

## MORPHOLOGICAL DEVELOPMENT OF HATCHERY-REARED LARVAL AND JUVENILE MEKONG GIANT CATFISH *PANGASIANODON GIGAS*

*Yushiro Kinoshita*<sup>1</sup>, *Viseth Hav*<sup>1,2</sup>, *Fumihito Akishinonomiya*<sup>3</sup>,  
*Yasuhiko Taki*<sup>4</sup> and *Hiroshi Kohno*<sup>1\*</sup>

### ABSTRACT

Morphological development of *Pangasianodon gigas* was described for hatchery-reared 111 larvae and juveniles of 3.41–65.5 mm in body length (BL) sampled from day 0 to day 35. BLs of larvae and juveniles on day 0 were 3.87±0.30 (mean±SD) mm, reaching 22.1±0.53 mm on day 14 and 58.8±5.58 mm on day 35. Notochord tip flexion began in the smallest larva of 3.41 mm BL, and the completely flexed notochord tip was evident in specimens of about 9.00 mm BL. Melanophores were scarcely developed in the yolk sac larvae smaller than 7.13 mm BL and started appearing on many body parts in the flexion larvae 6.93–9.00 mm BL. The juvenile stage started at 20.9 mm BL, at which point some of the body proportions relative to BL were in the process of developmental change, while others had reached peak or constant values. The results were compared with those of such confamilial species as *Pangasianodon hypophthalmus*, *Pangasius bocourti* and *Pangasius larnaudii*.

Key words: Pangasiidae, morphology, ontogeny, developmental stage

### INTRODUCTION

The Mekong giant catfish *Pangasianodon gigas*, a species of the siluriform family Pangasiidae, is one of the world's largest freshwater fishes, attaining about 3 m in total length and 300 kg in body weight (SMITH, 1945; RAINBOTH, 1996; HOGAN, 2004). This species is endemic to the Mekong River basin (RAINBOTH, 1996; HOGAN, 2004; POULSEN *ET AL.*, 2004), occurring sporadically in the main channels of the Mekong River and its tributaries in Thailand, Lao PDR and Cambodia at present (HOGAN, 2013). Due to the deterioration of habitat conditions and insufficient stock management for the fish, its natural population has declined steeply (HOGAN *ET AL.*, 2001; POULSEN & VIRAVONG, 2002; MITCHELL & BRAUN, 2003; HOGAN, 2004; POULSEN *ET AL.*, 2004; FROESE & PAULY, 2014). Therefore, the fish is now categorized as “critically endangered” in IUCN Red List (IUCN, 2014), and its international trade is controlled by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), listed in Appendix I (CITES, 2014). The Department of Fisheries of the

---

<sup>1</sup> Laboratory of Ichthyology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

<sup>2</sup> Fisheries Administration, Ministry of Agriculture, Forestry and Fisheries, Cambodia

<sup>3</sup> Tokyo University of Agriculture, 1737 Funako, Atsugi-shi, Kanagawa 243-0034, Japan

<sup>4</sup> Nagao Natural Environment Foundation, 3-3-7 Kotobashi, Sumida-ku, Tokyo 130-0022, Japan

\* Corresponding author. E-mail: hirokun@kaiyodai.ac.jp

Received 6 April 2015; accepted 28 July 2015.

Ministry of Agriculture and Cooperatives of Thailand succeeded in the artificial propagation of the fish using wild-caught adult specimens in 1983, and the seed production of the fish has been conducted also in private fish farms using pond-raised broodstocks (ROBERTS & VIDTHAYANON, 1991; Prachya Musikasinthorn, 2014, personal communication).

The establishment of artificial propagation systems, while being a great contribution to the conservation of this endangered species, has resulted in the occurrence of the fish outside its original distribution range due to arbitrary releasing of fingerlings. This fish has been seen in such areas as the Mekong Delta in Vietnam and the Chao Phraya River system in Thailand in recent years, and hybridization with other local pangasiid species is a concern (Prachya Musikasinthorn, 2014, personal communication). Now local pangasiid faunas in these river systems are in need of strict management, and the early morphological development and larval/juvenile taxonomy of pangasiids will be among the basic data necessary for their stock management in natural waters as well as for their ontogenetic and phylogenetic investigations.

As a part of our research series on the morphological development of pangasiids, we described the larvae and juveniles of *Pangasius larnaudii* (VISETH ET AL., 2011) and *P. bocourti* (VISETH ET AL., 2013), and those of *Pangasianodon hypophthalmus* were described by ISLAM (2005), BARAS ET AL. (2010) and MORIOKA ET AL. (2010). However, as to the larval and juvenile morphology of *Pangasianodon gigas*, little information is available: the presence of mandibular barbels and teeth on jaws and palate in early developmental stages was confirmed by MEENAKARN (1984) and FUMIHITO (1989); some figures were drawn by Apichart Termvidchakorn in the work of ROBERTS & VIDTHAYANON (1991) without any additional comments. The purpose of this study is thus to describe the external morphologies of larval and juvenile *Pangasianodon gigas* and to compare them with those of other pangasiids.

## MATERIALS AND METHODS

The *Pangasianodon gigas* larvae and juveniles used in this study originated at the spawning and rearing facilities of Phayao Inland Fisheries Station of Department of Fisheries, Phayao Province, Thailand. The larvae and juveniles were reared at ambient water temperature. Samplings of 6-10 fish larvae and juveniles were carried out every day from hatching (day 0) to day 8 and on days 10, 12, 14, 21, 28 and 35; these specimens were fixed and preserved in 5% formalin solution immediately after collection. A total of 111 specimens 3.41–65.5 mm in body length (BL) used in this study are deposited in the Museum of the Tokyo University of Marine Science and Technology under catalog numbers MTUF-P(L) 26641 and 26642.

The general morphology, fin development and pigmentation of all of the specimens were observed, and the following nine body dimensions were measured and expressed as a percentage of BL: eye diameter, head length, maxillary barbel length, maximum body depth and its position, mouth width, pre-anal length, upper jaw length, and snout length. The nine body dimensions of the specimens sampled from day 0 to day 12 (3.41–16.0 mm BL) were measured under a binocular microscope with an ocular micrometer, and those from day 14 and older (20.9–65.5 mm BL) were measured by using a dial caliper; both types of measurement were recorded to the nearest 0.01 mm. The myomeres were counted on the specimens from days 1 to 12 ( $n = 52$ ). The volume of the yolk and oil globule was computed by applying the equation of BLAXTER & HEMPEL (1963):  $V = \pi/6 \cdot l \cdot h^2$ , where  $l$  is length and  $h$  is height. “Yolk volume” in this study refers to the combined volume of the yolk and oil globule. Measurement and counting methods mainly followed those of LEIS & TRNSKI (1989), VIDTHAYANON (1993), POUYAUD ET AL. (1999) and HUBBS & LAGLER (2004).

