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Larval Development of the Japanese Whiting, *Sillago japonica*

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Abstract Larval development of laboratory reared Japanese whiting, *Sillago japonica*, was described according to morphological and histological observations of the development of organ systems. The development of larvae from hatching to the early phase of the juvenile period was classified into ten stages. These ten stages were grouped into three phases that were characterized by the marked development of certain organ systems: Phase I—the digestive organs, Phase II—the locomotor and respiratory organs, and Phase III—other morphological features that are characteristic of adult fish. These phases corresponded to yolk-sac larval stage, larval stage and transformation stage, respectively. Dry weights and six specified linear dimensions of the larvae were measured throughout the larval period. A power function best described the relationship between the total length and the dry weight from day 5 to day 27. This detailed serial description of the stages of larval development should be useful for future experimental studies on the Japanese whiting.

Japanese whiting, *Sillago japonica* Temminck et Schlegel, is a common species of Sillaginidae inhabiting shallow coastal waters in the northwestern Pacific. In Japan it is found on sandy bottoms from southern Hokkaido to Kyushu (Mochizuki, 1984). The developmental process of the eggs were described by Ueno and Fujita (1954) and Oozeki and Hirano (1985), but there is little published information about larval development. Earlier reports on larval development have been limited to the stages from hatching to several day-old larvae and to sea-caught larvae (Kamiya, 1925; Ueno and Fujita, 1954; Ueno et al., 1958).

This paper presents a detailed description of the larval development of laboratory reared Japanese whiting from hatching to 27-days old. Special attention was paid to the development of organ systems in order to describe the developmental process.

Materials and methods

Larval rearing and observations were conducted from June to September in 1980 and 1985, at the Fisheries Laboratory, University of Tokyo. Naturally spawned eggs of the Japanese whiting were collected from the tanks of brood stocks (Oozeki and Hirano, 1985). Thirty thousand fertilized eggs were transferred and incubated in a 1,000 liter polycarbonate tank. The temperature during the rearing trial was 25.1±1.0°C. From day 2 to day 7, the larvae were fed with neonates of the small type (S-type; Fu et al., 1991a, b) rotifer *Brachionus plicatilis*. The neonates were selected by using 108 μm mesh. After day 7 unfiltered S-type rotifers were fed to larvae. After the 14th day, the larvae were also fed with large type rotifers (Fu et al., 1991a, b). Both types of rotifers were cultured with ω-Yeast (Kyowa Hakko Kogyo Co., Ltd., Japan; Imada et al., 1979). The density of the rotifers in the larval rearing tank was maintained at 5 to 10 individuals/ml during the rearing period.

Larvae were sampled daily and fixed with 10% buffered formalin or 2.5% glutaraldehyde in phosphate buffer (pH=7.3; Oozeki and Hirano, 1988) for measurement of body lengths and other morphometric analyses. Six measurements were made on each formalin-fixed specimen, i.e., total length (TL), notochord length (NL), snout to anus distance (SA), snout to dorsal fin distance (SD), eye diameter (ED) and body depth at anus (BD). Shrinkage in 10% buffered formalin was measured at the same time as sampling in order to make back-calculations from measured values to live ones. Osteological development was observed in 2.5% glutaraldehyde-fixed specimens using the enzyme clearing method (Dingerkus and Uhler, 1977). After drying 2.5% glutaraldehyde-fixed specimens at 60°C for 24 hours, the dry weight of each larva was measured using a

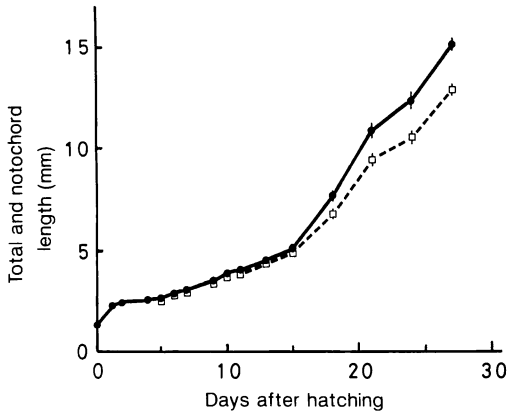

Fig. 1. Larval growth of total and notochord length in *Sillago japonica*. (closed circles: total length, open squares: notochord length, vertical bar: standard error of the mean, n=20).

