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Development of Mixed-feeding Stage Larvae of Japanese Flounder, *Paralichthys olivaceus* (Pisces: Paralichthyidae)

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Early growth, yolk and oil exhaustion, early morphological development and initial feeding were examined in laboratory-reared larvae of the Japanese flounder, *Paralichthys olivaceus*. Based on nutritional transition, the early larval stage of Japanese flounder was divided as follows: the endogenous nutritional phase from hatching (mean total length 2.2 mm) to 90 hours after hatching (HAH) (3.6 mm); a mixed-feeding phase from 90 to 140 HAH (4.2 mm); and an exogenous feeding phase after 140 HAH. Furthermore, the endogenous nutritional phase was divided into two sub-phases at around 50–60 HAH (3.4–3.5 mm), at which time flexion points were apparent on the semi-logarithmic plots of yolk and oil globule absorption. This border coincided with the first observations of mouth opening. In the pre-mouth-opening phase from hatching to 50–60 HAH, a rapid increase in larval growth rate was recorded, while growth rate was slower in the post-mouth-opening phase. Based on comparisons with early-life-stage features of other marine fish species, Japanese flounder larvae retained very few endogenous nutritional resources at the onset of feeding, a characteristic that is disadvantageous for survival in spite of a long mixed feeding period. However, the high rate of feeding at the exhaustion of endogenous nutrition, the reduced time from initial feeding to 100% feeding, and the large larval size at the onset of feeding found in this study are considered to advantageous features supporting the nutritional requirements of larvae.

Key words: flounder, *Paralichthys olivaceus*, nutritional resources, larval development, oil globule, yolk, mixed feeding period

In the early larval stage of fish, the transition from endogenous to exogenous nutrition is referred to as a "critical period" because it is during this time that a large percentage of the overall mortality occurs[1-4]. This transition process has been studied in several marine fish species, particularly in cultivated tropical fish[5]. Kohno *et al.*[5,6] compared the transition process in several tropical marine

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fish species to evaluate the vulnerability of early stage larvae. Moteki *et al.*[7] investigated this transition process in the red sea bream (*Pagrus major*) as a representative temperate species.

The Japanese flounder (*Paralichthys olivaceus*) is one of the most important commercial fish found in the coastal waters of Japan, and is extensively propagated artificially for commercial purpose. Due to the commercial importance of this species, and the resulting interest in its growth and development, the larval development and early life history of this species have been well studied in larvae raised in artificial environment as well as in the wild [8-12]. To understand the events related to the critical, mixed-feeding period better, Fukuhara[13] studied the yolk absorption rate and growth of four marine fish species, including the Japanese flounder, under different temperature conditions. Except for that study[13], little attention has been paid to the development of the Japanese flounder larvae in mixed-feeding phase. Studies of development during the critical, mixed-feeding period in this fish would contribute to the basic understanding of the larval biology of the other temperate species. The objective of this study was to describe events pertaining to the transition from endogenous to exogenous nutrition in laboratory-reared larvae of the Japanese flounder. Characteristics of the transition process were compared with those of several other marine fish.

Materials and Methods

The larvae used in this study originated from eggs that were spawned naturally on 23 May 2001 at the Hakata-jima Station of the Japan Sea-Farming Association, in Ehime. The eggs were placed in a 1000-L tank in the rearing facilities of Fukuyama University, Innoshima, Hiroshima, at a density of about 30 eggs/L. The eggs began hatching at 16:00 on 25 May, and hatching ended at 22:00 on the same day; the peak of hatching was at 19:00. In this experiment, the time of hatching was considered to be 19:00. During the experiment, a still-water system was employed, with the water temperature ranging from 18.3 to 21.2°C (mean ± SD: 20.2 ± 0.58°C) and the salinity from 34.5 to 35.0 psu. Rotifers, *Brachionus plicatilis*, were added to maintain a density exceeding 10 individuals/L. Green algae, *Nannochloropsis* sp., was introduced into the water.

In the period between hatching and 158 hours after hatching (HAH), 15 larvae were removed one to four times each day and preserved in 5% formalin. Before fixation, the following data were recorded: total length; oil globule diameter; yolk length and height; rotifer numbers in the gut; and eye pigmentation. The number of rotifers in the gut was counted once or twice each day between 10:00 and 16:00. Mouth width was measured for 10 preserved larvae. The method of calculating yolk and oil globule volumes followed the methods of Blaxter and Hempel[14].

