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Aspects of the Early Life History of the Japanese Eel *Anguilla japonica* Determined from Otolith Microstructure

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Otoliths (sagittae) of 22 elvers of *Anguilla japonica* which were collected in southern Kyushu, Japan, in December 1981 and January and March 1982, and in northern Taiwan in November 1981, and of two artificially hatched larvae were examined using scanning electron microscopy. Otoliths of five elvers of *A. marmorata* from the same places were also observed. The arrangement and other characteristics of otolith rings in both species were similar to those of the daily rings known for other species. The total number of rings in *A. japonica* for the November, December, January and March samples averaged 130.8, 155.2, 163.7 and 169.8, and that in *A. marmorata* for the November and January samples was 146.3 (mean) and 133, respectively. The otolith of 6 day old larvae indicated that the first ring in the elvers was not formed at hatching. The spawning time was estimated to be June–October 1981 for *A. japonica*, and May–August 1981 for *A. marmorata*, respectively. The three samples from southern Kyushu differed with each other in the time of spawning, the location of and/or the route from the waters they were spawned. The December elvers may have grown faster than the March elvers.

Since 1961, a total of 78 larvae of the Japanese eel (*Anguilla japonica* Temminck et Schlegel) have been collected mainly in the seas south of Okinawa and east of Luzon, the Philippines.[1-7] All the larvae were in the middle and latest stages of developing leptocephalus or in the metamorphic stage. Their early life history is poorly known, particularly the time and location of spawning and the larval life in the ocean. Recent development in the technology of induced spawning, on the other hand, has made available laboratory-hatched larvae almost every year.[8]

Daily growth increments in the otolith have been found in more than 20 species of fish larvae. The otolith microstructure, including changes in width and optical density of growth increments, has been shown to be related to a variety of morphological and ecological events in the early life history of fishes.[9-20] The otoliths of *A. japonica* elvers have rings similar to the daily growth increments observed in other fish larvae. As far as we know, age of elvers of *A. japonica* has not been studied, while Tsukamoto *et al.*[7] tried to demonstate the age of leptocephali of *A. japonica* from the otolith microstructure.

The present paper deals with scanning electron microscope (SEM) observations on the otolith microstructure of elvers and artificially hatched larvae of *A. japonica* with reference to the early life in the sea and estuary. Otoliths of elvers of *A. marmorata* were also observed for comparison.

Materials and Methods

Eel elvers, about 30 specimens in each sample, were collected in the mouth of the Sendai River, Kagoshima Prefecture, southern Kyushu, Japan, on December 11–13, 1981, and January 6 and March 25, 1982, and in the mouth of the Shih-Ting River, northern Taiwan, on November 16, 1981. All the Japanese elvers consisted of *A. japonica* except one specimen of *A. marmorata* from the January sample, while the Taiwanese elvers consisted of both *A. japonica* and *A. marmorata*. The elvers from the November, December and January samples were very young, and belonged to the same developmental stages of