## RESULTS

### Growth

The mean BL ( $\pm$  SD) of newly hatched larvae on day 0 was  $3.87 \pm 0.30$  mm ( $n = 7$ ) (Fig. 1), and the larvae grew slowly from  $6.93 \pm 0.14$  mm BL (6) at day 2 to  $12.3 \pm 3.51$  mm BL (3) at day 12. Thereafter, the larvae/juveniles grew rapidly to  $22.1 \pm 0.53$  mm BL ( $n = 8$ ) at day 14, to  $31.3 \pm 3.54$  mm BL (9) at day 21, and to  $58.8 \pm 5.58$  mm BL (7) at day 35.

### Notochord flexion

The smallest specimen (3.41 mm BL, day 0) had a slightly upward-bent notochord tip ( $30^\circ$  angle, Fig. 2). The notochord flexion angle remained between  $20^\circ$  and  $40^\circ$  at 7–8 mm BL. Subsequently, the notochord flexion progressed with larval growth to  $40$ – $50^\circ$  at 8–9 mm BL and was complete, about  $45^\circ$  to  $54^\circ$  in specimens larger than about 9 mm BL (day 10). The angle could not be measured in specimens larger than about 16 mm BL (day 12) because of heavy pigmentation on the caudal fin base (Figs. 2, 3h).

### General morphology

Drawings of larvae and juveniles collected on days 0, 1, 2, 3, 5, 7, 10, 12 and 21 are shown in Figure 3, and photos of specimens on days 21, 28 and 35 are presented in Figure 4, showing the gross morphology.

The head and body of newly hatched larvae (3.41 to 4.14 mm BL) were transparent and compressed laterally, and a large spherical yolk sac was located at the anterior part of the body (Fig. 3a); their yolk sac length and height ranged from 1.06 to 1.30 mm (mean  $\pm$  SD =  $1.21 \pm 0.08$  mm;  $n = 7$ ) and from 0.75 to 1.20 mm ( $1.02 \pm 0.15$  mm), respectively, and the yolk volume was  $0.69 \pm 0.21$  mm<sup>3</sup>. The yolk volume decreased to  $0.37 \pm 0.10$  mm<sup>3</sup> at day 1 (6.65 to 6.98 mm BL,  $n = 10$ , Fig. 3b),  $0.04 \pm 0.03$  mm<sup>3</sup> at day 2 (6.70 to 7.13 mm BL,  $n = 6$ , Fig. 3c), and was completely absorbed by day 3 at a BL of 6.93 to 7.65 mm ( $n = 8$ , Fig. 3d).

The mouth and anus were open, and the eyes were well pigmented in the day 1 specimens (Fig. 3b), in which the posterior end of upper jaw reached beyond the vertical line of the posterior edge of eyes. The nostril buds were well developed at day 1 (Fig. 3b), their shape having changed from round to slender gourd-shaped with larval growth, and they were divided into two portions by 8.64 mm BL (day 7, Fig. 3f).

The buds of maxillary barbel appeared on day 0, whereas those of mandibular barbel were observed first on day 1 (Fig. 3b, c). The maxillary and mandibular barbels began to develop through elongation at day 1. The lengths of both maxillary and mandibular barbels increased with increasing size of the larvae/juveniles (Fig. 3b–j).

Myomeres numbered 17–19 + 26–29 = 44–48 in the day 1 to 12 specimens. In larger/older specimens, myomeres were not countable because of lack of body transparency.

### Fin development

The day 0 specimens (3.41 to 4.14 mm BL) had a fin-fold originating from the central part of the dorsal contour, continuing around the caudal region and ending at the yolk sac; the height of the fin-fold was almost constant, except for a tapering at the anterior starting point on the dorsal side (Fig. 3a). The fin-fold was constricted around the caudal peduncle

