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Early Development of Laboratory–reared Yellow Croaker, *Nibea albiflora*

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Morphological development of larvae and juveniles of laboratory–reared yellow croaker *Nibea albiflora* are described from a series of specimens sampled daily. Details on the early developmental stages are illustrated, with special reference to morphological transformations and the developments of digestive tract, fins and scales.

Artificially fertilized eggs were 836.5±38.2 μm in mean diameter. Hatching occurred by 24–26 h after the fertilization at water temperatures of 18.2–21.1 °C. On the 3 rd day after hatching, larvae completed yolk absorption and started feeding rotifers at 2.70±0.08 mm in body length (BL). Notochord flexion started on the 19 th day at 5.30±0.33 mm BL. Transformation from the larval to the juvenile stage occurred between 7.6 and 12.0 mm BL. Then all fin rays attained the adult complement. Squamation completed at 8.9–11.8 mm BL, and rudimentary pyloric caeca appeared when the larvae transformed into juveniles. Between 12.6 and 14.9 mm BL, juveniles completed the formation of adult–like digestive system. Three marked changes appeared in the relative growth at approximately 5–7 mm, 10–14 mm and 40–50 mm BL. These morphological changes were closely related to the notochord flexion and two important changes, i.e. the beginning and ending of the juvenile stage.

INTRODUCTION

The yellow croaker *Nibea albiflora* is a sciaenid fish which reaches about 50 cm in total length, distributed the coasts of Japan, the south–east area of the Korean peninsula, the Yellow Sea, and the East China Sea (Matsubara, 1937; Takita, 1974). Some of sciaenid species are commercially important, particularly as a material of surimi–based products in Japan. Also in Korea, dried croaker *Larimichthys polyactis* is known to be indispensable for a material of happy events. But recently, natural resources of sciaenids have been decreased mainly due to over fishing. Therefore the rapid establishment of culture technology for them is strongly expected. Nevertheless, in spite of their importance in fishery industry, little biological knowledge on seedling fry production (Tabaru *et al.*, 1988) and mariculture (Takeda *et al.*, 1994; Han *et al.*, 1994; El–Zibdeh *et al.*, 1995abc; Ide *et al.*, 1998) exist in Japan so far.

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In this report, the ontogeny and morphological characters at their early developmental stages with special reference to fin development, squamation and the development of digestive organs are described in detail from a series of reared specimens to provide more information on the early life history of *N. albiflora*.

MATERIALS AND METHODS

Artificial fertilization of eggs

Fertilized eggs were obtained by hand stripping mature adults caught with set-nets on May 20, 1994, at Takezaki, the Ariake Sound of Kyushu. After fertilization the eggs were transported to the Fishery Research Laboratory of Kyushu University and introduced into1kl-capacity polycarbonate tanks with an initial density of 30,000–40,000 eggs. The eggs were incubated successively with sand-filtered sea water (salinity:30–33 ppt).

Larval and juvenile rearing

The newly hatched larvae were reared in still sea-water for the first 3 days, whereafter a running water system was employed. Larvae were fed with so-called S type marine rotifer *Brachionus rotundiformis* enriched with n-3 HUFAs (Yoshimatsu *et al.*, 1997) until the 29 th day after hatching. From 22 nd to 39 th day after hatching, they were fed with *Artemia* nauplii. Subsequently they were fed with a commercial artificial feed for marine fish. Deposits from the tank bottom were removed every morning by syphoning. The water temperature during the experimental period ranged from 19.2 to 29.5 °C.

Observations and measurements

The observations of embryonic and early developments were conducted under dissecting and/or high-power microscopes. After being anesthetized with MS222, morphological observations were carried out on live specimens sampled every day until 33 days after hatching, and thereafter with a few days' intervals. After preserving in 5–10% neutralized formalin solutions, the fish were measured for total length (TL), body length (BL), head length (HL), body depth at the portion of pectoral fin (BD), upper jaw length (UJL), eye diameter (ED), pre-anal length (PAL), the distance between ventral fin base and anus (DVA), and pre-dorsal fin length (PDL). The body weight was measured by weighting five preserved specimens together (duplicate, <ca. 5 mm TL) or individually (>ca. 5 mm TL), after removing body surface moisture with filter paper carefully. Preserved specimens were also used for studying the fin development and squamation, and the development of digestive tract . Fin development and squamation were inspected with the specimens stained by Alizarin Red-S. After observation, used specimens were registered as Larval Specimen Collection of the Fishery Research Laboratory, Kyushu University.

RESULTS

Embryonic development

The eggs were transparent, non-adhesive, pelagic, and spherical in shape, measuring 836.5±38.2 μm (n=100, mean±SD) in diameter with a single oil globule (201.7±5.8 μm