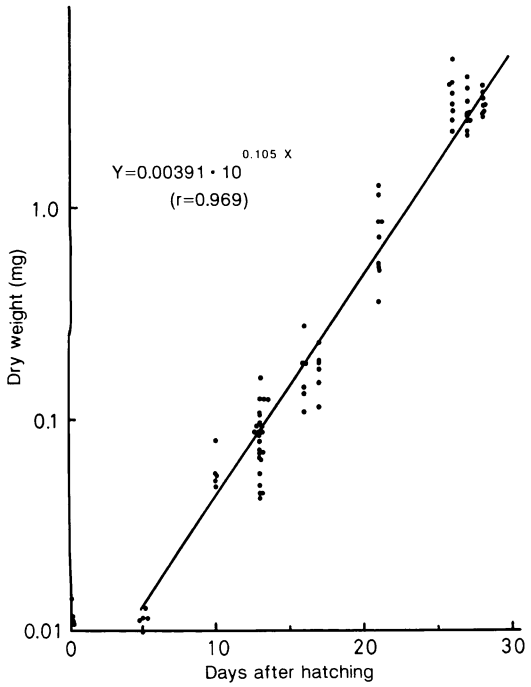

Fig. 2. Relationship between days after hatching and body weight (dry weight) of larval *Sillago japonica*.

micro chemical balance (Inaba Co., Ltd., M-1A).

Larvae fixed daily with Bouin's solution or Zenker-formol fixative (Luna, 1968) were prepared for histological observations. These specimens were embedded in paraffin, then serially sectioned at 5 to 6

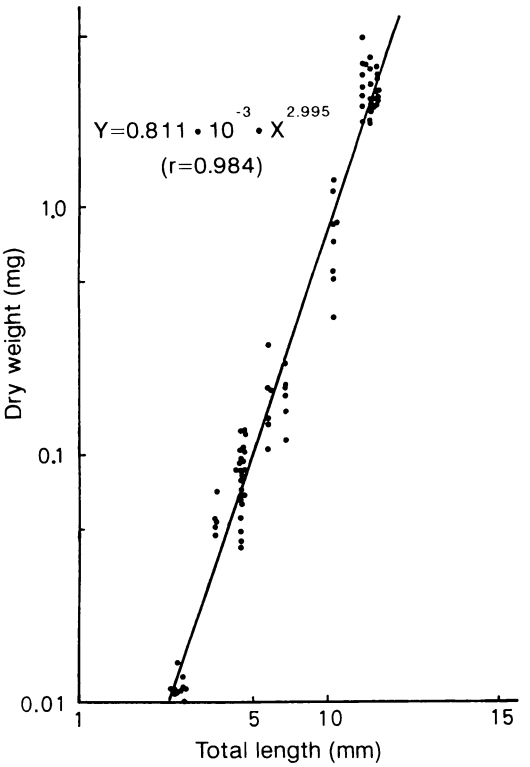

Fig. 3. Relationship between the total length and body weight (dry weight) of larval *Sillago japonica*.

μm in sagittal and cross sections, and treated with hematoxylin-eosin stain, Masson's trichromatic stain or periodic acid-Schiff stain. Figures were drawn with a camera lucida at ten and twenty magnifications.

Results

Total length of the larvae increased only slightly until the larvae were 15 days old after which time the increase in length was rapid (Fig. 1). The difference between the total and the notochord lengths became marked after 18 days old. The relationship between days after hatching and the dry weight per larva from day 5 to day 27 was expressed by the following regression (Fig. 2, n=89, r=0.969):

$$y=0.00391\cdot 10^{0.105x}$$

where x was the number of days after hatching and y was the dry weight (mg). The relationship between the total length and the dry weight was expressed by