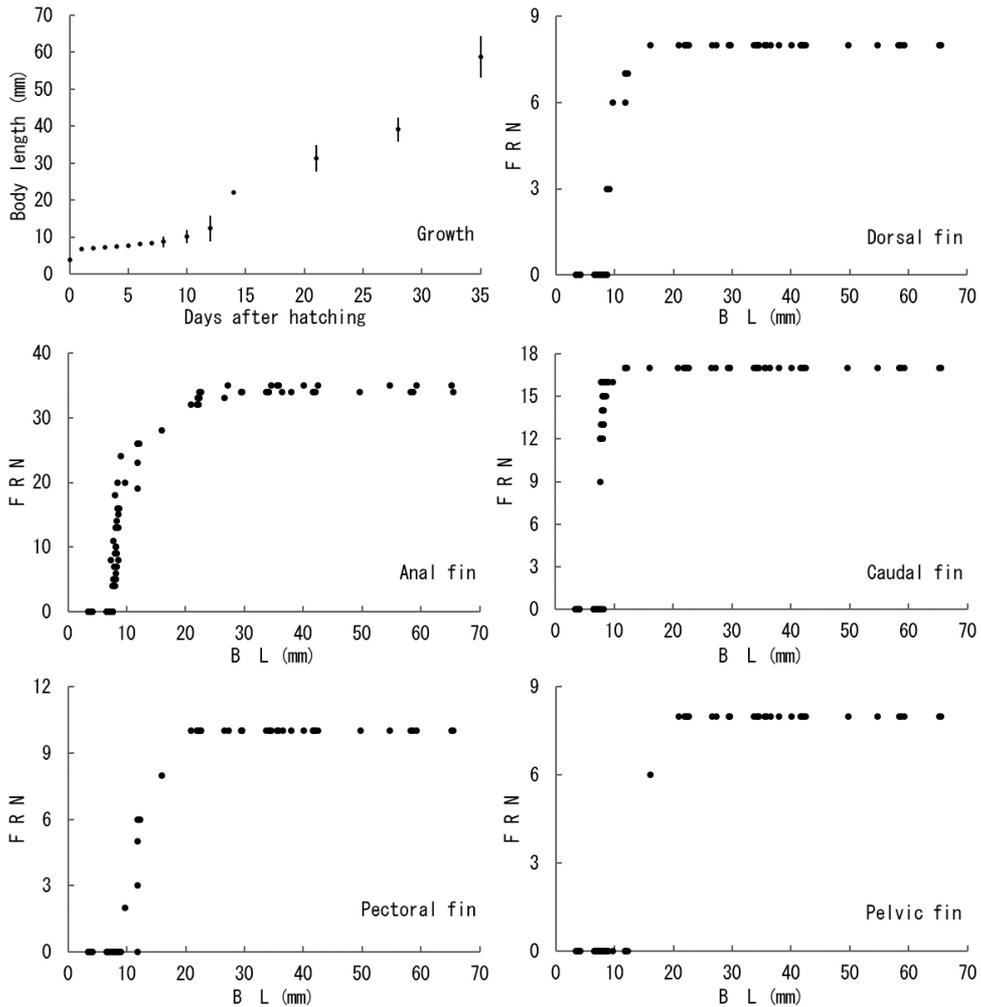


Figure 1. Growth and relationships between body length (BL, mm) and fin ray numbers (FRN) in hatchery-reared larval and juvenile *Pangasianodon gigas* from day 0 to day 35 after hatching. Solid circles indicate means and vertical bars standard deviations.

in specimens of 6.55 mm BL and larger (Fig. 3b), and it became separated dorsally into the adipose and caudal-anal fin-folds at 8.28 mm BL (day 7, Fig. 3f for 8.64 mm BL). The anal and caudal fins became separated at 20.9 mm BL on day 14 (Fig. 3i), in which the pre-anal fin-fold was completely lost.

The dorsal fin bud first appeared at 6.84 mm BL on day 2 in a position anterior to the adipose fin-fold (Fig. 3c). The first dorsal fin-ray formation was apparent at 8.70 mm BL, when 3 soft fin rays were observed (Fig. 1); a full complement of fin rays (I, 7) was attained at 16.0 mm BL (day 12, Figs. 1, 3h).

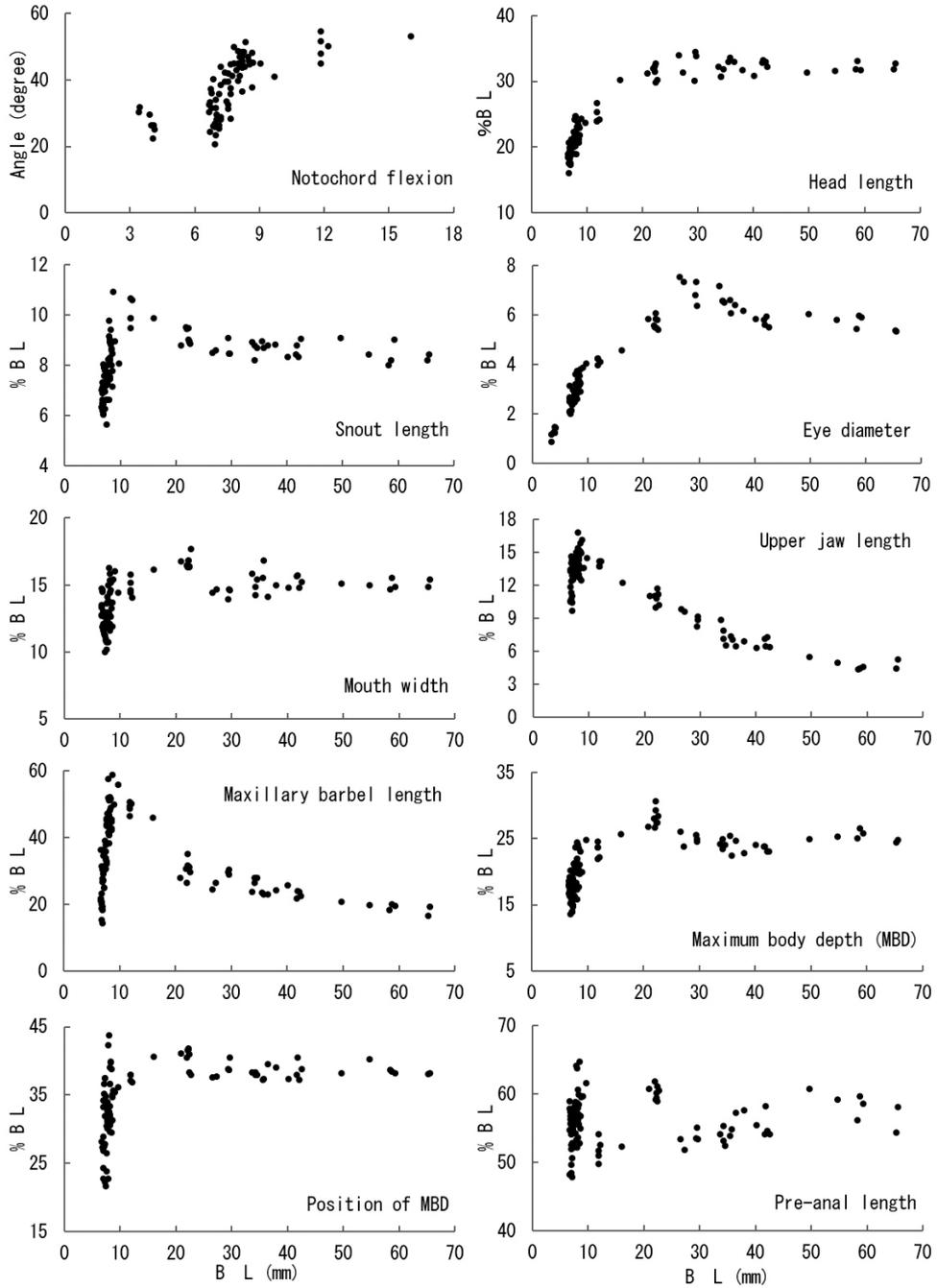


Figure 2. Angle (degree) of notochord flexion and nine body parts dimensions expressed as a percentage of body length (%BL) in hatchery-reared larval and juvenile *Pangasianodon gigas* from day 0 to day 35 after hatching.