Table 1. Embryonic development of *Nibea albiflora*

Time (hr : min)	WT (°C)	Figure (Fig. 1)	Descriptions
0:00	18.2	A	Fertilized egg
3:50	20.3	B	Late morula
4:10	20.3	C	Blastula
5:00	20.3		Germ ring appeared
6:30	19.8		Gastrula
7:30	20.0		Blastoderm 1/4 of yolk–sac
10:00	20.0	D	Blastoderm 2/3 of yolk–sac
12:00	20.5		Blastoderm 4/5 of yolk–sac, formation of embryonal body
13:00	20.5	E	Embryonal body 1/2 of yolk–sac, blastopore closed
15:50	21.1	F	Formations of eye vesicles, Kupffer's vesicles and 10 somites, melanophores appeared on dorsal part of embryo and oil globule and xanthophores appeared on almost whole part of embryo and lower part of oil globule
22:00	21.0	G	Embryonal body 3/4 of yolk–sac, 20 somites, disappearance of Kupffer's vesicles, formation of auditory vesicles and eye lenses
23:30	21.0		Heart pulsation starts, embryo wriggles occasionally
24:15	21.0		Free larva, hatching began
26:15	21.0		Hatching completed

in diameter). The perivitelline space was narrow. The diameters of eggs and oil globules obtained in the present study were almost the same as those in the previous study by Takita (1974). The embryonic development is summarized and shown in Table 1 and Fig. 1, respectively. Most of the eggs were hatched successfully with 24–26 h incubation at 18.2–21.1 °C.

General morphology and behavior of larvae and juveniles

The change in mean body length of the first 114 rearing days is shown in Fig. 2. The body length of newly hatched larvae (Fig. 3A) was 1.70±0.05 mm BL. The anus was situated slightly posterior to the middle of the body. The number of somites was mostly 27 (8+19; pre–anal+post–anal) in total. The oil globule was situated posterior to the yolk–sac. Melanophores were present on the top of head, the snout–tip, the trunk and caudal regions, and the dorsal side of oil globule. Xanthophores were present around eyes, trunk, and caudal regions, and the ventral side of oil globule. The eyes were not yet pigmented. Larvae had weak swimming ability and they were suspended in the water column with the portion of ventral side up.

The 1–day yolk–sac larvae (Fig. 3B) were 2.39±0.27 mm BL. The number of somites was 6+19=25. The eyes of larvae were pigmented at this stage.

The 3–day yolk–sac larvae (Fig. 3C) were 2.70±0.0 mm BL. The mouth was open, but not yet functioning. Larvae had fan–shaped pectoral fins. The first inflation of the gas bladder was observed among almost all individuals. As larvae completed yolk absorption,

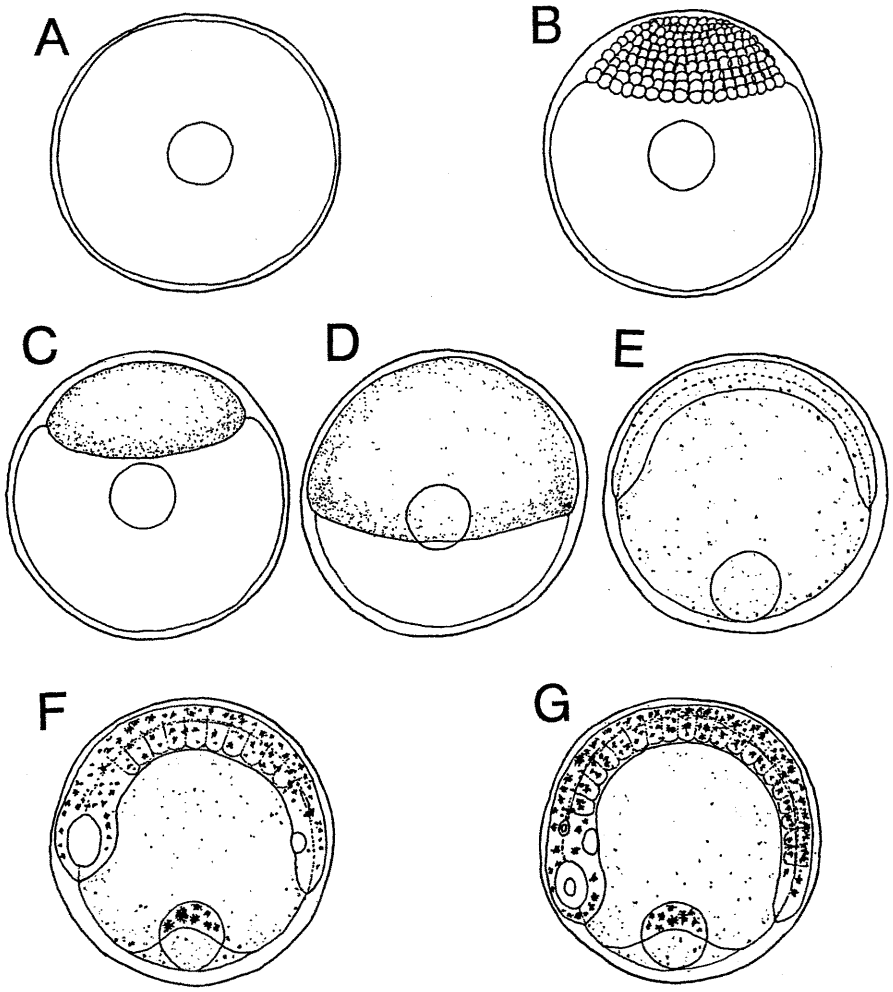

Fig. 1. Embryonic development of *Nibea albiflora* at 18.2–21.1 °C. A, immediately after fertilization; B, 3 h50 min; C, 4 h10 min; D, 10 h; E, 13 h; F, 15 h50 min; G, 22 h.

they started feeding rotifers.