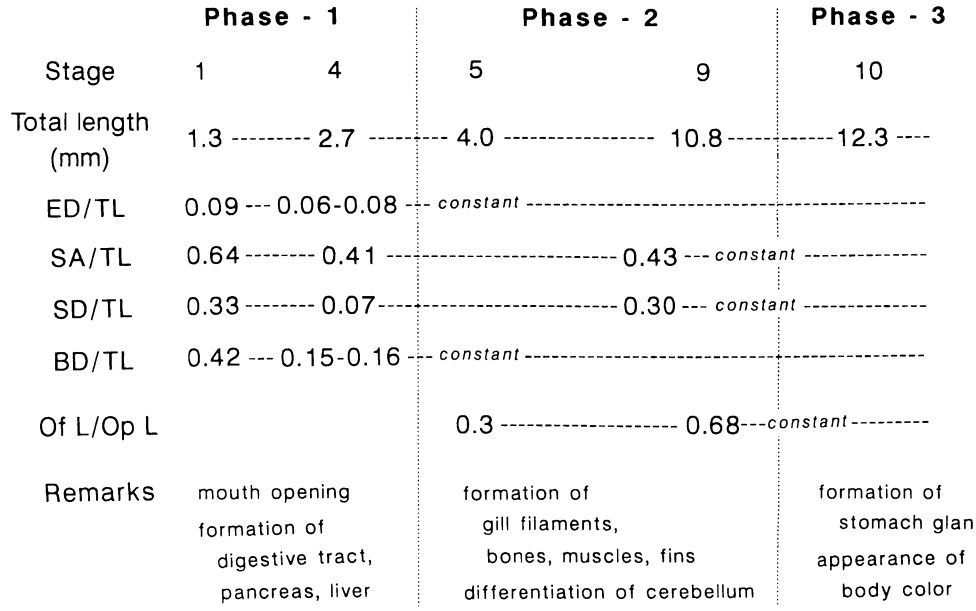

Fig. 4. Schematic representation of the development of morphological ratio and the developmental characters of organs in *Sillago japonica* larvae. TL: total length, ED: eye diameter, SA: snout to anus distance, SD: snout to dorsal fin distance, BD: body depth at anus, OlL: length of olfactory lobe, OpL: length of optic lobe.

the following regression (Fig. 3, n=89, r=0.984):

$$y=0.811\cdot10^{-3}\cdot x^{2.995}$$

where x was the total length of larvae in mm and y was the dry weight (mg).

According to observations made on morphology and histology of the organ system, the development of larvae from hatching to the early phase of the juvenile period was divided into ten stages. These ten stages could be grouped into three phases upon observations made on the development of organ systems (Fig. 4). Phase I was characterized by development of the digestive organs, i.e., the mouth, digestive tract, liver and pancreas (stage 1–4). Phase II was characterized by the development of the locomotor and respiratory organs, i.e., gill filaments, bones, muscles and fins (stage 5–9). Phase III was characterized by the change of body shape from larval to juvenile types (stage 10). The larval period was described as follows and a typical specimen at each stage illustrated in Figs. 5 and 6.

1) Post-hatching (Fig. 5-A)

Newly hatched larvae (1.2 mm NL, 1.3 mm TL) already had a digestive tract that was visible as a thin duct. The end of the anterior alimentary canal was connected to the anus, but the tract was not yet evident at the ventral side of the brain. The mouth had not opened, but the liver and pancreas appeared as rudimentary tissues. The yolk space was large and occupied most of the abdominal cavity. Morphometric ratios at this stage indicated maximum values throughout the developmental process; these were ED/TL=0.09, SA/TL=0.64, SD/TL=0.33 and BD/TL=0.42. The neurocoele was not divided into ventricles and the brain appeared as a neural tube (Fig. 7-A). Pectoral fins had begun to differentiate as small wing-like projections. The eyes were unpigmented. Body pigmentation of newly hatched larvae consisted of stellate melanophores and xanthophores scattered sparsely on the head and trunk. Two transverse bands of pigment extended onto the trunk at the vent and about half-way between the vent and notochord tip. Stellate melanophores were evident on the surface of an oil droplet. A scattered row of xanthophores was present along the lateral side of the notochord from the head to the tip of the notochord. This stage lasted until the formation of the tract at the ventral side of the brain.

2) Digestive tract formation (Fig. 5-B)

One day after hatching (2.2 mm NL, 2.4 mm TL), the digestive tract had developed into a thick duct and extended to the ventral side of the brain. The

