body, while *G. fuliginosa* had chromatophore pigments, which looked like a small dark band on the dorsal part of the body. On the other hand, the absence of the dark band in the middle of the body was observed in *G. notata*. Therefore, the complete development of the juvenile stage of the fishes was verified by the complete development of the morphology of the head, mouth, barbells, fins, and chromatophore pigment patterns. Interestingly, the scales were the last developmental part. Therefore, the fishes at the juvenile stage look like adult fishes.

The duration of the larval development in the fishes was based on the water temperature in the artificial breeding tanks and rearing ponds. In this study, the data indicated that *G. cambodgiensi* showed the shortest duration of the larval development from larvae to juvenile stages. The water temperature was the main factor affecting the larval development. The optimum temperature, a good condition for the development of the fish larvae, ranged from 28 – 30 ˚C (Safran, 2009).

5. Conclusion

Studies on the developmental stage and identification of fish larvae in the genus *Garra* in Thailand were carried out using artificial breeding specimens collected from the Mae Hong Son and Nan Fisheries Research Stations, and at the ages from just hatching to 50 day-old juvenile. At each larval stage, the differences among the morphometric and meristic characteristics, and chromotophore pigment patterns were observed in the different fish species. Therefore, the morphometric and meristic characteristics and chromotophore pigment patterns used in the present study can be applied for the identification of other fish species.

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