The 10–day larvae (Fig. 3D) were 3.66 ± 0.82 mm BL. Melanophores were present on the shoulder, the surfaces of gas bladder and digestive tract, the ventral side of caudal region.

The 13–day larvae (Fig. 3E) were 4.27 ± 0.15 mm BL. A rudimentary caudal fin appeared. Marked spot of melanophores which were reported to be characteristic of the present species were prominent around rudimentary caudal fin.

The 19–day larvae (Fig. 3F) were 5.30 ± 0.33 mm BL. The notochord started to flex upwards. The anlagen of dorsal and anal fin rays appeared.

The 23–day larvae (Fig. 3G) were 5.41 ± 0.40 mm BL. Melanophores of the dorsal

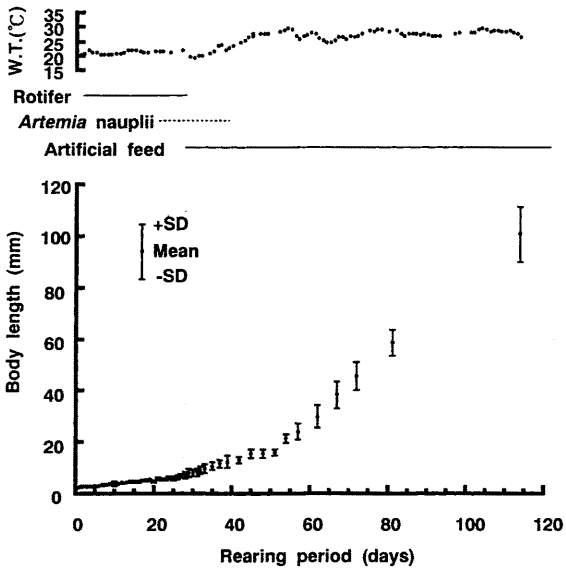

Fig. 2. Mean growth of *Nibea albiflora* in body length, feeding schedule, and water temperature (W. T.) during the first 114 days of the rearing experiment.

surface of visceral cavity became heavy. The nostril transformed to comma-shaped.

The 28-day larvae and juveniles (Fig. 3H) were 7.21±1.18 mm BL. Most individuals had fully developed fin rays except the lower part of the pectoral fins, and all the fin ray counts completed. Only caudal fin was segmented. Initial scales appeared along the anterior portion of the tail at an approximate size of 7 mm BL. Fish began to change their swimming stratum from the surface to the more benthic position in the rearing tank at this stage.

The 37-day juveniles (Fig. 3I) were 11.6±1.4 mm BL. The anus sifted to backward. The nostril separated into two pairs.

The 48-day juveniles (Fig. 3J) were 15.6±1.6 mm BL. Melanophores on their body surface became more remarkable that made a strong impression on the appearance of fish of this stage.

The 54-day juveniles (Fig. 3K) were 21.5±1.7 mm BL. The melanophore spots still remained on the dorsal side of their body. After this developmental stage, the snout projected and became overhanging beyond the mouth.

The 72-day young (Fig. 3L) were 45.5±5.4 mm BL. Melanophores on their body surface were not clear any more in appearance, compared with those of the fish in the previous stages. Their body were covered with guanine and looked externally silvery. The proportion of caudal fin closed to that of adult after this stage.

Fin development

A full complement of fin ray counts occurred at the size of 7.6 mm BL for the smallest