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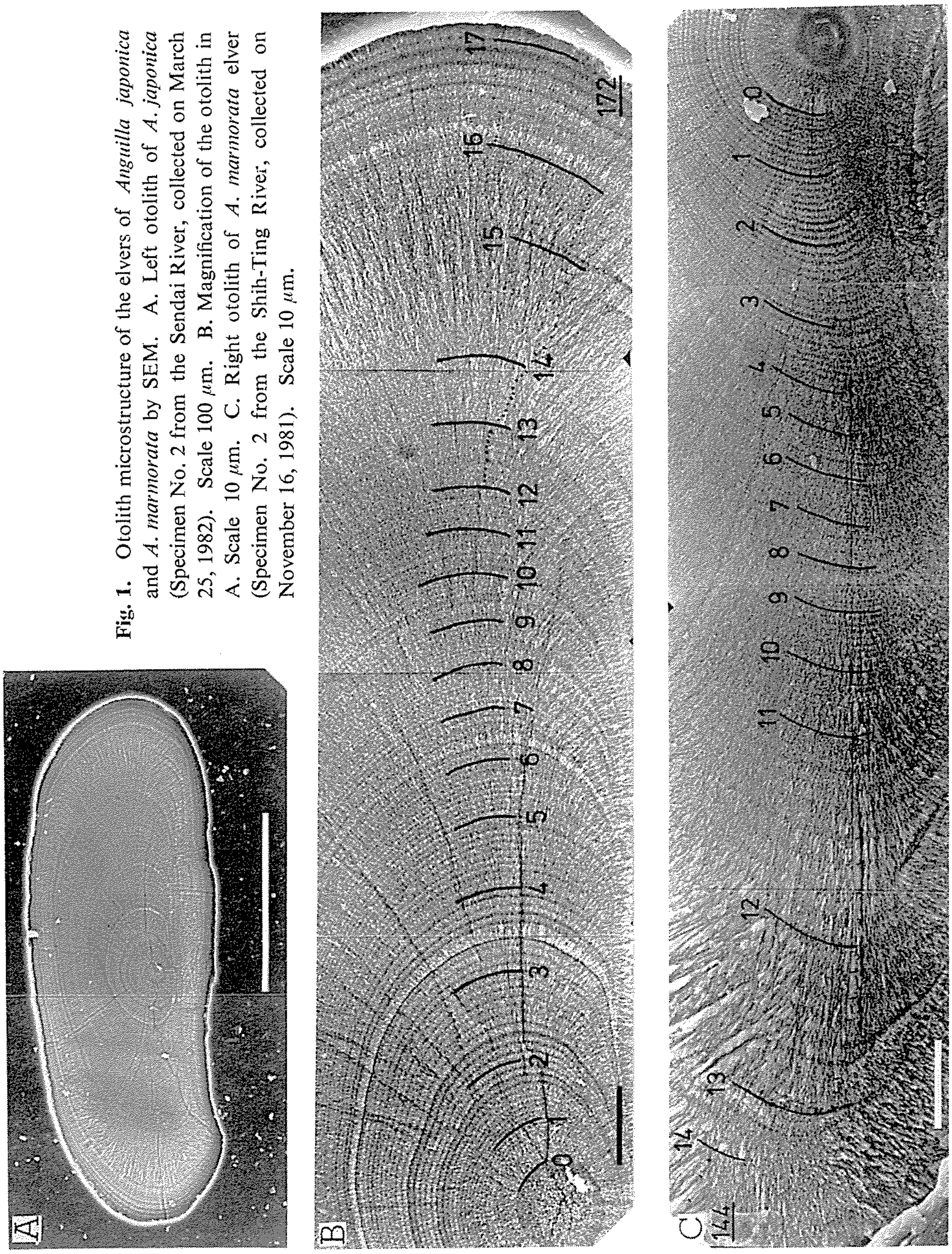

Fig. 1. Otolith microstructure of the elvers of *Anguilla japonica* and *A. marmorata* by SEM. A. Left otolith of *A. japonica* (Specimen No. 2 from the Sendai River, collected on March 25, 1982). Scale 100 μm. B. Magnification of the otolith in A. Scale 10 μm. C. Right otolith of *A. marmorata* elver (Specimen No. 2 from the Shih-Ting River, collected on November 16, 1981). Scale 10 μm.

pigmentation (Stage VB–VI A_{II} by Bertin[21]). Among the elvers from the March sample, however, pigmented elvers older than Stage VI A_{III} were found as well as the young elvers mentioned above. Such later stage pigmented elvers were excluded from the present examination. Eighteen specimens from the Sendai River and nine specimens from the Shih-Ting River (five *A. japonica* and four *A. marmorata*) were dissected for otolith collection.

Chiba Prefectural Inlandwater Fisheries Experimental Station, Japan, succeeded in inducing spawning of Japanese eel on February 6, 1985 and raised the larvae for 8 days after hatching which occurred a day after the spawning. We observed the otoliths of two 6 day old specimens (about 5.8 mm TL) from these reared larvae.

Sagittal otoliths were removed from the elvers and the artificially hatched larvae under a dissecting microscope, embedded in a few drops of