Results

Growth

The mean total length of larvae at hatching was 2.17 mm (SD = 0.10 mm). Thereafter the larvae grew rapidly until about 40 HAH, with a mean total length of 3.42 mm (0.09) at 42 HAH (Fig. 1). After 40–50 HAH, the growth rate slowed until about 110 HAH, and mean total length was 3.73 mm (0.14) at 109 HAH. Subsequently, the growth rate accelerated, and mean total length was 4.62 mm (0.15) at 158 HAH.

Endogenous nutrition

Newly hatched larvae had a mean yolk volume of 2226 x 10^{-4} mm^3 (SD = 327 x 10^{-4} mm^3), including an oil globule volume of 24.4 x 10^{-4} mm^3 (4.2 x 10^{-4}). The yolk was consumed rapidly, decreasing to 717 x 10^{-4} mm^3 (166 x 10^{-4}) (32.2% of the volume in newly hatched larvae) at 22 HAH, 127 x 10^{-4} mm^3 (41.8 x 10^{-4}) (5.7%) at 49 HAH, and 3.8 x 10^{-4} mm^3 (2.1 x 10^{-4}) (0.2%) at 73 HAH (Fig.2). The yolk appeared to be completely absorbed in larvae at 90 HAH in 7 out of the 15 larvae examined. The yolk was consumed in all larvae by 137 HAH. Semi-logarithmic plots of mean yolk volume (Fig. 2) show that the slope of the regression line is gentle until about 60 HAH, with a mean of yolk volume of 72 x 10^{-4} mm^3 (31 x 10^{-4}) at 59 HAH. Absorption of the yolk became rapid after 60 HAH. The results of the regression analyses are shown in Table 1.

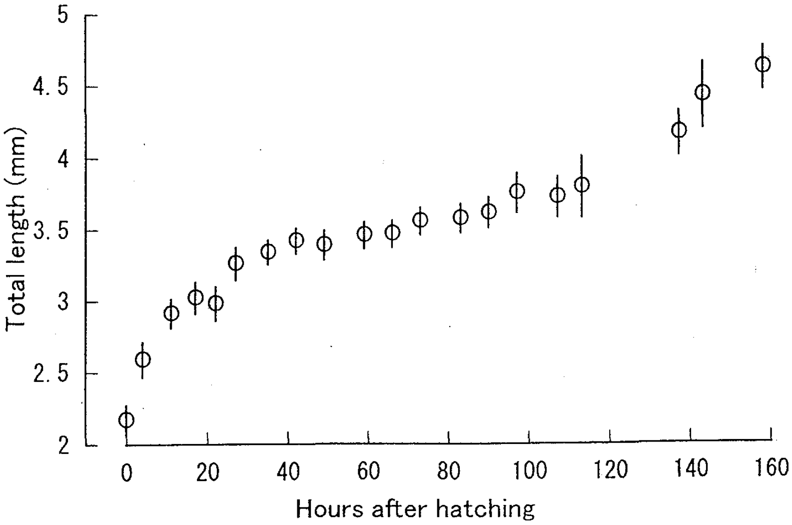

Fig.1. Growth (mean total lengths) with standard deviations (bars) in larval Japanese flounder, *Paralichthys olivaceus.*