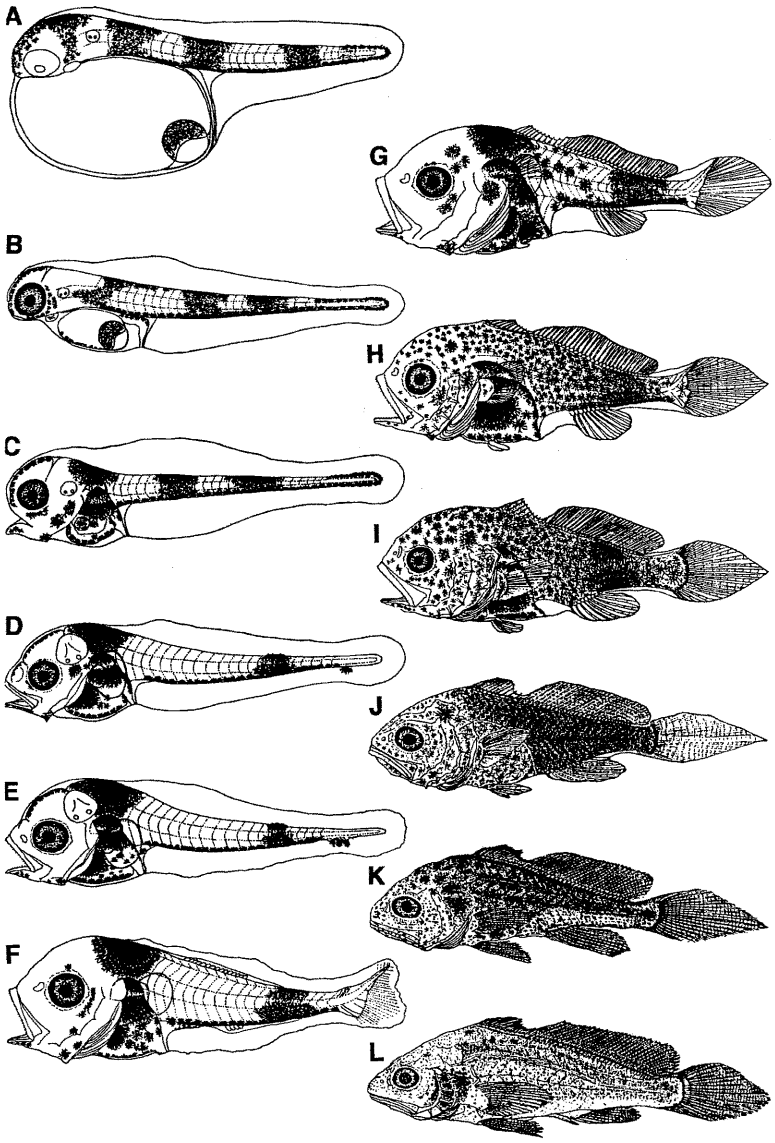

Fig. 3. *Nibea albiflora* reared in the laboratory.
A, 1.70 mm BL; B, 2.39 mm BL; C, 2.70 mm BL; D, 3.66 mm BL; E, 4.27 mm BL; F, 5.30 mm BL; G, 5.41 mm BL; H, 7.21 mm BL; I, 11.6 mm BL; J, 15.6 mm BL; K, 21.5 mm BL; L, 45.5 mm BL.

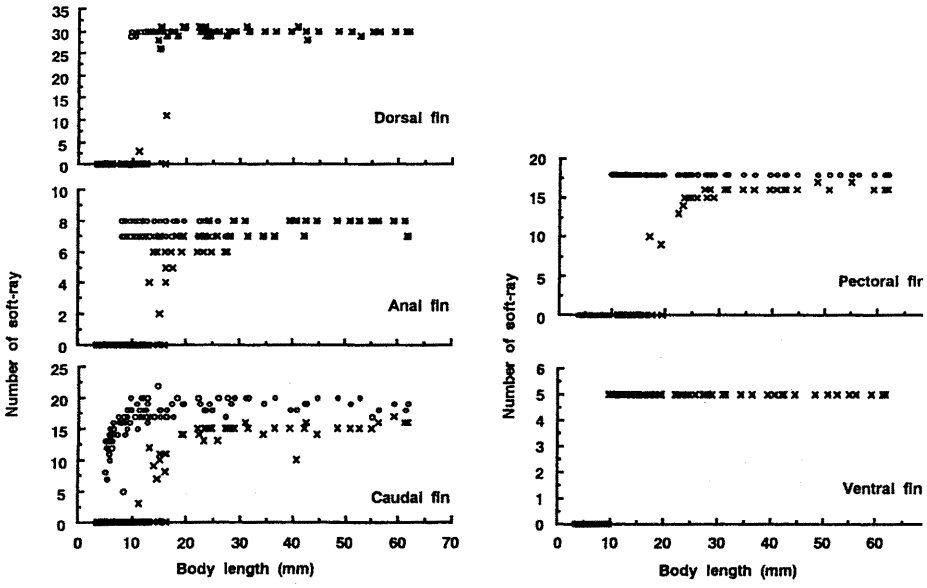

Fig. 4. The segmentation (open circles) and branching (cross) of soft ray on the unpaired and paired fins in *Nibea albiflora*.

specimens, and at 12.0 mm BL for the largest one, thus the transformation from the larval to juvenile stage occurred when fish were 7.6 to 12.0 mm BL (Fig. 3H, I) at 19.2–29.5 °C. After a full complement of soft rays in each fin, segmentation of fin–rays began, earlier in unpaired fins than in paired fins (Fig. 4). This phenomenon well agreed with those of other teleost fishes, such as *Engraulis japonica, Plecoglossus altivelis, Pagrus major, Acanthopagrus schlegeli* (Fukuhara, 1992). Caudal fin rays began to segment at about 5.0 mm BL, anal fin at 7.9 mm BL, dorsal fin at 8.0 mm BL, ventral and pectoral fins at 9.8 mm BL. The completion of segmentation in the fin was achieved at 9.0 mm BL in the anal, 10.4 mm BL in the ventral and the pectoral, 12.7 mm BL in the caudal and 12.9 mm BL in the dorsal, respectively under the present rearing condition.

Branching of soft rays began after the segmentation was completed, except for the ventral fins. Soft ray branching was observed at approximately 9.8 mm BL in the ventral, 11.2 mm BL in the caudal fin, 11.3 mm BL in the dorsal fin, 12.8 mm BL in the anal and 17.1 mm BL in the pectoral. Branching was completed at 24.1 mm BL in the caudal, 27.8 mm BL in the anal and 16.2 mm BL in the dorsal, 10.4 mm BL in the ventral and 48.5 mm BL in the pectoral, respectively. Consequently all fins were completely segmented by 12.9 mm BL, and were branched when fish reached 48.5 mm BL.