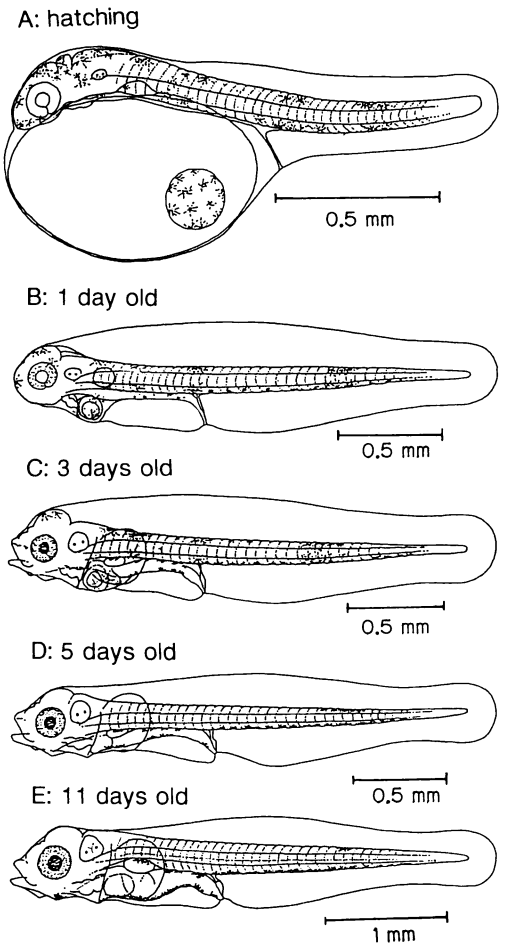

Fig. 5. Larval developmental stages of *Sillago japonica* (I).

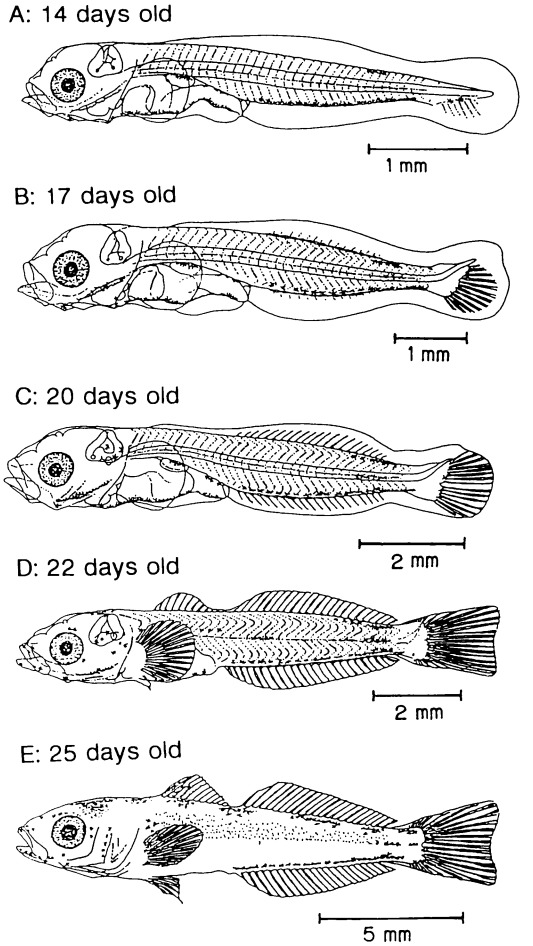

Fig. 6. Larval developmental stages of *Sillago japonica* (II).

liver and pancreas were evident in the abdominal region. The value of the yolk sac had decreased and the yolk sac was 0.12 mm in diameter. Fan-like pectoral fins were clearly observed. Body pigmentation consisted of melanophores only, as xanthophores were absent at this and later stages. One transverse band of pigments near the tip of the notochord had increased noticeably in intensity. Three heavily pigmented spots were evident on the dorsal side of the notochord at the yolk sac, anus and on the area between them. Three spots were also evident on the ventral side of the notochord at the anus, just behind the transverse band and at the mid-length of the intestine. This stage lasted until the opening of the mouth.

3) Opening of the mouth (Fig. 5-C)

Two days after hatching (2.3 mm NL, 2.5 mm TL), the upper and lower jaws were visible, and both the oral and gill chambers had opened. The retina was pigmented. The volume of the yolk sac had decreased further and was mainly occupied by an oil droplet. The liver, pancreas and gas bladder had developed and were visible from outside. A valve-like structure was observed between the mid and hind sections of the intestine. Fundamental components of the brain had formed (Fig. 7-C). Prosencephalon, mesencephalon and rhombencephalon were present. Telencephalon and diencephalon were noticed at the prosencephalon. The optic lobe occupied most of the mesencephalon. The medulla oblongata was evident in the rhombencephalon

region. An atrium and ventricle were visible as a connected duct with a constriction in the pericardial cavity. Pigmentation of the trunk was similar to that of the previous stage. Stellate melanophores occurred on the ventral surface of the notochord and on the dorsal and ventral surface of the intestine. This stage lasted until the complete yolk absorption.