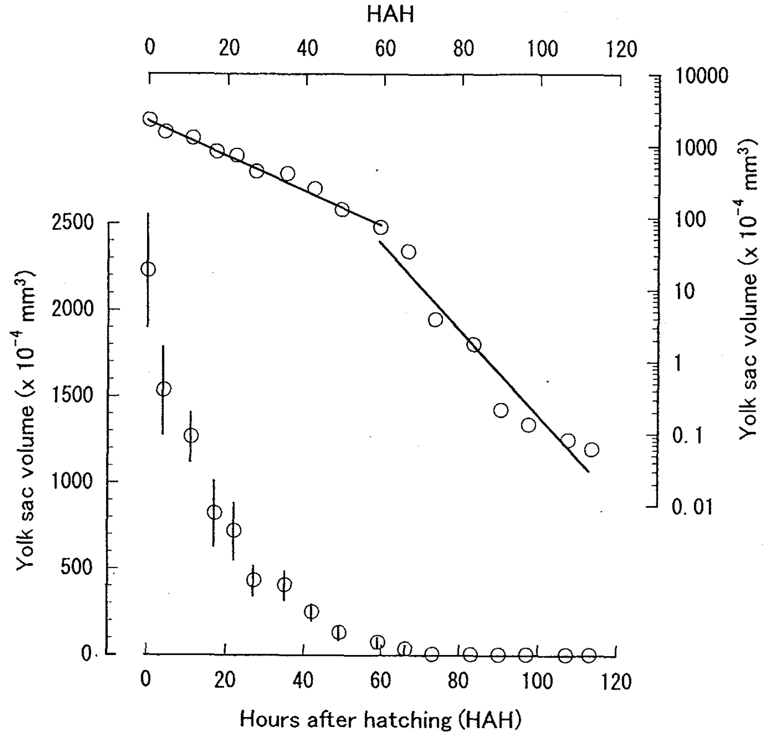

Fig. 2. Yolk absorption in larval Japanese flounder, *Paralichthys olivaceus.*

Top right: Semi-logarithmic plots of mean volumes; bottom left: mean volumes with standard deviations (bars).

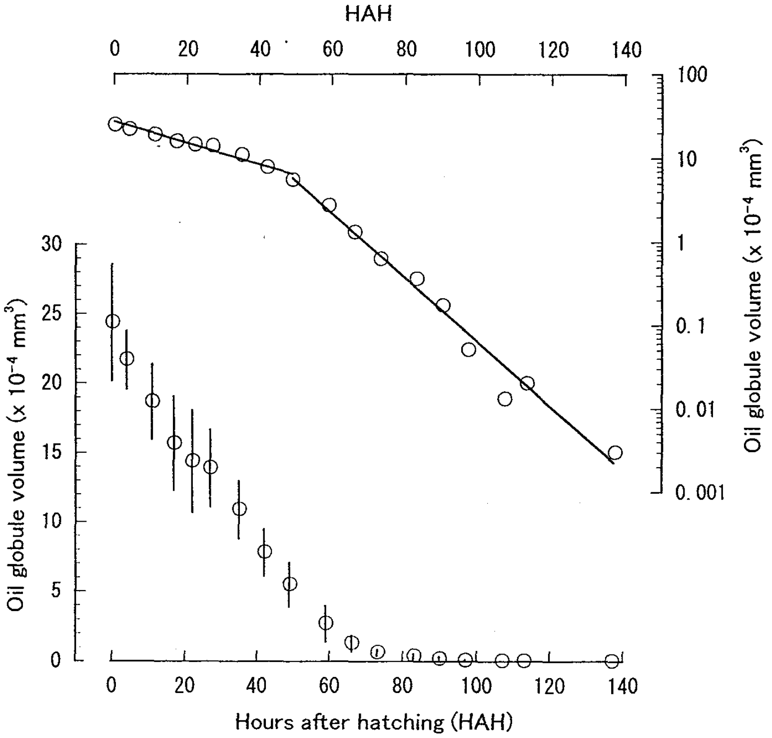

Fig. 3. Oil globule absorption in larval Japanese flounder, *Paralichthys olivaceus.*

Top right: Semi-logarithmic plots of mean volumes; bottom left: mean volumes with standard deviations (bars).

The oil globule was consumed relatively slowly, decreasing to 14.4 x 10^{-4} mm^3 (SD = 3.7 x 10^{-4} mm^3) (59.0%) at 22 HAH, 5.5 x 10^{-4} mm^3 (1.6 x 10^{-4}) (22.5%) at 49 HAH and 0.6 x 10^{-4} mm^3 (0.2 x 10^{-4}) (2.5%) at 73 HAH (Fig. 3). At 90 HAH, 13 out of 15 larvae possessed an oil globule, ranging from 0.03

Table 1. Regression analyses of yolk sac and oil globule volumes in Japanese flounder larvae, *Paralichthys olivaceus*

	HAH*	n	a	b	r
Yolk sac volume	0-59	10	-0.056	7.704	-0.992
	59-113	8	-0.139	11.998	-0.969
Oil globule volume	0-49	9	-0.028	3.246	-0.981
	49-137	10	-0.091	6.181	-0.989

Equations, ln (vol.) = $a \cdot$(HAH) + b. r, correlation coefficient.
*Hours after hatching.

