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Age and Birth Date of *Anguilla japonica* Leptocephali Collected in Western North Pacific in September 1986*1

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In order to determine the spawning season of the Japanese eel *Anguilla japonica*, age and birth date of the leptocephali were estimated with the daily growth increment in otoliths of 14 specimens collected in Western North Pacific in September 1986.

The sagittae of the leptocephali (40.5–47.0 mm in TL) were translucent, laterally compressed oval structures of about 155 μm diameter with a dark opaque core (6 μm in long axis) in the center. Distinct concentric growth increments (ring) were observed around the core from the center till the edge of otolith. About 80% specimens examined had a distinct dark ring (heavy discontinuous zone) with about 11 μm diameter near the core, which seemed to be a "hatch check" formed at hatching.

The age of leptocephali was nearly uniform, 72±3 days (mean±SD). Their birth date ranged from June 28 to July 18, 1986 and the mean was July 13, suggesting that this species spawns in summer.

During the fourth survey cruise on biology of the Japanese eel by R. V. Hakuho Maru, Ocean Research Institute, University of Tokyo,[1] a total of 21 leptocephali of *Anguilla japonica* Temminck et Schlegel were obtained,[2,3] and their vertical distribution was investigated.[4] The present study was focused on the age of *A. japonica* leptocephali collected during the cruise in order to estimate the spawning season of this species.

Recently, Tsukamoto[5] validated the daily growth increment in sagitta of the elver of *A. japonica* by otolith tagging[6] with tetracycline as a time marker. Umezawa *et al.*[7] also confirmed daily deposition of sagittal increments using sequential samples of artificially hatched larvae of this species (0–6 days). In the present study, assuming a daily increment deposition in leptocephali as well as hatched larvae and elvers, we estimated the age and the birth date of leptocephali.

Methods

Fish

Fourteen specimens of 21 *A. japonica* leptocephali in total collected by IKMT, Hexagon Net and ORI Net in the Western North Pacific east to Taiwan and Luzon in 1986[1,2] were used (Table 1). The sizes ranged from 40.5 to 47.0 mm in total length and the mean±SD was 43.5±1.9 mm. Most of specimens (12 fish) were obtained at Station C (Fig. 1, Table 1). The other 2 specimens were collected at Station E and Station 31 (Fig. 1).

Otolith Preparation

Sagitta and lapillus of right side were removed under a dessecting microscope from a freshly caught specimen before preservation. Some specimens (3 fish) were used after preservation in 10% (v/v) formalin buffered with sodium tetraborate for 1–2 days.*6 Asteriscus was not examined here,

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*6 Etching or dissolution of otoliths did not seem to occur during preservation in formalin, since the outermost ring at the edge of otolith was complete and distinct.

Table 1. *Anguilla japonica* leptocephali used in the otolith study

Fish No.*1	Sampling			TL (mm)	Sagitta (μm)		Age (day)	Birth Date*2
	Date*2	Stn.	Location		Dia.	Radius		
6	Sep 11	31	22°02.3′N 122°24.9′E	40.5	154.0	84.9	75	Jun 28
7	Sep 23	C-G7	19°02.8′N 129°25.5′E	43.1	148.2	84.1	74	Jul 11
8	Sep 23	C13	18°58.7′N 129°12.3′E	43.0	152.3	81.3	67	Jul 18
9	Sep 23	C14	18°59.1′N 129°13.5′E	42.7	160.0	88.7	78	Jul 07
11	Sep 23	C15	18°58.5′N 129°15.2′E	43.4	154.0	85.3	70	Jul 15
12	Sep 23	C15	18°58.5′N 129°15.2′E	42.8	154.2	85.2	70	Jul 15
13	Sep 23	C15	18°58.5′N 129°15.2′E	41.8	151.9	82.2	74	Jul 11
14	Sep 23	C15	18°58.5′N 129°15.2′E	43.4	161.2	87.8	70	Jul 15
15	Sep 23	C15	18°58.5′N 129°15.2′E	44.0	152.2	78.8	70	Jul 15
16	Sep 23	C15	18°58.5′N 129°15.2′E	45.4	149.2	82.1	70	Jul 15
17	Sep 23	C15	18°58.5′N 129°15.2′E	45.9	165.5	87.9	78	Jul 07
18	Sep 24	C19	18°55.7′N 129°10.5′E	40.5	147.9	85.7	72	Jul 14
19	Sep 24	C19	18°55.7′N 129°10.5′E	47.0	162.6	87.3	69	Jul 17
21	Sep 25	E2	20°18.5′N 125°58.1′E	44.8	151.9	81.7	69	Jul 18
mean	—	—	—	43.5	154.7	84.5	71.9	Jul 13
SD	—	—	—	1.9	5.5	2.9	3.4	5.3
max	Sep 25	—	22°02.3′N 129°25.5′E	47.0	165.5	88.7	78	Jul 18
min	Sep 11	—	18°55.7′N 122°24.9′E	40.5	147.9	78.8	67	Jun 28