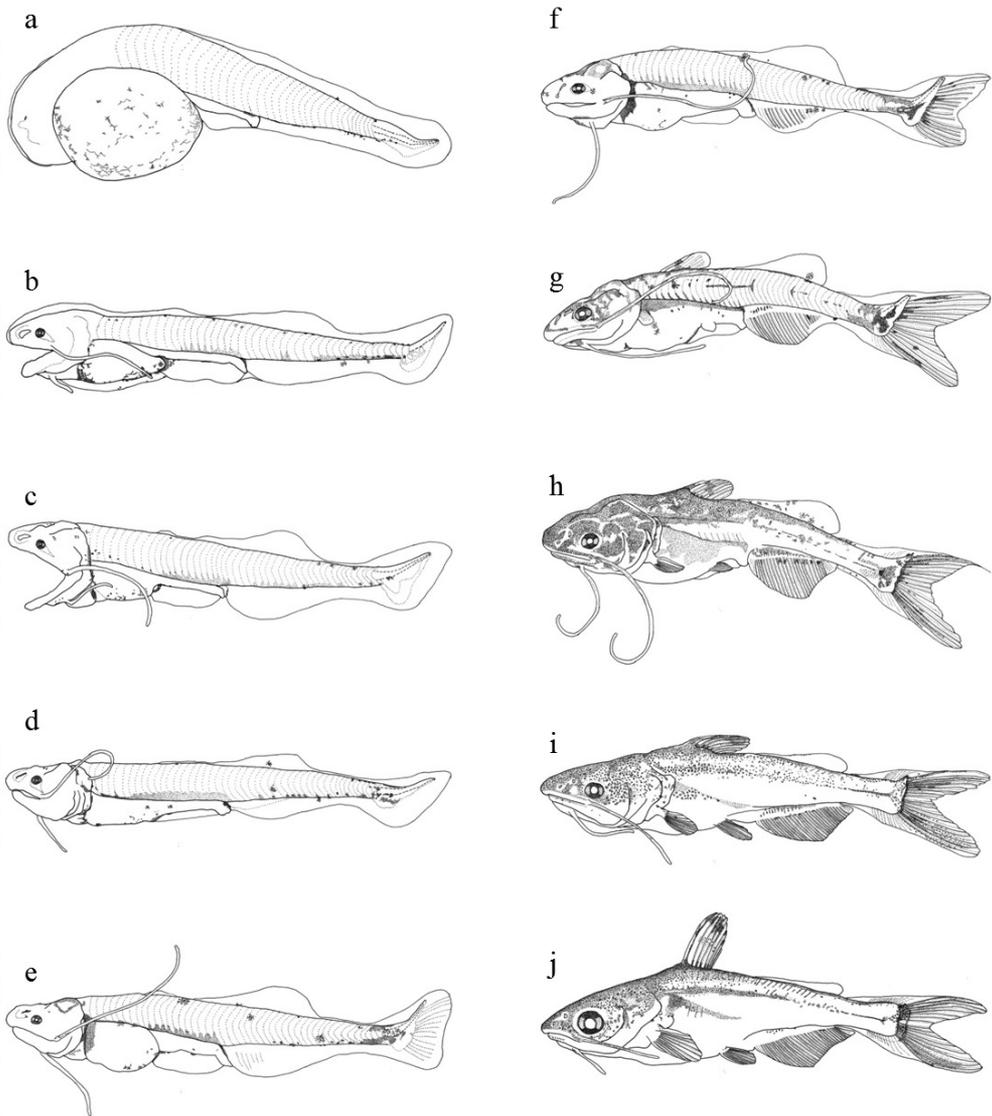


Figure 3. Hatchery-reared larval and juvenile *Pangasiodon gigas* from day 0 to day 21 after hatching. a, Yolk sac (YS)/ flexion larva (day 0, 4.09 mm BL); b, YS/flexion larva (day 1, 6.55 mm BL); c, Flexion larva (day 2, 6.84 mm BL); d, Flexion larva (day 3, 7.65 mm BL); e, Flexion larva (day 5, 8.19 mm BL); f, Flexion larva (day 7, 8.64 mm BL); g, Postflexion larva (day 12, 11.9 mm BL); h, Postflexion larva (day 12, 16.0 mm BL); i, Juvenile (day 14, 20.9 mm BL); j, Juvenile (day 21, 26.6 mm BL).