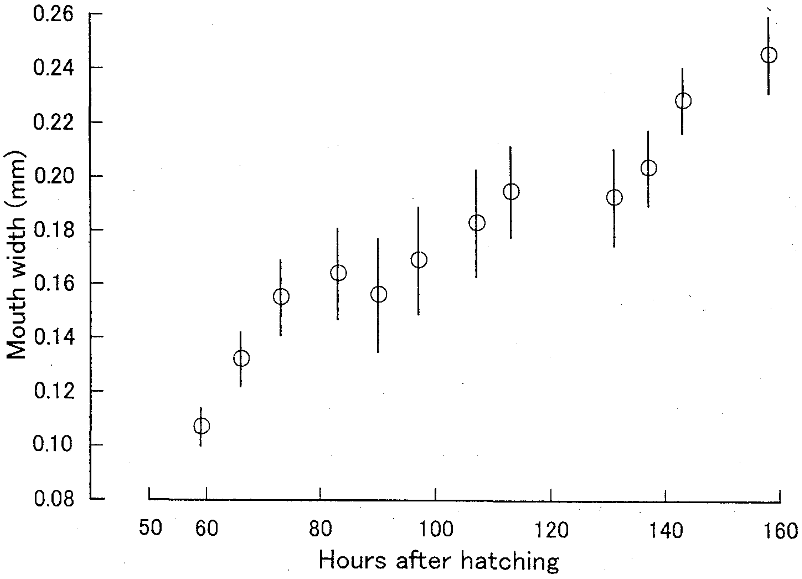

Fig.4. Mouth width (circles) with standard deviations (bars) in larval Japanese flounder, *Paralichthys olivaceus.*

to 0.79 x 10^{-4} mm^3. The oil globule was entirely consumed in all larvae by 143 HAH. On the semi-logarithmic plots of the mean volume, one flexion point was apparent at about 50 HAH (Fig. 3), after which the slope of the regression line became steep (Table 1).

Morphological development

Eyes began to become pigmented at 49 HAH and pigmentation was complete by 66 HAH in all larvae. The larvae started moving their pectoral fins at 49 HAH. The larvae started opening their mouths at 59 HAH, when mean mouth width was 0.107 mm (SD = 0.007 mm, n = 9). The mouth width initially increased rapidly until about 80 HAH, with a mean width of 0.164 mm (0.017, 10) at 83 HAH (Fig.4). Thereafter, the increase in width slowed somewhat until about 140 HAH (mean 0.204, SD = 0.014 at 137 HAH), after which the mouth width again increased rapidly (mean 0.246, SD = 0.014 at 158 HAH).

Exogenous feeding

Feeding on rotifers was first observed at 90 HAH, at which time 11 out of 15 larvae contained 1 to 9 rotifers (mean = 3.5 individuals) in their gut. By 110 HAH, all larvae had ingested rotifers. At this time, the larval digestive tracts contained 1 to 13 rotifers (mean = 6.9 individuals). The number of rotifers ingested by the larvae increased slowly, ranging from 2 to 21 individuals (mean = 10.0 individuals) at 158 HAH.

Discussion

Scheme of development

The transition from endogenous to exogenous nutrition dependence and the developmental events of the early larval Japanese flounder are schematically represented in Fig. 5. The transition from endogenous to exogenous nutrition occurred in three phases: the period from hatching to about 90 HAH was a purely endogenous nutritional phase; the second phase was a mixed-feeding phase, and occurred between about 90 to 140 HAH; the third phase occurred after about 140 HAH, and was a purely exogenous phase. Flexion points were observed on the semi-logarithmic plots of the absorption of the yolk sac and oil globule volumes at around 50–60 HAH, which coincided with the time of mouth opening. This suggests that some physiological changes occurred at 50–60 HAH that might be related to mouth opening. Before 50–60 HAH, a rapid growth in total length was recorded, while growth slowed after 50–60 HAH. Due to these two morphological changes that occurred at 50–60 HAH, we divided the endogenous nutritional phase into two sub-phases: the pre-mouth-opening phase and the post-mouth-opening phase (Fig. 5). In the latter phase, a rapid increase of mouth width was recorded, in spite of slow total length growth. During this phase, development of the