4) Yolk extinction (Fig. 5-D)

Five days after hatching (2.5 mm NL, 2.7 mm TL), the yolk was completely absorbed. The ratios of ED, SA, SD and BD to TL decreased from hatching to this stage and indicated minimum values, i.e., ED/TL=0.06, SA/TL=0.41, SD/TL=0.07 and BD/TL=0.15. Two morphometric ratios increased again during this stage and became stable at ED/TL =0.08 and BD/TL=0.16. The liver had increased in volume and occupied the anterior part of the abdominal cavity. Torsion had not yet occurred at the middle section of the intestine. The fundamental development of the digestive organs was completed at this stage. A row of melanophores was evident at the ventral side of the notochord. Pigmentation on the dorsal side of the notochord had disappeared except at the transverse band. The pigmentation on the lateral side of the trunk disappeared. This stage lasted until the occurrence of torsion at the middle section of the intestine.

5) Development of gills (Fig. 5-E)

By 11 days after hatching (3.9 mm NL, 4.0 mm TL), the formation of gill filaments had proceeded rapidly following the previous stage, and rudimentary gill lamellae were observable as wart-like protrusions on the gill filaments. Torsion had occurred at the middle section of the intestine. The development of the brain had proceeded and the ratio of the olfactory lobe to the optic lobe in sagittal length was 0.3 (Fig. 7-E). The gut and the ventral side of the trunk were more heavily pigmented than in the previous stage. A single large melanophore was evident below the eyes at the angular of the jaw. This stage lasted until the appearance of a mass of cells on the ventral side of the notochord in the tail.

6) Formation of vertebra (Fig. 6-A)

Thirteen days after hatching (4.4 mm NL, 4.5 mm TL), a mass of cells which later differentiated into fin rays and the early appearance of the hypural bone were noticed on the ventral side of notochord in the tail. The fin rays had begun to differentiate at the caudal fin. Formation of the vertebrae was initiated in the area surrounding the notochord in the trunk. The cerebellum had begun to differentiate. The ratio

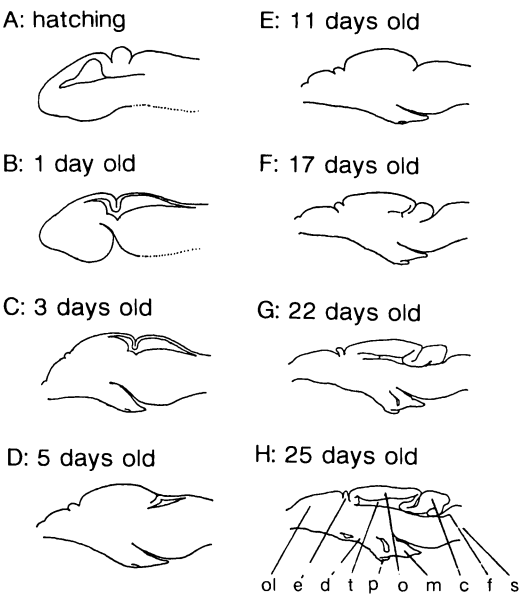

Fig. 7. Morphological changes of the brain of *Sillago japonica* larvae (c: cerebellum, d: diencephalon, e: epiphysis, f: fourth ventricle, p: pituitary gland, m: medulla oblongata, o: optic lobe, ol: olfactory lobe, s: saccus vasculosus, t: third ventricle).

of the olfactory lobe to the optic lobe was 0.43. Formation of the gill lamellae continued and the gill structure was visible from outside. This stage lasted until the start of flexion of the notochord and changes in muscle structure.

7) Changes in muscle structure (Fig. 6-B)

Fifteen days after hatching (4.9 mm NL, 5.1 mm TL), the form of the myomeres changed from an echelon (<) shape to a reverse sigma (ℨ) shape (Fig. 6-A, B); that is, the myomere structure had changed from the larval type to the adult type. Flexion of the notochord was seen at the caudal region. Neural and hemal spines were present on the vertebra, even though some spines remained as cartilage (Fig. 8-A). Masses of cells, which would later differentiate into fin rays and pterygiophores, had formed in the area of the dorsal and anal fins. The shape of the middle intestine changed from the torsional trunk to a looped one. The cerebellum was present and the ratio of the olfactory lobe to the optic lobe was 0.55 (Fig. 7-F). This stage lasted until the completion of the flexion of the notochord.