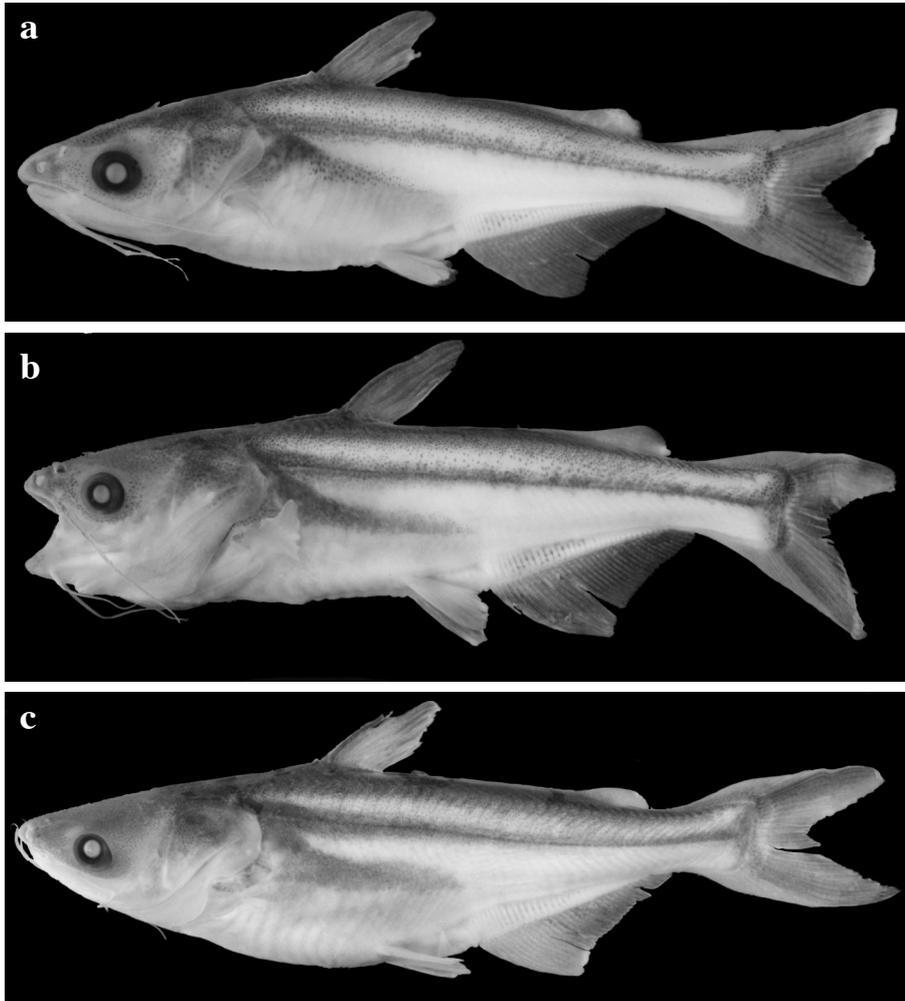


Figure 4. Photos of hatchery-reared juvenile *Pangasanodon gigas* from day 21 to day 35. a, day 21, 34.6 mm BL; b, day 28, 42.5 mm BL; c, day 35, 65.5 mm BL.

The anal fin rays first appeared at 7.56 mm BL on day 5, in which 5 soft fin rays were observed (Fig. 1). The full complement of 32 to 35 soft fin rays was attained at 20.9 mm BL on day 14 (Figs. 1, 3i).

The caudal fin was round, fan-shaped in the day 0 specimens of 3.41 to 4.14 mm BL (Fig. 3a). The dorsal part extended posteriorly, becoming pointed with the progress of notochord flexion at 7.02 mm BL (day 3, Fig. 3d for 7.65 mm BL), and subsequently the lower part extended posteriorly to form an asymmetrical forked caudal fin at 7.74 mm BL on day 5 (Fig. 3f for 8.64 mm BL). The lower lobe extended to almost the same length as the upper one in specimens of 9.05 mm BL and larger (Figs. 3g–j, 4). The principal caudal fin rays first appeared at 7.65 mm BL, with 4+5 rays being observed (Fig. 1). The full complement of 8+9 principal caudal fin rays was attained at 11.9 mm BL (day 10, Figs. 1, 3g, 4).

The pectoral fin buds first appeared at 6.84 mm BL on day 2 (Fig. 3c). The first pectoral fin rays to appear were 2 soft fin rays at 9.70 mm BL on day 10, and a full complement of fin rays (I, 9) was attained at 20.9 mm BL (day 14, Figs. 1, 3i).

The smallest specimen having pelvic fin buds was 8.70 mm BL on day 10, and the first pelvic fin rays to appear were 6 soft fin rays at 16.0 mm BL on day 12 (Fig. 3h). The full complement of 8 soft rays was attained at 20.9 mm BL (day 14, Figs. 1, 3i).

### Relative growth

The ratio of head length to BL increased from 16.0% at 6.75 mm BL and reached a constant level of about 30% at about 16 mm BL and larger (Fig. 2). The ratio of snout length increased rapidly from 5.6% at 7.47 mm BL to a peak of 10.9% at 8.70 mm BL, and subsequently declined gently to a constant level of 8-9% at 20.9 mm BL and larger (Fig. 2). The ratio of eye diameter increased rapidly from 0.9% at 3.41 mm to about 4% at about 10 mm BL and then gently to a peak of 7.5% at 26.6 mm BL, and thereafter declined, reaching a constant level of about 6% at about 40 mm BL and larger (Fig. 2). The ratio of mouth width increased rapidly from 10.0% at 7.20 mm BL to a constant level of about 15% at 26.6 mm BL and larger (Fig. 2). The ratio of upper jaw length increased rapidly from 9.7% at 6.93 mm BL to a peak of 16.7% at 8.00 mm BL, and subsequently declined gently to a constant level of about 5% at 49.7 mm BL and larger (Fig. 2). The ratio of maxillary barbel length increased rapidly from 15.5% at 6.80 mm BL to a peak of 58.7% at 8.70 mm BL, and thereafter declined, reaching a constant level of 17-21% at 50 mm BL and larger (Fig. 2). The ratio of maximum body depth increased rapidly from 13.6% at 6.93 mm BL and then gently to a peak of 30.6% at 22.2 mm BL, and thereafter declined, reaching a constant level of about 25% at 27 mm BL and larger (Fig. 2). The ratio of maximum body depth position increased rapidly from 22.2% at 7.20 mm BL to a peak of 43.7% at 8.00 mm BL, and thereafter declined rapidly to a constant level of about 40% at 16 mm BL and larger (Fig. 2). No clear inflection point was observed for the ratio of pre-anal length; however, the ratio varied slightly from 45-65% in the specimens of 10 mm BL and smaller, being steady thereafter at 50-60% (Fig. 2).

### Pigmentation

Many melanophores were scattered on the lateral-ventral surface of the yolk sac of newly hatched larvae of 3.41 to 4.14 mm BL (Fig. 3a), and the yolk sac melanophores decreased as the yolk was absorbed in specimens of days 1 and 2 (6.55 to 7.13 mm BL; Fig. 3b, c). Several small melanophores started appearing on the dorsal surface of the gut at 6.55 mm BL (Fig. 3b). The number of melanophores increased to form the pigmented cap of the gut in specimens of 6.84 mm BL and larger (Figs. 3c-j, 4a). A small number of melanophores appeared along the dorsal side of the rectum at 3.41 mm BL on day 0 (Fig. 3a for 4.09 mm BL), and the rectum melanophores disappeared in specimens of 16.0 mm BL on day 12 (Fig. 3h) and larger.

Melanophores were detected on the eyes of the smallest specimen, 3.41 mm BL on day 0 (Fig. 3a for 4.09 mm BL). All the specimens had fully pigmented eyes at day 1 (6.55 to 6.98 mm BL) and later (Figs. 3b). One small melanophore started appearing on the snout, opercle, and cheek at 7.11, 6.66, and 6.65 mm BL, respectively. These melanophores did not develop well, but these body areas were more or less heavily pigmented in specimens larger than 11.9 mm BL (Figs. 3g-j, 4). Some small melanophores appeared on the top of the head at 6.70

mm BL (Fig. 3c for 6.84 mm BL). Melanophores on the head increased in number and size with growth, covering the entire head in specimens of 9.05 mm BL and larger (Figs. 3g–j, 4).