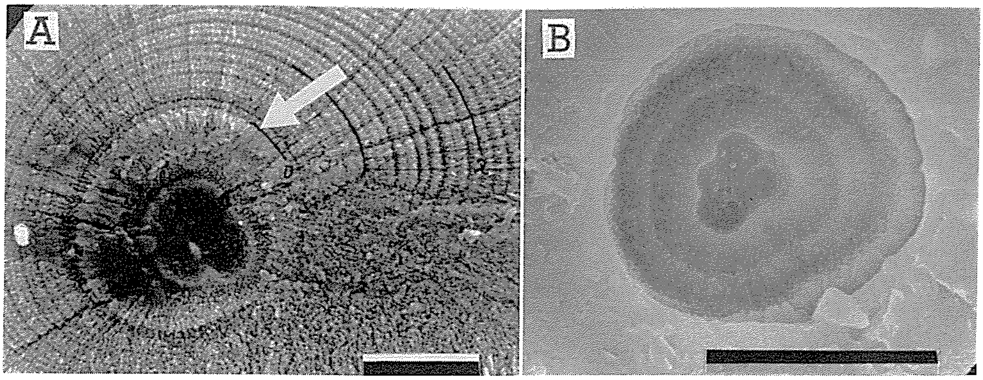

Fig. 2. Core region of the otolith of *Anguilla japonica*. A. From an elver (Specimen No. 2 from the Sendai River collected on January 6, 1982). Arrow shows the first ring. B. From a larva of 6 days old. Scales 10 μm.

epoxy resin, and placed on a glass slide. A longitudinal section passing through the otolith center was obtained by grinding with a whetstone; resulting sections were etched with a 0.5% HCl solution. Sections were dried and prepared for SEM by coating with gold. The gold-coated surface was examined and photographed with a JSM-25 SEM at 15 KV. Measurements and counts of the otolith microstructure were made on SEM photographs. The radii, total number of rings and distances between neighboring rings of otoliths were determined from both right and left otoliths, and the average was calculated for each. The maximum radius of the ground otoliths was defined as the otolith radius.

Results

General Description of the Microstructure of Otoliths

The ground and etched otoliths of the elvers in both species were elongated ovals in shape (Fig. 1 A), and showed concentric ring patterns with the core region delimited by the innermost etched groove (discontinuous zone[11]) (Fig. 1 B and C). All the otoliths of the elvers showed the same pattern of ring arrangement except in the core region. The nucleus appeared as a deeply etched hole, and was observed in the core region in 10 elvers out of the 27 elvers examined (Fig. 2 A). Rings were sometimes unclear along the line of maximum radius in a range from about the 100th to the margin, but could easily be traced from other regions where rings were clearly recognizable.

Longitudinal sections of the otolith of the 6 day old larvae were more rounder in shape, and the nucleus was observed in the core region (Fig. 2 B). It was characteristic for the 6 day old larvae that the otolith diameters (13 and 17 μm) were smaller than those of the first ring in the elvers (18–24 μm, mean 20 μm); also, the rings were widely spaced than those in the elvers.

Table 1. Collection date, total length, maximum radius, and number of rings of otoliths in *A. japonica* elvers from the Sendai River

Date	Specimen No.	TL (mm)	Radius (μm)	Number of rings
December 11–13, 1981	1	54.0	136	163
	2	57.0	132	161
	3	58.0	123	153
	4	56.0	142	151
	5	58.8	151	148
	6	57.0	134	—*1
	(mean)	56.8	136.3	155.2
January 6, 1982	1	57.0	134	171
	2	54.5	137	168
	3	57.0	136	152
	4	55.5	135	—
	5	58.5	146	—
	6*2	48.0	137	133
	(mean)	56.5	137.5	163.7
March 25, 1982	1	56.5	159	173
	2	55.5	153	172
	3	58.5	150	172
	4	53.0	141	168
	5	53.0	152	164
	6	56.5	156	—
	(mean)	55.5	151.8	169.8

*1 could not be counted.
*2 *Anguilla marmorata*, which was excluded from calculation of mean value.