8) Flexion of notochord (Fig. 6-C)

Eighteen days after hatching (6.7 mm NL, 7.6 mm

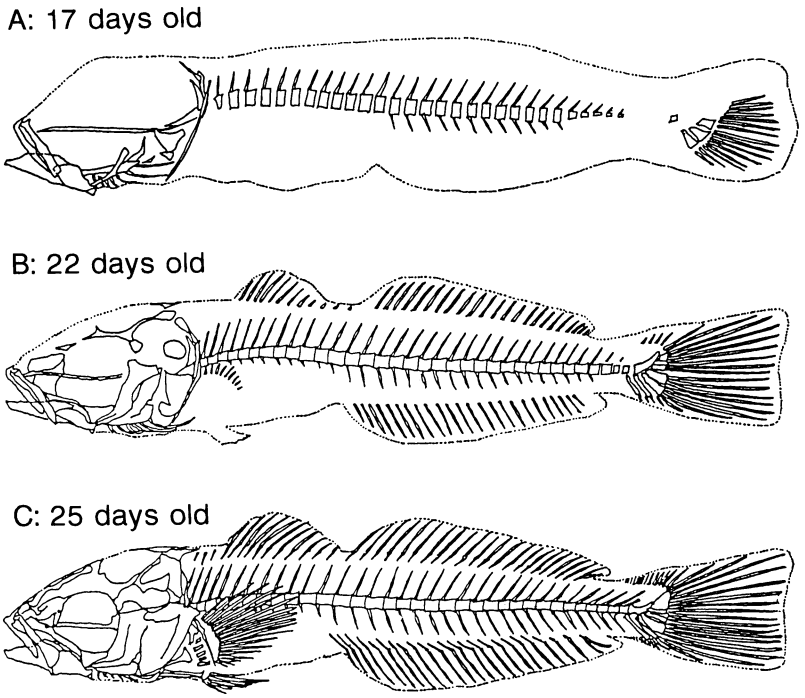

Fig. 8. Osteological development in *Sillago japonica* larvae.

TL), the flexion of the notochord was complete. The caudal fin rays had increased in length. The full complement of fin rays had developed as well as the formation of the fin rays of the second dorsal fin and the anal fin. The finfold of the second dorsal fin separated from the caudal fin. Two morphometric ratios became stable at SD/TL=0.30 and SA/TL=0.43 at this stage. Ventricle cavities were evident in the brain. The teeth were visible on both the upper and lower jaws. Pigmentation was similar to that of the previous stage except at the end of the trunk region, where large melanophores appeared. This stage lasted until the formation of the pectoral fin rays.

9) Formation of pectoral fin rays (Fig. 6-D)

Twenty-one days after hatching (9.4 mm NL, 10.8 mm TL), the pectoral fin rays were formed and rudimentary pelvic fins had appeared. The dorsal and anal fins were completely formed. The vertebral column was ossified (Fig. 8-B). The shape of the caudal fin had changed from the "rounded type" to the "emarginate type" (Bond, 1979). The ratio of the olfactory lobe to the optic lobe was 0.68 (Fig. 7-G). The differentiation of pyloric caeca was evident. Trunk pigment had increased at the transverse band. Melanophores were evident at the base of the caudal fin. This stage lasted until the formation of the pelvic fin rays.

10) Formation of stomach (Fig. 6-E)

Twenty four days after hatching (10.5 mm NL, 12.3 mm TL), gastric glands began to differentiate. Pelvic fin rays were visible. The nostril was divided into two pores, the anterior and the posterior nostrils. The dorsal surface of the head was heavily pigmented, particularly behind the eyes. Small external melanophores and iridophores covered the dorsal half of the trunk. The body coloration had changed from the larval type to that of the adult.

Discussion

Many staging categories have been proposed for different fish larvae (e. g., Doyle, 1977; Ryland, 1963). A staging system based on notochord flexion was generally used, i.e., yolk-sac larvae, preflexion larvae, flexion larvae, postflexion larvae and transformation larvae (Kendall et al., 1984). The developmental stages based on organ development correspond to usual terminology for early development. Yolk-sac larvae, preflexion to postflexion larvae and

transformation larvae correspond exactly to Periods I, II and III in this paper, respectively. The same phenomena were reported for the developmental process of *Engraulis mordax* (O'Connell, 1981). Formation of the digestive organs precede formation of the locomotor and respiratory organs and are observed until 4 mm in standard length, that is, the yolk absorption stage. After yolk absorption, the locomotor and respiratory organs are formed (O'Connell, 1981). Studies of larval energetics and metabolism require not only a general staging scheme which is convenient for taxonomic and systematic analyses, but also detailed knowledge of the morphological and functional development of organ systems.