A small melanophore appeared on the lower jaw at 6.84 mm BL (Fig. 3c), and all specimens larger than 8.50 mm BL possessed the lower jaw melanophores (Figs. 3f–j, 4). Two small melanophores appeared on the upper jaw at 7.20 mm BL, and all specimens larger than 7.92 mm BL possessed the upper jaw melanophores. The upper jaw was more or less heavily pigmented in specimens of 11.9 mm BL and larger (Figs. 3g–j, 4).

A small melanophore appeared on the base of both maxillary and mandibular barbels at 6.75 and 7.80 mm BL, respectively, and all specimens larger than 7.65 and 11.8 mm BL possessed them (Figs. 3e–j, 4). These melanophores increased in number and expanded distally to about 70 to 90% of barbel lengths in specimens larger than 9.70 and 16.0 mm BL for the maxillary and mandibular barbels, respectively.

Melanophores started appearing on the pre-dorsal fin area at 7.20 mm BL. The pre-dorsal fin area became heavily pigmented with growth, and the pigmented area was fully connected with the head melanophores at 11.9 mm BL and larger (Fig. 3g). The pre-dorsal fin pigment also expanded down- and posterior-ward to connect with the lateral line melanophores at 16.0 mm BL and larger (Fig. 3h).

A few, small melanophores appeared on the dorsal contour of body and tail at 6.55 mm BL (Fig. 3b), and the pigment area from the dorsal fin base to the end of tail was fully pigmented at 16.0 mm BL and larger (Figs. 3h–j, 4). Eight to 15 small melanophores appeared on the ventral contour of the tail along the anal fin-fold in all of the larvae on day 0 (3.41 to 4.14 mm BL, Fig. 3a). With growth, the ventral contour melanophores intruded into the tail along the myoseptum and formed inner melanophores cluster, the number of which decreased and became difficult to see from the outside in specimens of 12.2 mm BL and larger.

A small melanophore appeared on the posterior part of lateral line area at 6.70 mm BL, and all specimens larger than 8.70 mm BL possessed the lateral line melanophores (Figs. 3g–j, 4). The lateral line melanophores increased in number with growth to form a broad lateral band from above the pectoral fin base to the caudal peduncle in specimens of 16.0 mm BL and larger (Figs. 3h–j, 4).

The lateral sides of the caudal peduncle were externally pigmented at 7.02 mm BL and larger (Fig. 3d–j). The pigmented area expanded and encircled the caudal peduncle in specimens between 8.19 and 12.2 mm BL (Fig. 3e–g). The encircled melanophores reduced gradually and were more concentrated on the mid-lateral area of the caudal peduncle in specimens between 16.0 and 27.3 mm BL (Fig. 3h–j). In specimens larger than 29.4 mm BL, the caudal peduncle melanophores were connected to the dorsal contour and the lateral body band (Fig. 4).

Melanophores appeared on the anterior part of the dorsal fin at 11.9 mm BL on day 12 (Fig. 3g), and the dorsal fin melanophores increased in number and scattered over the dorsal fin with growth. Melanophores appeared on the pre-anal fin-fold at 6.55 mm BL (Fig. 3b) but disappeared at 11.9 mm BL (Fig. 3g). Melanophores on the anal fin-fold appeared at 4.05 mm BL, and all specimens larger than 8.10 mm BL possessed the melanophores on the anal fin-fold or fin (Figs. 3e–j, 4). A melanophore appeared on the anal fin base at 8.20 mm BL, and all specimens of 8.70 mm BL and larger had the melanophores (Figs. 3g–j, 4). The anal fin base melanophores increased in number and expanded posteriorly to 70 to 80% of the anal fin base length in specimens between 16.0 and 42.5 mm BL (Figs. 3h–j, 4).

On the anterior and posterior areas of the ventral side of notochord tip, developing later to the upper- and lower-hypural areas, melanophores appeared at 6.65 and 6.75 mm BL,

respectively, and all specimens larger than 16.0 and 7.74 mm BL had the melanophores on the hypural areas. Melanophores appeared first on the base of caudal fin rays at 7.13 mm BL, and all specimens larger than 8.30 mm BL possessed the melanophores (Figs. 3f–j, 4). The upper and lower lobes of caudal fin started to be pigmented at 7.38 and 7.20 mm BL, respectively, and all specimens larger than 8.70 mm BL possessed the melanophores (Figs. 3g–j, 4). These melanophores were not scattered over the caudal fin lobes but concentrated on the upper and lower areas of each caudal fin lobe to form the wide, black stripes.

The pectoral fin was pigmented in specimens of 20.9 mm BL and larger (Figs. 3i–j, 4). However, the melanophores were not spread over the pectoral fin but concentrated on the base and anterior area of the pectoral spine. No melanophores were observed on the pelvic fin in all specimens examined in this study.

A small melanophore appeared on the adipose fin-fold at 6.72 mm BL, and all specimens larger than 8.00 mm BL possessed the melanophores (Figs. 3e–j, 4). Melanophores appeared on the pectoral symphysis in all specimens examined, although the number varied from 4 to 35. One to 8 melanophores appeared on the isthmus at 6.55 mm BL (Fig. 3b).

## DISCUSSION

As discussed by VISETH *ET AL.* (2011, 2013), information on the morphological development of larval and juvenile pangasiid species is limited to *Pangasianodon hypophthalmus* described by MORIOKA *ET AL.* (2010), to *Pangasius larnaudii* by VISETH *ET AL.* (2011), and to *Pangasius bocourti* by VISETH *ET AL.* (2013). Therefore, the morphological characteristics of *Pangasianodon gigas* described in this study are compared with those of the three pangasiid species.