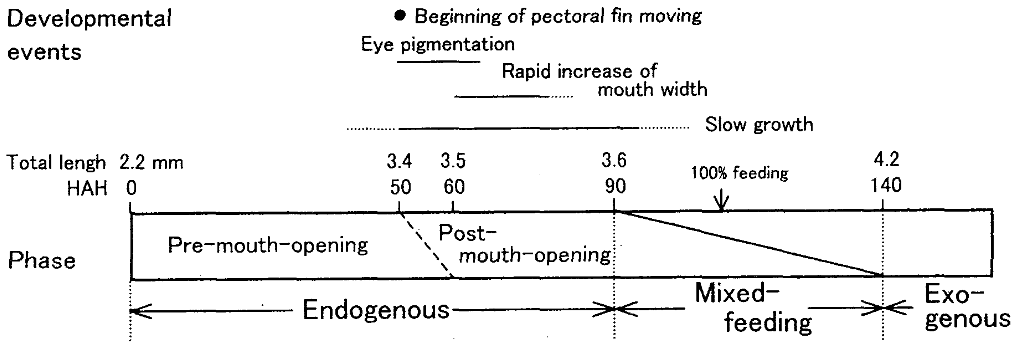

Fig. 5. Diagrammatic outline of the development of larval Japanese flounder, *Paralichthys olivaceus*. Phases are explained in the text. 100% feeding, time by which all larvae had started feeding. HAH, hours after hatching.

feeding apparatus before the onset of feeding at 90 HAH is thought to have a higher priority than growth in total length in Japanese flounder.

Comparisons with other marine fish

Many studies have described the transitional process from endogenous nutrition to exogenous feeding in several marine fish. Kohno[5)] and Moteki *et al.*[15)] summarized the characters pertaining to the transition for the following species: sand eel (*Ammodytes personatus*), American shad (*Alosa sappidissima*), milkfish (*Chanos chanos*), seabass (*Lates calcarifer*), groupers (*Epinephelus fuscoguttatus* and *E. coioides*), red snapper (*Lutjanus argentimaculatus*), little tuna (*Euthynnus affinis*), rabbitfish (*Siganus guttatus* and *S. javus*) and Black Sea turbot (*Psetta maxima*). Furthermore Moteki *et al.*[7)] studied the nutritional transition in the larvae of the red sea bream (*Pagrus major*). We compared several selected characters pertaining to the nutritional transition in Japanese flounder larvae with those in the above noted fish. The larval size at the onset of feeding for little tuna and seabass were obtained from the data of Kohno *et al.*[16,17)].

The time from the onset of feeding to exhaustion of endogenous nutrition sources was 53 h for the Japanese flounder, which was the moderate value among the compared species. This time was similar to that in the following species: American shad (48 h), milkfish (48 h), little tuna (47 h), and red sea bream (48.5 h). However, Japanese flounder larvae had the smallest volume of endogenous nutrition at the onset of feeding, 0.39×10^{-4} mm^3, followed by the red snapper and red sea bream (0.4 and 1.5×10^{-4} mm^3, respectively). In the other species, the volume ranged from 2.7 to 130.6×10^{-4} mm^3. Energy deficiency at around the time that endogenous nutrition resources are depleted is thought to be one of the main causes of mortality in early stage larvae[4,18)]. Although the mixed-feeding period is somewhat long in Japanese flounder, the small volume of endogenous nutrition that is present at the onset of feeding is thought to be disadvantageous in terms of nutritional transition. However, the number of rotifers in the gut at the exhaustion of endogenous nutrition was fourth highest in Japanese flounder larvae among the compared species, with a mean of 11.5 individuals. The mean number of rotifers in the gut for species with a larger number was 43.1 for the Black Sea turbot, 12.7 for the red sea bream, and 12.4 for the seabass. In the remaining species, the number of rotifers in the gut at this time ranged from 1.6 to 8.8 individuals. The Japanese flounder had the third shortest time from the onset of feeding to the time when all larvae were ingesting rotifers (100% feeding), with a time of 20 h. The shortest time was found in the red sea bream (10 h), followed by seabass (15.5 h). When compared to fish that hatch at about the same size, Japanese flounder had the largest larval size (total length) at the onset of feeding (3.62 mm). The other compared species and their total lengths were: Black Sea turbot (3.5 mm), little tuna (3.5 mm), red sea bream (3.3 mm), *Epinephelus coioides* (2.75 mm) and *E. fuscoguttatus* (2.81 mm), *Siganus guttatus* (2.95 mm), *S. javus* (2.85 mm), and seabass (2.59 mm). Larger larval size is thought to result in better swimming ability and wider selectivity in prey size, and therefor result in a higher feeding ability[4,19].