*1 Corresponding to Table 3 of The Scientific Member of the Cruise[1] and to Table 1 of Tabeta and Mochioka.[3]
*2 in the Year 1986.

although we could obtained them from some specimens. Total length was measured after preservation in formalin. Otoliths were mounted in Euparal (Chroma-Gesellshaft) on a glass microscope slide.

Otolith Measurement

The core and edge of sagitta were traced through a light microscope (Nikon Optiphoto) with a camera lucida. The longest part between the core and the edge was measured as a radius, and a diameter of otolith was defined as a extention of the radius to the opposite edge.

Age Determination

A bipartite structure of a narrow opaque band (discontinuous zone) and adjacent wider translucent band (incremental zone)[8] was regarded as one growth increment (ring). Sagittae of *A. japonica* larvae have diameter of about 8.3 μm at hatching with no embryonic increment.[7] Therefore, the number of rings outside 8.3 μm diameter were regarded as an age of the animal. They were traced and counted along the radius through a light microscope with a camera lucida at 1600× magnification. The birth date was calculated from the age and the sampling date.

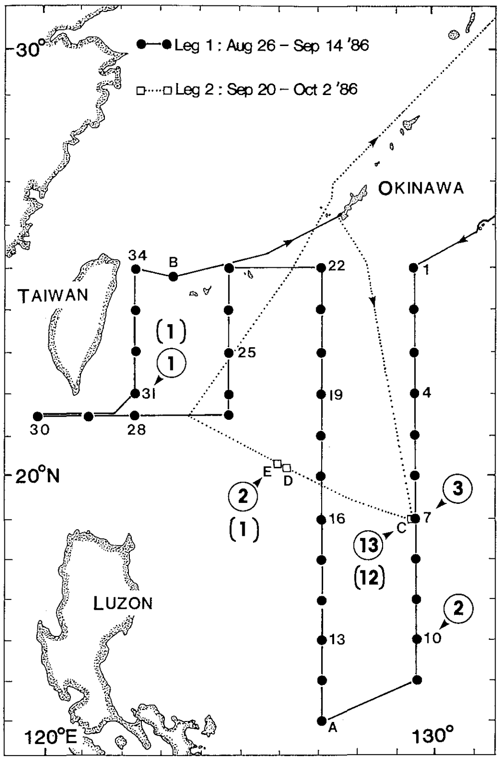

Fig. 1. Sampling location of *Anguilla japonica* leptocephali during the R.V. Hakuho Maru Cruise KH-86-4, August 26–October 2, 1986. Numerals in a circle and parentheses indicate the number of specimen collected in the cruise and the number of fish examined in otolith analysis, respectively.

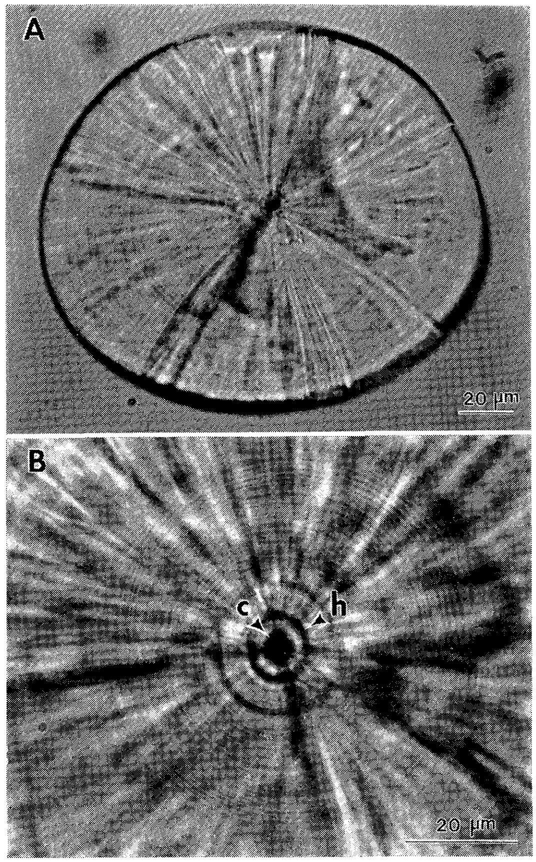

Fig. 2. Otolith of *Anguilla japonica* leptocephalus. **A**: Sagitta of the leptocephalus, Fish No. 17, TL: 45.9 mm. **B**: High magnification of the central part of sagitta of Fish No. 19, TL: 47.0 mm. Arrow with "**h**" indicates "hatch check", a heavy discontinuous zone formed at hatching and the arrow with "**c**" is the core of otolith.