The largest specimen of *Pangasianodon gigas* possessing the yolk sac was 7.13 mm BL on day 2, although the smallest one in which the yolk was completely absorbed was 6.93 mm BL on day 3. The notochord tip was bent upward even in the smallest specimen examined (3.41 mm BL on day 0), and none of the specimens observed had a straight notochord. Therefore, the yolk sac and preflexion larval stages were not recognized in this study; the 3.41 to 7.13 mm BL larvae (day 0–2) were classified in the yolk sac/ flexion larva stage (Table 1). Similarly, the yolk sac/flexion larva stage was reported by MORIOKA *ET AL.* (2010: *Pangasianodon hypophthalmus*, 3.30–6.40 mm BL on day 0–2), VISETH *ET AL.* (2011: *Pangasius larnaudii*, 3.42–6.03 mm BL on day 0–2) and VISETH *ET AL.* (2013: *Pangasius bocourti*, 5.35–8.10 mm BL on day 1–3). Although MORIOKA *ET AL.* (2010) reported the stage of “yolk sac/preflexion larvae” (= yolk-sac larva stage of KENDALL *ET AL.* (1984)) in *P. hypophthalmus*, we could not observe the stage not only in *P. gigas* (this study) but also in *P. larnaudii* and *P. bocourti* (VISETH *ET AL.*, 2011, 2013); the body size of the stage was limited to 2.8–3.2 mm BL on day 0 in *P. hypophthalmus*, and thus, as pointed out by VISETH *ET AL.* (2011), this stage would last a surprisingly short time.

We could not exactly determine the size of *Pangasianodon gigas* specimens with a completely flexed notochord tip, but it was about 9 mm BL on day 10, and thus the flexion larva stage was defined as from 6.93 mm BL (day 3) to about 9 mm BL (day 10) (Table 1). Thereafter, the larvae entered the postflexion larva stage, which lasted until the completion of fin ray numbers at 20.9 mm BL on day 14, after which the larvae became juveniles (Table 1). However, no sampling was done on day 13 in this study, and the largest specimen with incomplete fin ray numbers sampled on day 12 was 16.0 mm BL; thus the *P. gigas* larvae would

Table 1. Body length (BL) and age for each developmental stage of *Pangasianodon gigas*.

Stage	BL (mm)	Age (days after hatching)
Yolk sac/flexion larva	3.41–7.13	0–2
Flexion larva	6.93–ca. 9.00	3–10
Postflexion larva	ca. 9.00–16.0	10–12
Juvenile	≥ 20.9	≥14

enter the juvenile stage between 16.0–20.9 mm BL on day 12–14. The larval size of 16.0–20.9 mm BL, when they entered the juvenile stage, in *P. gigas* is larger than the other pangasiids; 14.4 mm BL in *Pangasius larnaudii*, 12.8 mm BL in *Pangasianodon hypophthalmus*, and 12.2 mm BL in *Pangasius bocourti*. These size differences are basically caused by the rapid growth of *P. gigas*, even though the rearing methods were various by species. The mean BLs of the pangasiids are summarized as follows: (day 14–18) 22.1 mm in *P. gigas* and 13.3 mm in *P. bocourti* on day 14, 15.9 mm in *P. larnaudii* on day 15, and 17.9 mm in *P. hypophthalmus* on day 18; (day 21–28) 31.3 mm in *P. gigas* and 20.6 mm in *P. bocourti* on day 21, 23.4 mm in *P. hypophthalmus* on day 25, and 29.0 and 30.0 mm in *P. larnaudii* and *P. bocourti* on day 28; (day 35) 58.8 mm in *P. gigas*, 39.4 mm in *P. larnaudii* and 33.5 mm in *P. bocourti*.

The appearance and completion orders of fin rays were compared between the three pangasiids, *Pangasius bocourti*, *Pangasius larnaudii* and *Pangasianodon hypophthalmus* by VISETH ET AL. (2013). The appearance order in *Pangasianodon gigas* in this study was anal (7.56 mm BL) - caudal (7.65 mm) - dorsal (8.70 mm) - pectoral (9.70 mm) - pelvic (16.0 mm) fins, and this order is the same as those in the three pangasiids except for the following: the caudal fin appeared first in *P. larnaudii*, and the caudal and anal fins appeared at the same body size in *P. hypophthalmus*; the pectoral and pelvic fins appeared at the same body size in *P. bocourti* and *P. larnaudii*. However, we could not compare the completion order of *P. gigas* with those of the other pangasiids, because we could not determine the exact body size when the anal, pectoral and pelvic fins becoming complete, the completion of these fins occurring between 16.0 and 20.9 mm BL.

The head length is about 30% of BL, once it reaches a constant level, in *Pangasianodon gigas*, *Pangasianodon hypophthalmus* and *Pangasius larnaudii*, which is a little larger than that of *Pangasius bocourti*, 21–24%; however, a peak is detected in the latter two species, declining and stable thereafter, but no peak is observed in the former two species. The snout length is clearly shorter in the two *Pangasianodon* species (smaller than 10% of BL) than in the two *Pangasius* species (10–15% of BL). Although the stable eye diameter is not over about 6% of BL in all the four pangasiid species, the eye diameter is temporarily 6–8% of BL during the larval size intervals of 20 to 40 mm BL in *P. gigas*. The upper jaw length reaches 15–20% of BL in the four pangasiids, while the stable one is smaller in the two *Pangasianodon* species (about 5% of BL) than in the two *Pangasius* species (about 10% of BL).

We compared the morphological differences of larvae and juveniles between the four pangasiid species, two each belonging to two genera, *Pangasianodon* and *Pangasius*. However, it is difficult to conclude anything about generic relationships and larval/juvenile identification, because of the lack of substantial information; therefore, we emphasize the need to collect more information about the morphological development of pangasiid larvae and juveniles.

## ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Dr. Jaranthada Karnasuta, Advisor of Office of His Majesty's Principal Private Secretary and the staff members of the Division of Freshwater Fisheries, Department of Fisheries, Royal Thai Government, for their help in the collection of the specimens. We also wish to thank Dr. Prachya Musikasinthorn, Kasetsart University, Thailand, for his valuable comments on this paper and two anonymous reviewers for their helpful comments.