Conclusions

Japanese flounder larvae are thought to have a high ability to feed at the transition stage from reliance on endogenous to exogenous nutrition resources. This conclusion is supported by the high rate of feeding at the stage of exhaustion of endogenous nutrition, the reduced time from the onset of feeding to 100% feeding, and the large larval size at the onset of feeding found in this study. The larvae started to grow rapidly at the 100% feeding stage (110 HAH), suggesting a smooth transition of nutritional resources. This smooth transition occurred despite the fact that little endogenous nutrition available at around the onset of feeding as a backup nutritional source. The high feeding ability and lack of endogenous energy at the onset of feeding at the transition phase in Japanese flounder were similar to those characteristics in another temperate species, the red sea bream[7].

In the present study, we examined the biological natures of Japanese flounder larvae at a water temperature of about 20°C. However, this temperature is appreciably higher than the spawning temperatures in the coastal waters of Japan (12–17°C)[20]. It is well known that temperatures influence the larval growth, yolk absorption rate, and initial feeding rate[11,13,18,21,22]. Examinations under lower temperature conditions would result in different transition patterns. Thus, to understand the early life history of the Japanese flounder better, it is necessary to consider the effects of temperature on the nutritional transitional process.

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References

1) J. C. Marr: The “critical period” in the early life history of marine fishes. *J. Cons. perm. int. Explor. Mer*, **21**, 160-170 (1956).

2) J. R. Hunter: The feeding behavior and ecology of marine fish larvae, in “Fish behavior and its

use in the capture of fishes" (ed. by J. E. Bardach, J. J. Magnuson, R. C. May, and M. Reinhart), ICLARM, Manila, 1980, pp. 287-330.

3) B. A. Rogers and D. T. Westin: Laboratory studies on the effects of temperature and delayed initial feeding on development of striped bass larvae. *Trans. Am. Fish. Soc.*, **110**, 100-110 (1981).

4) E. Kamler: Early Life History of Fish: An energetics approach. Chapman and Hall, London. 1992.

5) H. Kohno: Early life history features influencing larval survival of cultivated tropical finfish, in "Tropical mariculture" (ed. by S. S. de Silva), Academic Press, London. 1998, pp. 71-111.

6) H. Kohno, A. Ohno, and Y. Taki: Why is grouper larval rearing difficult ?: a comparison of the biological natures of early larvae of four tropical marine fish species, in "The Third Asian Fisheries Forum" (ed. by L. K. Chou, A. D. Munro, T. J. Lam, T. W. Chen, L. K. K. Cheong, D. K. Ding, K. K. Hooi, H. W. Khoo, V. P. E. Phang, K. F. Shim, and C. H. Tan), Asian Fish. Soc., Manila, 1994, pp. 450-453.

7) M. Moteki, T. Ishikawa, N. Teraoka, and H. Fushimi: Transition from endogenous to exogenous nutritional sources in larval red sea bream, *Pagrus major. Suisanzoshoku*, **49**, 323-328 (2001).

8) T. Minami: The early life history of a flounder *Paralichthys olivaceus. Nippon Suisan Gakkaishi*, **48**, 1581-1588 (1982).

9) Y. Takahashi: Morphological and behavioral changes with growth in reared larvae and juveniles of a flounder, *Paralichthys olivaceus. Suisanzoshoku*, **33**, 43-52 (1985).