The thresholds between stages were defined by marked organ developments in this study. Balon (1979) insisted that a threshold should be defined as an abrupt functional change, and that the theory of saltation required detailed analyses of all biological aspects. Staging and description of larval development should be essential as a scale for biochemical, physiological and ecological analyses, although the descriptions in this paper would not be sufficient from Balon's point of view.

The histological observations demonstrated that there was an important relationship between the development of the brain and the behavior of the Japanese whiting larvae which involved olfaction. Japanese whiting is a pelagic feeder during the larval period, but changes to a demersal feeder upon becoming juveniles. The olfactory sense for Japanese whiting probably increases in importance at this time. The ratio of the olfactory lobe to the optic lobe increased from 0.3 (stage 5) to 0.68 (stage 9) in sagittal length. The ratios of pelagic feeding fishes indicate low values, i.e., Japanese flyingfish *Cypselurus agoo agoo*, 0.30; chub mackerel *Scomber japonicus*, 0.37; yellowtail *Seriola quinqueradiata*, 0.48, whereas the ratios are high in demersal feeders, i.e., black sea bream *Acanthopagrus schlegeli*, 1.43; catfish *Silurus asotus*, 1.89; rock cod *Epinephelus fario*, 0.92 (calculated from the descriptions in Uchihashi, 1953). The increase of this ratio in the Japanese whiting, and its values in other species, suggest that high values are associated with an acute olfactory sense which is necessary in demersal feeding.

There was a noticeable size difference found between the present results and the description of sea-caught larvae reported by Ueno et al. (1958). Although the specimens were originally identified as *S. sihama*, *S. sihama* is considered to be a misidentification of *S. japonica* (Sano and Mochizuki, 1984). Plates 6, 7, 8 and 9 in Ueno's report correspond to stages 6, 8, 9 and 10 in this paper from the point of view of organ development. The total lengths of these specimens were reported as 3.1 mm, 5.9 mm, 9.0 mm and 15.5 mm on plates 6, 7, 8 and 9, respectively, whereas the values at the lower 90% confidence limit of the total lengths at respective stages were 4.24 mm, 7.08 mm, 9.98 mm and 11.47 mm for stages 6, 8, 9 and 10 in this study. Hence the total lengths of the sea-caught larvae described by Ueno et al. were smaller than those of the reared larvae in this study except for stage 10. These differences might be attributed to shrinkage in the process of net capture and fixation. Formaldehyde fixation usually causes tissue shrinkage, particularly in fish larvae (Blaxter, 1971; Rosenthal and von Westernhagen, 1976; Fukuhara, 1979; Oozeki and Hirano, 1988). The size differences in larval stages smaller than 10 mm TL might be due to shrinkage caused by formaldehyde fixation and preservation.

The descriptive information of the development of organs and morphological characteristics of Japanese whiting larvae, as described in this paper, would be useful for future studies of their metabolic development, and in comparative studies of the developmental physiology of larval fish.

Acknowledgments

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Literature cited

Balon, E. K. 1979. The theory of saltation and its application in the ontogeny of fishes: steps and thresholds. Env. Biol. Fish., 4(2): 97–101.

Blaxter, J. H. S. 1971. Feeding and condition of Clyde herring larvae. Rapp. P.-v. Reún., Cons. Int. Explor. Mer, 160: 128–136.

Bond, C. E. 1979. Biology of fishes. W. B. Saunders Company, Philadelphia, London, Toronto, 253 pp.