Numbers of Increments on Otoliths

The total number of rings for *A. japonica* of the

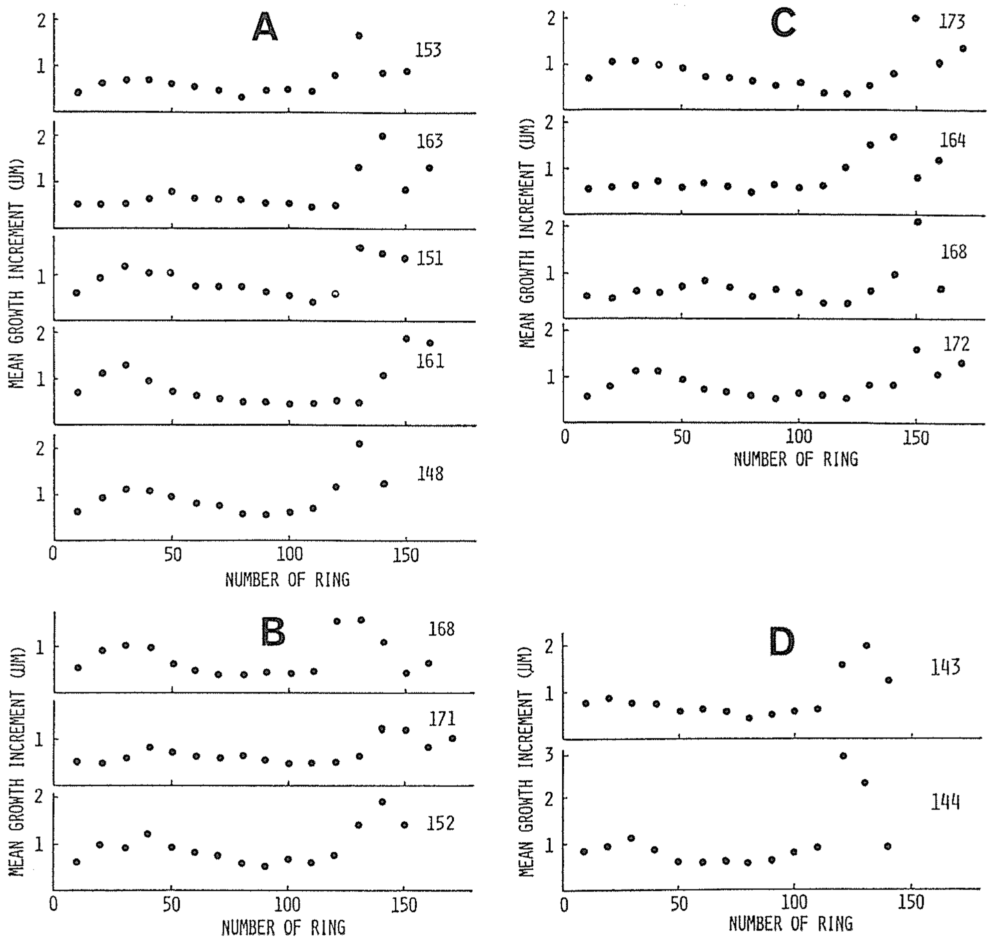

Fig. 3. Changes in mean width of rings averaged over ten succeeding rings in *Anguilla* elver otoliths. A. *A. japonica* from the December sample of the Sendai River. B. *A. japonica* from the January sample of the Sendai River. C. *A. japonica* from the March sample of the Sendai River. D. *A. marmorata* from the November sample of the Shih-Ting River.

Table 2. Maximum radius and number of rings of the otolith from the elvers of *A. japonica* and *A. marmorata* collected from the Shih-Ting River, northern Taiwan, on November 16, 1981

Specimen No.	Radius (μm)	Number of rings
Anguilla japonica		
1	138	144
2	134	135
3	130	124
4	143	120
5	147	—*
(mean)	138.4	130.8
Anguilla marmorata		
1	148	165
2	140	144
3	139	143
4	117	—
(mean)	136.0	150.7

*could not be counted.

December, January and March samples of the Sendai River averaged 155.2, 163.7 and 169.8, respectively (Table 1). An elver of *A. marmorata* from the January sample had 133 rings. The total number of rings for the Taiwanese elvers averaged 130.8 in *A. japonica* and 150.7 in *A. marmorata*, respectively (Table 2).

Time lags of collection dates were compared with differences in mean number of rings between the samples (Table 3), suggesting an extended spawning season. Time lags in days did not agree with differences in mean number of rings between the samples from the Sendai River. Between *A. japonica* elvers from Taiwan (November sample) and the December sample from the Sendai River, however, the time lag was about 26 days and the difference in mean ring number was 24.4, indicating good accordance between days and rings.