Results

Morphology of Otolith

Sagitta is a translucent, laterally compressed oval structure with a dark opaque core in the center (Fig. 2). The diameter of sagitta ranged from 147.9 to 165.5 μm and the mean$\pm$SD was 154.7$\pm$5.53 μm, whereas the mean of radius$\pm$SD was 84.5$\pm$2.94 μm (Table 1). Otolith diameter and radius presented a significant linear relationship ($p<0.005$, $r=0.687$). Relationship between TL and diameter was significant ($p<0.05$, $r=0.480$), whereas TL and radius did not show a significant relationship ($p>0.05$, $r=0.049$). The core of sagitta is roughly circular, 6 μm in the long axis, and some of them have 1–3 light spots in it. Clear concentric rings (increments) are observed arround the core. In most of specimens examined (11 out of 14 fish; 79%), a distinct dark ring (heavy discontinuous zone[8]) with ca. 11 μm diameter was observed outside the core. This seems to be a "hatch check" formed at hatching, although its diameter is slightly larger than the otolith size at hatching of artificially hatched larvae (8.3 μm).[7] The mean ring width of the first 10 layers outside 8.3 μm diameter was 0.74 μm. Between the core and the ring with 8.3 μm diameter, 1–4 faint and rather broader layers were observed, which were conventionally interpreted as embryonic increments according to the definition in the present study. Subdaily ring, faint increment with a half or one third width of a daily one's, did not occur in all specimens examined.

Lapillus is also a translucent disk, but circular in shape, with clear concentric rings around the core in the center. It was smaller than sagitta and ranged from 83.0 to 109.6 μm in diameter. This otolith was not used for age determination, because of the small size and the narrow incremental width, although increment count was also possible through light microscopy.

Age and Birth Date

Ages of the leptocephali were estimated as 67–78 days old after hatching and the mean±SD was 71.9±3.4 days (Table 1). Fish with an age of 70 days were the most (5 individuals) and exceeded 35% of examined fish. Relationships between TL and age, and between otolith diameter and age were not significant ($p>0.05$, $r=-0.223$ and $r=0.317$, respectively), suggesting that we could not estimate the fish age from TL or otolith diameter. However, a weak linear relationship ($p<0.05$, $r=0.490$) was observed between otolith radius and age. In any case, we should not conclude whether or not a fish age could be estimated from the otolith diameter or radius, since the samples used in the study were only 14 individuals and were rather uniform.

Birth dates ranged from June 28 to July 18, 1986 and the mean±SD was July 13, 1986±5.3 days (Table 1). The range of the birth dates was narrow, *i.e.* only 20 days, concentrating in mid July. Five out of 10 specimens (50%) collected at the same station C on September 23 had the same age (70 days) and thus, the same birth date (July 15) suggesting that a batch of larvae were transported or migrated all together in the same water mass. No. 6 specimen with the earlies birth date (June 28) was collected at Station 31, the westernmost positive station near Taiwan, where the Kuroshio Current flowed northward, whereas the youngest one (No. 8, 67 days old) with the latest birth date (July 18) occurred at eastern station C-13 (Fig. 1). It is noteworthy that the fish born earliest (No. 6, June 28) was the smallest specimen examined here (40.5 mm TL, Table 1).

Discussion

Age Determination

The Japanese eel deposits daily increments in sagittae at larval and elver stages.[5,7] In a strict sence, however, daily deposition of otolith increment during leptocephalus stage has not been confirmed, yet. In the present study, we regarded the increment deposition rate of leptocephali as "1 ring/day" based on the analogy of microstructure of increment among newly-hatched larvae,[7] leptocephali and elvers.[5]

We conventionally defined 8.3 μm diameter ring as the ring deposited at hatching and use it as a bench mark in counting. This may cause 4 days underestimation at most, because 1–4 rings were observed inside 8.3 μm diameter in some specimens. This error does not seem to be serious and has little effect on the result obtained on 2–3 months old leptocephali.