Squamation

The squamation process of yellow croaker is shown in Fig. 5. Squamation proceeded as larvae grew. The largest individual without scales was 7.1 mm BL, and the smallest having scales was 6.3 mm BL. Squamation started from along the mid–lateral part of the

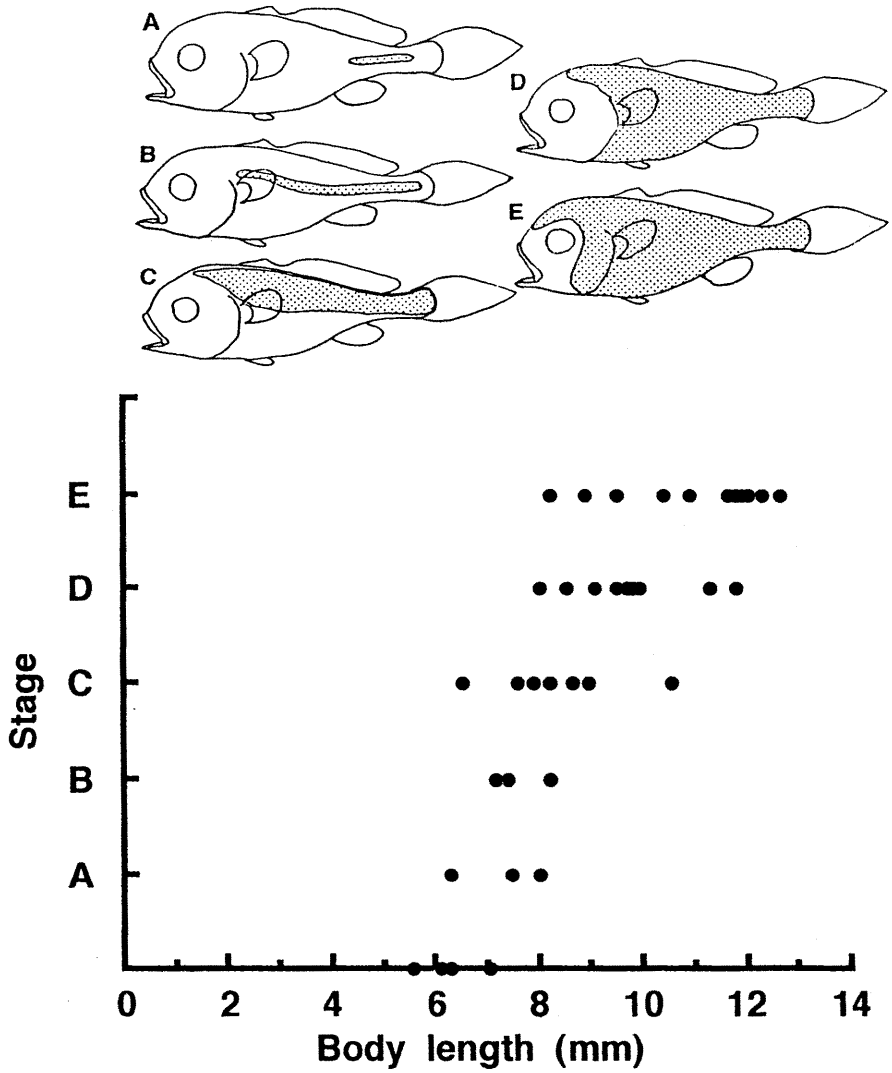

Fig. 5. Schematic illustrations showing developmental stages of squamation (upper), and plots of the stages against body length (lower) in *Nibea albiflora*.

body (Fig. 5A). The squameted area expanded rapidly to nearly the whole body surface including the opercular region in juveniles of 8.9–11.8 mm BL (Fig. 5B–E).

Development of digestive tract

The digestive system of a reared adult (250 mm BL, 170 g BW) is shown in Fig. 6. The stomach was Y-shaped. The number of pyloric caeca was eight. The intestine was coiled simply in the visceral cavity, and its convolution type was similar to those of *Pagrus*

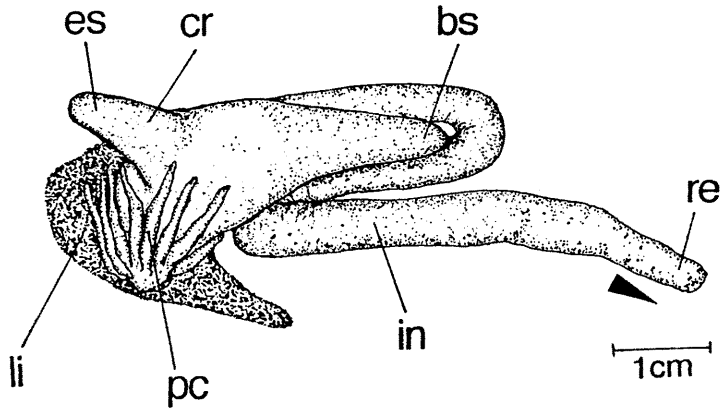

Fig. 6. Digestive system in adult *Nibea albiflora* (lateral side).
pc, pyloric caeca; es, esophagus; cr, cardiac region; bs, blind sac; li, liver; in, intestine; re, rectum. Arrow indicates the direction to the anus.