Table 3. Time lag of collection dates and difference in mean number of rings of otoliths between samples

Samples	Time lag in days	Difference in number of rings
Anguilla japonica		
Sendai R., Dec. vs. Jan.	ca. 25	+ 8.5
Dec. vs. Mar.	ca. 103	+14.6
Jan. vs. Mar.	78	+ 6.1
Shih-Ting R., Nov. vs. Sendai R., Dec.	ca. 26	+24.4
Anguilla marmorata		
Shih-Ting R. vs. Sendai R.	51	−17.7

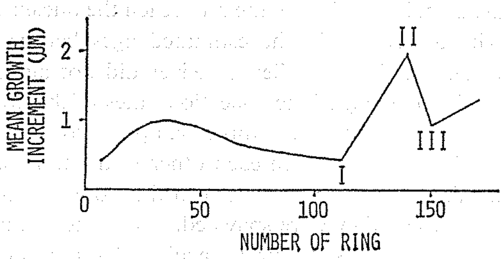

Fig. 4. Conceptual model to show the change in mean width of rings with the increase in number of rings in the otolith of *Anguilla* elvers. I, II and III are the steps that the width changes drastically.

Table 4. Position of each step in the conceptual growth increment of otolith (Fig. 4) expressed by the number of rings in the selected elvers of *A. japonica* from the Sendai River

Sample	Specimen No.	Step I	Step II	Step III
December	1	110	130	140
	2	120	140	150
	3	120	140	150
	4	130	150	
	5	110	130	
	(mean)	(118)	(138)	(147)
January	1	110	125	150
	2	130	145	160
	3	120	140	
	(mean)	(120)	(137)	(155)
March	1	140	150	160
	2	110	140	150
	3	140	150	(160)
	4	140	150	160
	(mean)	(133)	(148)	(158)

Two or three rings were counted in the otoliths of the 6 day old larvae (Fig. 2 B).

Microstructural Growth Pattern of Otoliths in Elvers

The relationship of the width of rings averaged for every ten increments from focus to margin of the otoliths is shown in Fig. 3. The ring width typically began to increase markedly after about the 110th ring (step I) to attain a maximum value (step II), then, in many cases, decreased to a lower value (step III), and increased again. This microstructural growth pattern from step I to step II was observed in all the elvers, irrespective of months and localities of collection, and species. In half of the elvers examined, however, step III was lacking. A conceptual model is illustrated in Fig. 4 to show the change in mean growth increments with an increase of rings in the elver otolith. Table 4 indicates positions of the three steps expressed by the ring number in selected elvers.

Discussion

Translucent growth zones and opaque border lines have been observed by light microscopy in the otoliths of the American eel *Anguilla rostrata* elvers by Liew[22] and in leptocephali and elvers of the European eel *A. anguilla* by Utrecht and Holleboom.[23] Irregular rings and clear marks occasionally appeared in the marginal region of the otolith in the present study by SEM (Fig. 1 A, B and C).

Since Pannella[9] first demonstrated the daily growth increments in marine fish otoliths, many workers have extended his technique to a wide variety of marine and freshwater fishes.[10-20] The present observation showed that the arrangements and other characteristics of the otolith rings in the *Anguilla* elvers were similar to those of the daily growth rings in other fishes. This suggests that the otoliths of both *A. japonica* and *A. marmorata* elvers also have daily growth rings. Prominent daily vertical migration in the leptocephalus stage[1-7] might be one of the environmental conditions which cause the formation of the daily growth increments.

The otolith diameters (13 and 17 μm) of the 6 day old larvae were smaller than those (18–24 μm, mean 20 μm) of the first ring of the elver otoliths. In addition, ring widths observed in the 6 day old larvae were different from those of the elvers. The first ring of the otoliths of the elvers is not considered, therefore, to have been formed at hatching.

Artificially hatched larvae of *A. japonica* were observed to exhaust their yolk 4 days after hatching at about 6.0 mm TL, and to perish usually within 8 days after hatching due to defect of initial food. The oldest reared larvae ever recorded is 14 days old (7.0–10.0 mm TL) in spite of consuming no food.[8,24–26] Natural larvae, on the other hand, are thought to change drastically in body form; for example, the anus changes position from about the 52nd to about the 78th myomere at a transitional stage from preleptocephalus to leptocephalus within 14 days after hatching,*1 which is derived from initial growth of the Japanese eel larvae[24–26] and morphology of the early larval stages of the European eel.[27]

Daily deposition of increments in the otoliths was reported to initiate with the start of feeding in reared Japanese anchovy.[28] Hatching, yolk absorption, changes in feeding and habitat, postlarval transformation and settlement in larval and juvenile stages can all potentially influence the deposition pattern of daily and subdaily growth increments.[17] If the first ring of the elver otoliths was formed at the start of feeding or at the transitional stage from preleptocephalus to leptocephalus, several days to about a fortnight more must be taken into consideration in addition to the days derived from the total number of rings in estimating their birth dates. Therefore, it is concluded that the birth dates for the December, January and March samples of *A. japonica* from the Sendai River would be June–July 1981, July–August 1981, and September–October 1981, respectively, and for *A. japonica* and *A. marmorata* from Taiwan June–July 1981 and May–June 1981, respectively. For one specimen of *A. marmorata* in the January sample from the Sendai River, the birth date is estimated to be August 1981.