10) O. Fukuhara: Morphological and functional development of Japanese flounder in early life stage. *Nippon Suisan Gakkaishi*, **52**, 81-91 (1986).

11) T. Seikai, J. B. Tanagonan, and M. Tanaka: Temperature influence on larval growth and metamorphosis of the Japanese flounder *Paralichthys olivaceus* in the laboratory. *Nippon Suisan Gakkaishi*, **52**, 977-982 (1986).

12) T. Noichi: Early life history, in "Biology and stock enhancement of Japanese flounder" (ed. by T. Minami and M. Tanaka), Koseisha-koseikaku, Tokyo, 1997, pp. 25-40.

13) O. Fukuhara: Effects of temperature on yolk utilization, initial growth, and behavior of unfed marine fish-larvae. *Mar. Biol.*, **106**, 169-174 (1990).

14) J. H. S. Blaxter and G. Hempel: The influence of egg size on herring larvae (*Clupea hurengus* L.). *J. Cons. perm. int. Explor. Mer*, **28**, 211-240 (1963).

15) M. Moteki, K. Yoseda, T. Sahin, C. Üstündag, and H. Kohno: Transition from endogenous to exogenous nutritional sources in larval Black Sea turbot, *Psetta maxima. Fisheries Sci.*, **67**, 571-578 (2001).

16) H. Kohno, A. Ohno, and T. Nakao: Transition from endogenous nutrition to exogenous food

sources in larval little tuna, *Euthynnus affinis*. *Suisanzoshoku,* **42**, 165-169 (1994).

17) H. Kohno, S. Hara, and Y. Taki: Early larval development of the seabass, *Lates calcarifer*, with emphasis on the transition of energy sources. *Nippon Suisan Gakkaishi,* **52**, 1719-1725 (1986).

18) G. C. Laurence: Influence of temperature on energy utilization of embryonic and prolarval tautog, *Tautoga onitis*. *J. Fish. Res. Board. Can.*, **30**, 435-442 (1973).

19) J. R. Hunter: Feeding ecology and predation of marine fish larvae, in “Marine fish larvae” (ed. by R. Lasker), Washington Sea Grant Program, 1981, pp. 33-77.

20) T. Minami: Life history, in “Biology and stock enhancement of Japanese flounder” (ed. by T. Minami and M. Tanaka), Koseisha-koseikaku, Tokyo, 1997, pp. 9-24.

21) J. R. Brett: Environmental factors and growth, in “Fish physiology Ⅷ” (ed. by W. S. Hoar, D. J. Randall, and J. R. Brett), Academic Press, London, pp. 599-675.

22) T. Fushimi: Ingestion by fish larvae and juveniles, in “The rotifer *Brachionus plicatilis* — Biology and mass culture” (ed. by Japanese Society of Fisheries Science), Koseisha-koseikaku, Tokyo, 1983, pp69-93.

ヒラメの混合栄養期仔魚の発育

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ヒラメ仔魚の成長，卵黄と油球の吸収様式，形態発育および初期摂餌量の変化を，孵化直後から 158 時間（158 HAH）調べた。その結果，栄養転換過程は以下のように分けられた： 1) 内部栄養期：孵化～90 HAH（全長 2.2～3.6 mm），2) 混合摂餌期：90～140 HAH (3.6～4.2 mm)，3) 外部栄養期：140 HAH～ (4.2 mm～)。また，50～60 HAH（3.4～3.5 mm）には内部栄養の吸収速度に変曲点が認められ，何らかの生理的な変化が起こることが示唆された。この時期は開口の時期とほぼ一致することから，内部栄養期をさらに前開口期と後開口期に分けた。ヒラメ仔魚の栄養転換過程を，これまでに知られているその他の海産魚種と比較したところ，ヒラメでは摂餌開始時の内部栄養量が少なく，これは初期仔魚の生残には不利な形質と考えられた。一方で，内部栄養の吸収完了時に摂餌量が多いこと，摂餌開始から 100%摂餌までの時間が短いこと，および摂餌開始時の全長が大きいことなど，初期摂餌に関しては，円滑な栄養転換をサポートする特徴をもつことも明らかとなった。