Dingerkus, G. and L. D. Uhler. 1977. Enzyme clearing of

alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Tech., 5(24): 229–232.
Doyle, M. J. 1977. A morphological staging system for the larvae development of the herring *Culpea harengus* L. J. Mar. Biol. Ass. U.K., 57: 859–867.
Fukuhara, O. 1979. On the shrinkage of eggs and larvae of red sea bream, *Chrysophrys major*, preserved in the formalin. Suisanzoshoku, 27(3): 129–136. (In Japanese with English abstract.)
Fu, Y., K. Hirayama and Y. Natsukari. 1991a. Morphological differences between two types of the rotifer *Brachionus plicatilis* O. F. Müller. J. Exp. Mar. Biol. Ecol., 151: 29–41.
Fu, Y., K. Hirayama and Y. Natsukari. 1991b. Genetic divergence between S and L type strains of the rotifer *Brachionus plicatilis* O. F. Müller. J. Exp. Mar. Biol. Ecol., 151: 43–56.
Imada, O., Y. Kageyama, T. Watanabe, C. Kitajima, S. Fujita and Y. Yone. 1979. Development of a new yeast as a culture medium for living feeds used in the production of fish seed. Bull. Japan. Soc. Sci. Fish., 45(8): 955–959.
Kamiya, N. 1925. Description of pelagic eggs and their larvae in Tateyama-bay. J. Imperial Fish. Inst., 21(3): 71–85. (In Japanese.)
Kendall, A. W., Jr., E. H. Ahlstrom and H. G. Moser. 1984. Early life history stages of fishes and their characters. Pages 11–22 *in* Ontogeny and systematics of fishes. Amer. Soc. Ichthyol. Herpetol., Spec. Publ. 1.
Luna, L. G. (Ed). 1968. Manual of histologic staining methods of the AFIP, 3rd ed. McGraw-Hill, New York, 258 pp.
Mochizuki, K. 1984. Family Sillaginidae. Page 151 *in* H. Masuda, K. Amaoka, C. Araga, T. Ueno and T. Yoshino, eds. The fishes of the Japanese Archipelago (English text volume). Tokai University Press, Tokyo.
O'Connell, C. P. 1981. Development of organ systems in the northern anchovy, *Engraulis mordax*, and other teleosts. Amer. Zool., 21: 429–446.
Oozeki, Y. and R. Hirano. 1985. Effects of temperature changes on the development of eggs on the Japanese whiting *Sillago japonica* Temminck et Schlegel. Bull. Japan. Soc. Sci. Fish., 51(4): 557–572.
Oozeki, Y. and R. Hirano. 1988. Effects of glutaraldehyde fixation on the body size of red sea bream (*Pagrus major*) larvae. Aquaculture, 71: 265–269.
Rosenthal, H. and H. von Westernhagen. 1976. Effects of formalin fixation on length, weight and condition factors of three siganid species. Arch. Fisch Wiss., 27(1): 37–43.
Ryland, J. S. 1963. The swimming speeds of plaice larvae. J. Exp. Biol., 40: 285–299.
Sano, M. and K. Mochizuki. 1984. A revision of the Japanese sillaginid fishes. Japan. J. Ichthyol., 31(2): 136–149.
Uchihashi, K. 1953. Ecological study of Japanese teleosts in relation to the brain morphology. Bull. Japan Sea Reg. Fish. Res. Lab., 2: 1–166. (In Japanese.)
Ueno, M. and S. Fujita. 1954. On the development of the egg of *Sillago sihama* (Forskal). Japan. J. Ichthyol., 3 (3,4,5): 118–120. (In Japanese with English abstract.)
Ueno, M., T. Senta and S. Fujita. 1958. *Sillago sihama* (Forskal). Pages 63–64 *in* Second laboratory of Fisheries Biology, Kyushu University, eds. Studies of the eggs, larvae and juvenile of Japanese fishes. Shyukousha, Fukuoka. (In Japanese.)

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飼育条件下におけるキス仔魚の発育過程

大関芳沖・黄　鵬鵬・平野禮次郎

キス (*Sillago japonica*) の受精卵を自然採卵法により採卵・孵化させた後，シオミズツボワムシを与えて 25.1±1.0°C で飼育した．仔稚魚の発育過程を外部形態ならびに各部分長・乾重量の測定結果および組織切片標本，透明骨格標本による内部観察を基に，10 段階に分けて記載した．この過程を急速に形成が進行する器官に注目して検討したところ，仔魚期全体を消化器官形成期，呼吸・運動器官形成期，稚魚移行期の 3 期に大別することが適当と考えられた．全長は孵化時 1.3 mm, 10 日齢 3.9 mm, 20 日齢 9.77 mm と増加し，全長と乾重量との関係は乾重量 (mg) = $0.811 \cdot 10^{-3} \times$全長 $(mm)^{2.995}$ で表された．

(大関：985 塩釜市新浜町 3-27-5　東北区水産研究所；黄：中華民国台北市南港区　中央研究院動物研究所；平野：岩手県気仙沼郡三陸町越喜来　北里大学水産学部)