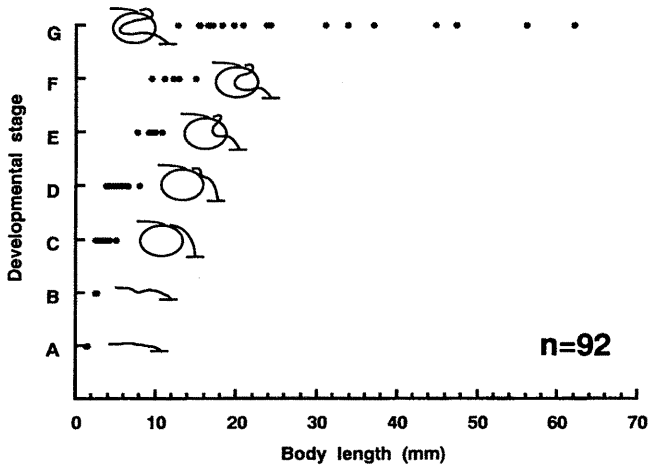

Fig. 7. Developmental stages of digestive tract plotted against body length in *Nibea albiflora* (n=92).

major, Acanthopagrus schlegeli (Fukuhara, 1987, 1992) and *Nibea japonica* (Ide *et al.*, 1998). The sequence of development of the digestive system during the early developmental stages and the relationship between body length are shown in Fig. 7. The digestive tract of newly hatched larvae was unlooped (Fig. 7A). The coiled digestive tract was formed when larvae attained more than 2.44 mm BL (Fig. 7C). The stomach was formed, and posterior portion of the digestive tract was curved slightly (Fig. 7D) when larvae reached the approximate size of 4 to 8 mm BL.

The pyloric caeca appeared (Fig. 7E), corresponding to the transformation from

larval to juvenile stages. The specimens over 12.0 mm BL had well-developed pyloric caeca (Fig. 7F). According to the progress in early development, pyloric caeca elongated and the shape of digestive tract became deeply rounded and curved form that almost similar to that of adults (Fig. 7G).

Relative growth

The body length (BL, mm)–body weight (BW, mg) relation is shown in Fig. 8. Allometric equations of the relationships are listed below. Inflexions nearly corresponding to the periods for the notochord-flexion and the two endings of larval and juvenile stages appeared at 5.06–7.79 mm, 13.2 mm and 42.4 mm BL, respectively.

$BW=4.425\times10^{-4}BL^{5.143}$ (r=0.901) 3.26 mm<BL<5.06 mm
$BW=1.543\times10^{-3}BL^{4.373}$ (r=0.935) 5.06 mm<BL<7.79 mm
$BW=3.629\times10^{-2}BL^{2.835}$ (r=0.955) 7.79 mm<BL<13.2 mm
$BW=4.172\times10^{-2}BL^{2.781}$ (r=0.990) 13.2 mm<BL<42.4 mm
$BW=1.145\times10^{-2}BL^{3.126}$ (r=0.995) 42.4 mm<BL<119.3 mm

Proportional changes of various parts of the body against body length are shown in Fig. 9, and the equations for each relative growth are listed below.

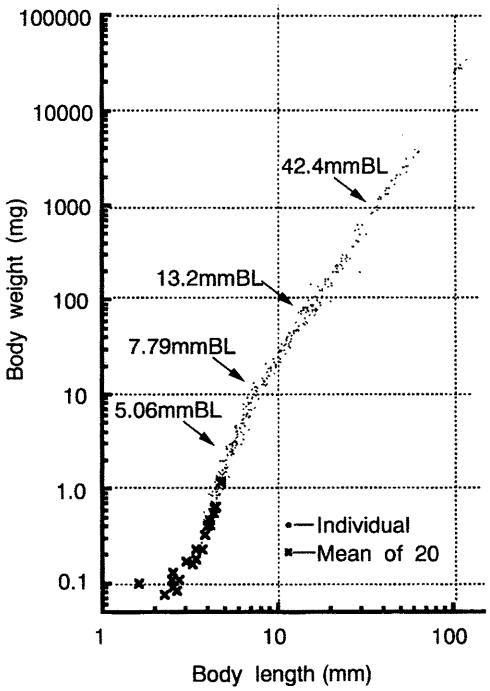

Fig. 8. Body length (BL, mm)–body weight (BW, mg) relationship of *Nibea albiflora*. Arrows show growth inflexions.