Studies of induced spawning have showed that *A. japonica* lays floating fertilized eggs, which need about 1–2 days to hatch at water temperature 22–23°C.[8,24–26] Taking this together with the above-mentioned estimate into consideration, the spawning time can be estimated as June–October 1981 for *A. japonica* and May–August 1981 for *A. marmorata*. Recently, Tsukamoto *et al.* counted 78–99 (mean 89) rings on the otoliths of 16 developing leptocephali of *A. japonica* collected mainly from the sea east of Luzon, the Philippines, in September 1986, and estimated that the larvae were spawned in June–July 1986.[7] The present results that the elvers of *A. japonica* collected in the estuaries of southern Japan and northern Taiwan might be 4–6 months old are in striking contrast to a common allegation that the eel spawns during winter and spring and elvers caught in Japanese waters are one year old.[29,30]

The nucleus in the core region of the otoliths was observed in the artificially hatched larvae as well as in 10 elvers out of the 27 elvers examined. The remnant 17 elvers' otoliths indicated no special structure in the core region. This may be caused by insufficient grinding of the core region of the otoliths. In preparing otolith samples for scanning electron microscopy, a special attention must be paid in grinding them to reach the nucleus.

The differences in the estimated ages between the samples from the Sendai River did not agree with the time lag of the collection dates (Table 3). This suggests that the three samples from the Sendai River differ with each other in the time of spawning, the location of and/orthe route from waters where they were spawned. It is interesting to note that Dr. N. Sakurai, Faculty of Agriculture and Veterinary Medicine, Nihon University, recognized three elver groups defined by frequencies of the body length and vertebral counts in the estuary of the Hikiji River, Kanagawa Prefecture, central Japan, during the same season.*2 On the other hand, the time lag of collection dates was well in accord with the difference in mean number of rings between *A. japonica* elvers of the November sample from Taiwan and those of the December sample from the Sendai River (Table 3). It seems possible that the elvers in these two samples were derived from the same spawning school, because they were judged to be the intial migrating groups in both rivers and were considered to have migrated into the waters of Taiwan and Japan, carried by the Kuroshio Current.[29]

Correlation of the steps I, II and III on the conceptual growth increment curve of the elver otoliths (Fig. 4) with morphological and ecological events is not clearly understood. The microstructural growth pattern of the otoliths in conger eel *Conger myriaster* larvae from the Seto Inland Sea, Japan,[31] was almost the same as that from the first ring to the vicinity of the step II in the present

*1 Tabeta *et al.*, unpublished.

*2 N. Sakurai, personal communication.

elvers. From the viewpoint of developmental stage, narrow and clear rings ranging from the first to about the 140–150th ring and wide rings from about the 150th ring to the otolith margin in the otoliths of the conger eel larvae were considered to have been formed in the period from hatching to developing stage of leptocephalus and the period from the latest stage of developing leptocephalus to the metamorphic stage, respectively.[31] The former ring range in the conger eel larvae corresponds to that from the first ring to step I in the present elvers, and the latter ring range from step I to the vicinity of step II. Morphological and ecological changes in the present elvers may be discussed by analogy with those in the conger eel, together with observations on leptocephali and elvers of *A. japonica* in the sea and estuary.[1–7,29] It is concluded that the elvers of *A. japonica* might have passed the period from the first ring to the step I in the waters far from land, begun the metamorphosis from leptocephalus to elver at around the step I, completed the metamorphosis before the step II in the offshore waters, and reached the coastal waters and estuaries after the steps II and III. The metamorphic period from leptocephalus to elver should therefore be within one month (Fig. 3).

It is supposed that elvers in the December sample had attained steps I, II and III at younger ages than those in the March sample (Table 4), suggesting that the former had grown faster than the latter.