## REFERENCES

- BARAS, E., J. SLEMBROUCK, C. COCHET, D. CARUSO, AND M. LEGENDRE. 2010. Morphological factors behind the early mortality of cultured larvae of the Asian catfish, *Pangasianodon hypophthalmus*. *Aquaculture*, 298: 211–219.
- BLAXTER, J. H. S., AND G. HEMPEL. 1963. The influence of egg size on herring larvae (*Clupea harengus* L.). *J. Cons. Int. Explor. Mer.*, 28: 211–244.
- CITES. 2014. Appendices I, II and III valid from 14 September 2014. <<http://www.cites.org/eng/app/appendices.php>>.
- FROESE, R., AND D. PAULY (eds.). 2014. FishBase, version (11/2014). <<http://www.fishbase.org/>>.
- FUMIHIITO, A. 1989. Morphological comparison of the Mekong giant catfish, *Pangasianodon gigas*, with other pangasiid species. *Japan. J. Ichthyol.*, 36: 113–119.
- HOGAN, Z. S. 2004. Threatened fishes of the world: *Pangasianodon gigas* Chevey, 1931 (Pangasiidae). *Environ. Biol. Fish.*, 70: 210.
- HOGAN, Z. 2013. A Mekong giant: Current status, threats and preliminary conservation measures for the critically endangered Mekong giant catfish. WWF Report, June 2013. <[http://www.wwf.at/de/view/files/download/showDownload/?tool=12&feld=download&sprach\\_connect=2403](http://www.wwf.at/de/view/files/download/showDownload/?tool=12&feld=download&sprach_connect=2403)>.
- HOGAN, Z. S., N. PENGUN, AND N. VAN ZALINGE. 2001. Status and conservation of two endangered fish species, the Mekong giant catfish *Pangasianodon gigas* and the giant carp *Catlocarpio siamensis*, in Cambodia's Tonle Sap River. *Nat. Hist. Bull. Siam Soc.*, 49: 269–282.
- HUBBS, C. L., AND K. F. LAGLER. 2004. Fishes of the Great Lakes Region. Revised by SMITH, G.R., University of Michigan Press.
- ISLAM, A. 2005. Embryonic and larval development of Thai Pangas (*Pangasius sutchi* Fowler, 1937). *Develop. Growth Differ.*, 47: 1–6.
- IUCN. 2014. The IUCN Red List of Threatened Species. Version 2014.3. <[www.iucnredlist.org](http://www.iucnredlist.org)>.
- KENDALL JR., A. W., E. H. AHLSTROM, AND H. G. MOSER. 1984. Early life history stages of fishes and their characters. Pages 11–22. in Moser, H. G., W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr. and S. L. Richardson (eds.). Ontogeny and systematics of fishes. Special Publication No. 1, American Society of Ichthyologists and Herpetologists.
- LEIS J. M., AND T. TRNSKI. 1989. The Larvae of Indo-Pacific Shorefishes. New South Wales University Press and University Press of Hawaii, Sydney and Honolulu. xii+ 371 pp.
- MEENAKARN, W. 1984. Taxonomically and behavioral difference of Pla buk, *Pangasianodon gigas* Chevey and pla sawai, *Pangasius sutchi* Fowler fingerling. Natn. Inland Fish. Inst., Thailand, Technical Paper No. 41. 17 pp.
- MITCHELL, R., AND D. BRAUN. 2003. Giant catfish critically endangered, group says. *National Geographic News*, November 18, 2003. <[http://news.nationalgeographic.com/news/2003/11/1118\\_031118\\_giantcatfish.html](http://news.nationalgeographic.com/news/2003/11/1118_031118_giantcatfish.html)>
- MORIOKA, S., K. SANO, P. PHOMMACHAN, AND B. VONGVICHITH. 2010. Growth and morphological development of laboratory-reared larval and juvenile *Pangasianodon hypophthalmus*. *Ichthyol. Res.*, 57:139–147.
- POULSEN A. F., AND S. VIRAVONG. 2002. Fish migration and the maintenance of biodiversity in the Mekong River basin. *Mekong Fish Catch and Culture*, 8: 1–7. <[http://www.mrcmekong.org/assets/Publications/Catch-and-Culture/catchsep02\\_vol8.1.pdf](http://www.mrcmekong.org/assets/Publications/Catch-and-Culture/catchsep02_vol8.1.pdf)>.
- POULSEN, A. F., K. G. HORTLE, J. VALBO-JORGENSEN, S. Chan, C. K. CHHUON, S. VIRAVONG, K. BOUAKHAMVONGSA, U. SONTORNARATANA, N. YOORONG, T. T. NGUYEN, AND B. Q. TRAN. 2004. Distribution and Ecology of Some Important Riverine Fish Species of the Mekong River Basin. MRC Technical Paper No. 10. ISSN: 1683-148. 116p. <<http://www.mrcmekong.org/assets/Publications/technical/tech-No10-distribution-n-ecology-of-important.pdf>>.

- POUYAUD, L., G. G. TEUGELS, AND M. LEGENRE. 1999. Description of a new pangasiid catfish from South-East Asia (Siluriformes, Pangasiidae). *Cybium*, 23: 247–258.
- RAINBOTH, W. J. 1996. Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. FAO, Rome. 265 pp.
- ROBERTS, T. R., AND C. VIDTHAYANON. 1991. Systematic revision of the Asian catfish family Pangasidae, with biological observations and descriptions of three new species. *Proc. Acad. Nat. Sci. Philadelphia*, 143: 97–144.
- SMITH H. M., 1945. The freshwater fishes of Siam, or Thailand. *Bulletin of the United States National Museum*, 188: 1–622.
- VIDTHAYANON, C. 1993. Taxonomic revision of catfish family Pangasiidae. Ph.D. thesis, Tokyo University of Fisheries, Tokyo, Japan. 203 pp.
- VISETH, H., R. UGAWA, Y. KINOSHITA, F. AKISHINOMIYA, Y. TAKI AND H. KOHNO. 2011. Morphological development of hatchery-reared larval and juvenile *Pangasius larnaudii*. *Aquaculture Sci.*, 59: 283–297. <[https://www.jstage.jst.go.jp/article/aquaculturesci/59/2/59\\_283/\\_pdf](https://www.jstage.jst.go.jp/article/aquaculturesci/59/2/59_283/_pdf)>
- VISETH, H., Y. KINOSHITA, F. AKISHINOMIYA, Y. TAKI AND H. KOHNO. 2013. Morphological development of hatchery-reared larval and juvenile *Pangasius bocourti*. *Nat Hist. Bull. Siam Soc.*, 59: 137–148. <[http://siamese-heritage.org/nhbsspdf/vol051-060/NHBSS\\_059\\_2h\\_Hav\\_MorphologicalDevelopment.pdf](http://siamese-heritage.org/nhbsspdf/vol051-060/NHBSS_059_2h_Hav_MorphologicalDevelopment.pdf)>