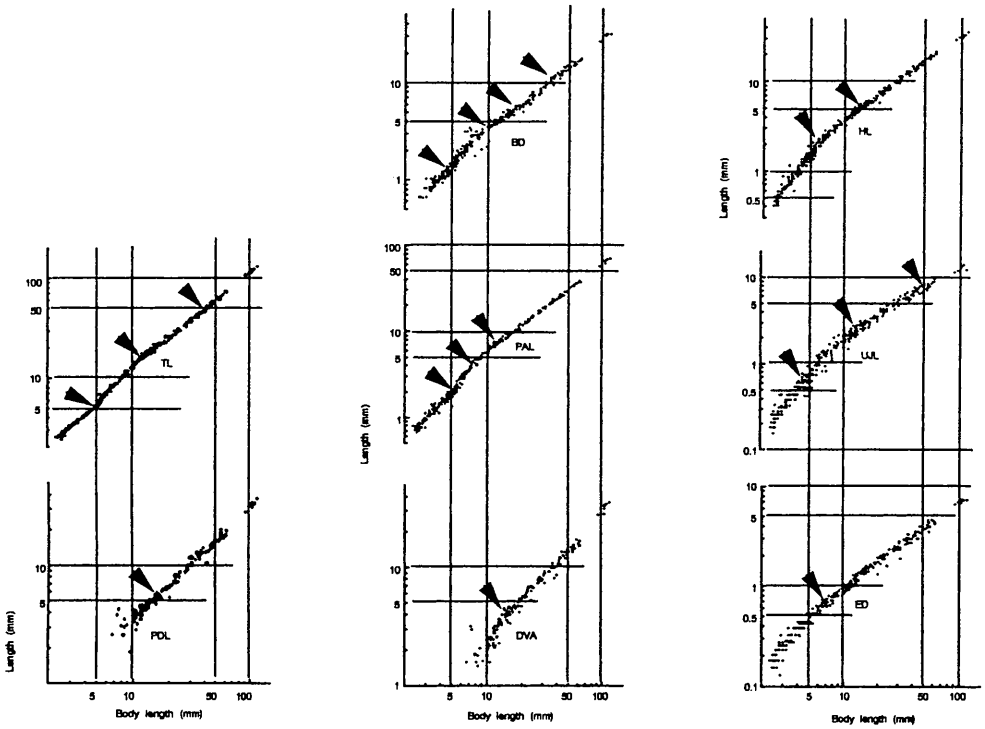

Fig. 9. Relative growth of total length (TL), body height at the portion of pectoral fin (BH), upper jaw length (UJL), eye diameter (ED), pre-anal length (PAL), The distance between ventral fin and anus (DVA), and pre-dorsal fin length (PDL) against body length (BL) of *Nibea albiflora* (n=431). Arrows show growth inflexions.

$TL=1.057BL^{1.003}$ (r=0.998) 1.24 mm < BL < 4.57 mm
$TL=6.951\times10^{-1}BL^{1.279}$ (r=0.997) 4.57 mm < BL < 11.6 mm
$TL=1.775BL^{0.896}$ (r=0.994) 11.6 mm < BL < 40.1 mm
$TL=1.387BL^{0.963}$ (r=0.997) 40.1 mm < BL < 115.2 mm

$HL=7.620\times10^{-2}BL^{1.918}$ (r=0.993) 2.41 mm < BL < 5.53 mm
$HL=3.510\times10^{-1}BL^{1.025}$ (r=0.987) 5.53 mm < BL < 11.6 mm
$HL=4.309\times10^{-1}BL^{0.941}$ (r=0.990) 11.6 mm < BL < 49.4 mm
$HL=5.571\times10^{-1}BL^{0.876}$ (r=0.996) 49.4 mm < BL < 115.2 mm

$BD=2.188\times10^{-1}BL^{1.141}$ (r=0.925) 2.63 mm < BL < 4.52 mm
$BD=1.343\times10^{-1}BL^{1.464}$ (r=0.931) 4.52 mm < BL < 8.82 mm
$BD=6.821\times10^{-1}BL^{0.718}$ (r=0.961) 8.82 mm < BL < 18.8 mm
$BD=3.262\times10^{-1}BL^{0.969}$ (r=0.949) 18.1 mm < BL < 39.1 mm
$BD=2.955\times10^{-1}BL^{0.996}$ (r=0.949) 39.1 mm < BL < 115 .2 mm

$UJL=6.308\times10^{-2}BL^{1.483}$ (r=0.909) 2.41 mm<BL<4.43 mm
$UJL=3.989\times10^{-2}BL^{1.791}$ (r=0.945) 4.43 mm<BL<10.3 mm
$UJL=2.612\times10^{-1}BL^{0.875}$ (r=0.978) 10.3 mm<BL<48.4 mm
$UJL=8.605\times10^{-1}BL^{0.568}$ (r=0.989) 48.4 mm<BL<115.2 mm

$ED=6.046\times10^{-2}BL^{1.237}$ (r=0.938) 2.34 mm<BL<7.80 mm
$ED=1.292\times10^{-1}BL^{0.864}$ (r=0.994) 7.80 mm<BL<115.2 mm

$PAL=1.922\times10^{-1}BL^{1.456}$ (r=0.932) 1.24 mm<BL<5.15 mm
$PAL=1.229\times10^{-1}BL^{1.728}$ (r=0.959) 5.15 mm<BL<7.48 mm
$PAL=2.633\times10^{-1}BL^{1.350}$ (r=0.986) 7.48 mm<BL<10.7 mm
$PAL=5.825\times10^{-1}BL^{1.014}$ (r=0.999) 10.7 mm<BL<115.2 mm

$DVA=4.467\times10^{-2}BL^{1.673}$ (r=0.898) 6.63 mm<BL<14.1 mm
$DVA=2.388\times10^{-1}BL^{1.040}$ (r=0.992) 14.1 mm<BL<115.2 mm

$HL=7.620\times10^{-2}BL^{1.918}$ (r=0.993) 2.41 mm<BL<5.53 mm
$HL=3.510\times10^{-1}BL^{1.025}$ (r=0.987) 5.53 mm<BL<12.2 mm
$HL=4.653\times10^{-1}BL^{0.918}$ (r=0.997) 12.2 mm <BL<115.2 mm

$PDL=5.836\times10^{-1}BL^{0.810}$ (r=0.904) 6.63 mm<BL<14.7 mm
$PDL=4.092\times10^{-1}BL^{0.942}$ (r=0.994) 14.7 mm<BL<115.2 mm

There appeared three-grouped marked changes in the body proportions corresponding to the morphological transitions. The changes in the first group were concentrated in about 5–7 mm, the second group in about 10–14 mm, and the third group in about 40–50 mm BL. Relative body proportions exhibited strong positive growth until the larvae attained about 5–7 mm BL where the flexion of notochord took place. After that, the development of the caudal fin followed by the notochord–flexion made the relative values small until they reached the juvenile stage.