Acknowledgments

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References

1) I. Matsui, T. Takai, and A. Kataoka: *J. Shimonoseki Univ. Fish.*, **17**, 17–23 (1968).
2) O. Tabeta and T. Takai: *Nippon Suisan Gakkaishi*, **41**, 137–145 (1975a).
3) O. Tabeta and T. Takai: *Japan. J. Ichthyol.*, **22**, 100–103 (1975b).
4) S. Tanaka: *Nippon Suisan Gakkaishi*, **41**, 129–136 (1975).
5) Ocean Research Institute, Univ. Tokyo: Preliminary report of the Hakuho Maru KH-75-1, 50 p., University of Tokyo, Tokyo (1976).
6) O. Tabeta and Y. Konishi: *Nippon Suisan Gakkaishi*, **52**, 1935–1937 (1986).
7) Ocean Research Institute, Univ. Tokyo: Preliminary report of the Hakuho Maru KH-86-4, University of Tokyo, Tokyo (in press).
8) Chiba Prefectural Inlandwater Fisheries Experimental Station: Report of the developmental study on the technique of production of seed for eel culture, Project of the Special Promotion in Study of Aquaculture, Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries, 1985, 4 p. (mimeograph) (1986) (in Japanese).
9) G. Pannella: *Science* (Washington, D. C.), **173**, 1124–1127 (1971).
10) B. D. Taubert and D. W. Coble: *J. Fish. Res. Board Can.*, **34**, 332–340 (1977).
11) K. Tanaka, Y. Mugiya, and J. Yamada: *Fish. Bull., U.S.*, **79**, 459–466 (1981).
12) J. D. Neilson and G. H. Geen: *Can. J. Fish. Aquat. Sci.*, **39**, 1340–1347 (1982).
13) B. C. Victor: *Mar. Biol.*, **71**, 203–208 (1982).
14) S. Ralston and G. T. Miyamoto: *Fish. Bull.*, **81**, 523–535 (1983).
15) S. E. Campana: *Mar. Biol.*, **80**, 239–246 (1984a).
16) S. E. Campana: *Can. J. Zool.*, **62**, 1507–1512 (1984b).
17) E. B. Brothers: in "Ontogeny and Systematics of Fishes" (ed. by H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, Jr., and S. L. Richardson), Allen Press Inc., Lawrence, KS, 1984, pp. 50–57.
18) K. Tanaka: *Aquabiology*, **6**, 250–256 (1984) (in Japanese with English summary).
19) A. Nishimura and J. Yamada: *J. Exp. Mar. Biol. Ecol.*, **82**, 191–205 (1984).
20) J. D. Neilson and G. H. Geen: *Fish. Bull.*, **83**, 91–101 (1985).
21) L. Bertin: Eels, A Biological Study, Cleaver-Hume Press Ltd., London, 1956, pp. 138–146.
22) P. K. L. Liew: in "The Proceedings of an International Symposium on the Ageing of Fish" (ed. by T. B. Bagenal), Unwin Brothers Ltd., The Gresham Press, Old Woking, Surrey, 1974, pp. 124–136.
23) W. L. van Utrecht and M. A. Holleboom: *Bijdragen tot de Dierkunde*, **55**, 249–262 (1985).
24) K. Yamauchi, M. Nakamura, H. Takahashi, and K. Takano: *Nature*, **263**, 412 (1976).
25) Research Group of Eel Reproduction Xiamen (Amoy) Fisheries College and Fujian Fisheries Institute: *Act. Zool. Sinica*, **24**, 399–401 (1978).
26) H. Sato: *Iden* (Heredity), **33**, 23–30 (1979) (in

Japanese).
27) J. Schmidt: *Meddelelser fra Kommissionen for Havundersorgelser*, Ser. Fiskeri, Bind V, 4, 1–20 (1916).
28) S. Tsuji and T. Aoyama: *Nippon Suisan Gakkaishi*, **50**, 1105–1108 (1984).
29) I. Matsui: *J. Shimonoseki Coll. Fish.*, **2**, 1–245 (1952).
30) O. Tabeta: *Aquabiology*, **3**, 412–417 (1981) (in Japanese with English summary).
31) K. Tanaka, O. Tabeta, N. Mochioka, J. Yamada, and S. Kakuda: *Nippon Suisan Gakkaishi*, **53**, 543–549 (1987).

Nippon Suisan Gakkaishi : Formerly *Bull. Japan. Soc. Sci. Fish.*