At the juvenile stage, the relative values of TL, BD, and PDL displayed almost constant levels, but the relative values of head organs (HL, ED, UJL) showed negative growth. On the other hand, the relative proportion of PAL and DVA, deeply related to the development of digestive organs, exhibited clear positive growth. After these growth inflexions, the relative growth showed a big change again when the fish reached 40–50 mm BL.

DISCUSSION

In order to conduct fry production successfully, basic biological studies about the sequence on the early development of the target fish must be done in advance. Generally, when most marine fish transform from the larval to juvenile stages, they show dramatic changes with morphological and organogenetic changes called metamorphosis (Tanaka, 1969ab, 1971; Kendall *et al.*, 1984; Fukuhara, 1992). Those morphological changes are also accompanied by a change from pelagic habits to more demersal habits that could be

observed in captivity as well. Also changes in body proportion take place simultaneously, i.e. growth inflexions concentrate at the transformation periods between larval to juvenile stages and juvenile to the next stages (Kitajima *et al.*, 1988, 1991; Fukuhara, 1992; Yoshimatsu *et al.*, 1992, 1993; Ide *et al.*, 1998). Fukuhara (1992) demonstrated that the change of life mode (habitation, feeding, and behavior) was linked closely with the morphological and ontogenetic development of teleost fishes. Therefore during these periods, fish with morphologically, at the same time physiologically, insecure condition should be reared and handled with great carefulness.

These critical changes were also observed clearly in laboratory–reared yellow croaker *Nibea albiflora* in the present study. As shown in Fig. 10, the result of the present study on relative growth also showed that big changes in morphometrical characteristics took place concurrently with the organogenesis and behavioral changes in the early life stage of *N. albiflora*. Consequently, the changes that were observed at about 10–14 mm and 40–50 mm BL could be regarded as corresponding to two important morphological transitions, namely the beginning and the end of the juvenile stage.

It is reported previously that melanophores on the body surface of sciaenid fish disappear at the transformation period from the juvenile to young stages (Taniguchi *et al.*, 1979; Taniguchi, 1982; Ide *et al.*, 1998). Our rearing results for *N. albiflora* well

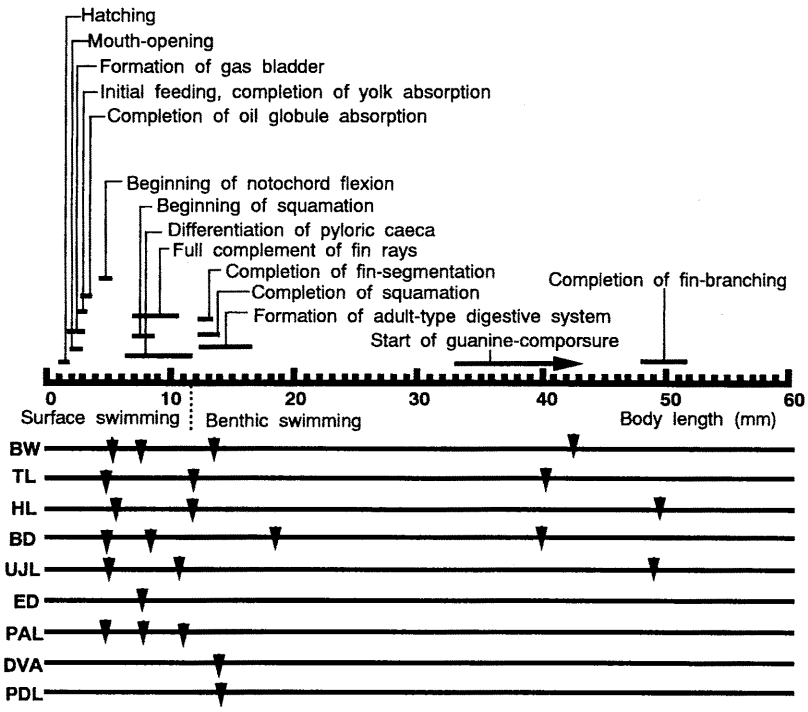

Fig. 10. The sequence of early development of reared *Nibea albiflora*.
Refer to Fig. 8 and 9 for BW, TL, HL, BD, UJL, ED, PAL, DVA and PDL. Arrows show growth inflexions.

agreed with those observations. This change in pigment pattern should be used as one of the important external criteria to distinguish juvenile from young sciaenid fish. From the viewpoint of ontogenesis, young with almost completed adult-like body systems must be stronger for rough-handling and starving, and more patient to environmental changes than those in earlier stages. Therefore the fry with silvery appearance might be ready for restocking or for moving to the successive intermediate rearing process.

To know morphological and behavioral changes on the early life history of the target species help us choosing suitable rearing conditions, feeding schedules for the successful seedling fry production. Nevertheless, still we have very limited knowledge on the early life history of sciaenid fishes so far. Therefore, further investigations about them would be necessary for establishing the seedling fry production technology of sciaenid fishes from now.

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