The Japanese eel hatched for 36–38 h after fertilization at 22–23°C.[9,10] Water temperatures at the stations positive for *A. japonica* leptocephali in the cruise were 27–28°C at surface, 24–26°C at 100 m deep, and even at 500 m deep, 23°C.[2,11] According to the above laboratory data, the incubation period in the field may be less than one and a half days, though the exact temperature at spawning area is still unclear. Furthermore, ear vesicle is formed 25 h after fertilization at 23°C in *A. japonica*.[9] These considerations suggest that otolith of the Japanese eel has no embryonic daily increment, or only one at most. The Japanese eel may form a hatch check in the otolith.[7] In order to obtain the conclusion, however, more number of specimens and more refined techniques, *i.e.* scanning electron microscopy, are needed, which enable more accurate measurement of otolith microstructure near the core.

We must know the age-associated bias in ageing fish at various developmental stages: *i.e.* hatched larvae, leptocephali, elvers and young eel. This problem is especially important in the eel species since they show a dramatic metamorphosis and diadromous migrarion. To solve the problem, we need a sequential sample of known age from hatched larvae, throughout leptocephalus stage, to elvers or young eel. However, we have not succeeded in rearing leptocephali, yet.

A set of a broad translucent zone and a narrow opaque zone observed under transmitted light in otolith of the Atlantic eels have been interpreted as an annual growth zone by Liew,[12] Utrecht and Holleboom[13] and many other workers.[14–18] Ehrenbaum and Marukawa[14] and Frost[15] interpreted the central dark area ("nucleus"[12]) of the European eel elver otoliths as the first year of leptocephali in the sea. Liew[12] validated the growth zones of the American eel otolith by the annual formation of the broad summer zone and the narrow winter zone for the young eel kept in a freshwater pond. However, the deposition rate of the growth zone is still unclear during leptocephalus stage in the sea: when do they start the formation of the "nucleus"[12)*1]? and at what age do they finish depositing the nucleus and "elver's ring"? Sagittae of leptocephali examined here have 84.5 μm radius and 72 "daily" growth increments (two and a half months old). Utrecht and Holleboom[13)] counted 2–6 growth zones (layers), which were interpreted to be annual, in otolith of leptocephali and glass eel of *A. anguilla*. Otolith size of *A. japonica* leptocephali (2.4 months old) in this study corresponds to the third growth zone of *A. anguilla* leptocephali (3 years old) and to the second one of glass eel (2 years old).[*2] Such great discrepancy in the estimated ages between *A. japonica* and *A. anguilla* (3 months *vs* 2–3 years old) seems to be derived from the difference in age character applied, growth zones and much finer increments, although it might be merely the species specific problem. Two types of age characters must be applied at the same time for both the Pacific and Altantic eels.

Spawning Season

The highlight of interests in biology of the eel is concentrated on the breeding place, spawning season and the time required for migration to freshwater. From the result obtained here that the specimen with the earlier birth date was collected at the western station, and those born later caught at the eastern station, the spawning ground of the Japanese eel seems to be located in the more eastern area than the stations studied in this cruise. Westward flow in the study area of this cruise which was determined through CTDO observation[1,11)] also supports this consideration.

Age determination of the eel is still confused: *i.e.* the European eel is supposed to spawn in spring-early summer[19,20)] and to metamorphose into glass eel for 2.5–3 years,[19,20)] 3–4 years,[13)] or 12–15 months.[21)] The American eel is considered to spawn in late winter,[19,20)] or in February[21)] and the leptocephalus phase lasts for 10–12 months,[18,19)] or 8–12 months.[22)] Liew[12)] suggested that the American eel spent 2 years in the sea, and the European eel, 3 years.

Age determination of the Japanese eel leptocephalus and elver with daily growth increments in otolith suggested that leptocephalus started metamorphosing to glass eel at an age of 3–4 months old[*3] which lasts for a month or more[*3] and migrated to the Japanese coast at an age of 7 months old.[*4] The spawning season of the Japanese eel estimated from the age of elvers caught in the Japanese coast ranged from April to November with the peak at July-August.[*4] The spawning seasons estimated for the elvers and the leptocephali completely overlapped each other, with small difference in their peaks of only 9 days.[*4] From these results obtained here, we might conculude that the Japanese eel spawned in summer, quite contrary to winter spawning which was generaly believed without any concrete evidences.

The spawning season, the duration of leptocephalus phase and the time required to migrate to freshwater are greatly different among the Japanese eel and both Atlantic eels. Such discrepancy would be a future subject of research.

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*1 Liew's[12)] "nucleus" is the central wide dark area with a focus, light spot in the center under transmitted light.

*2 See Figs. 5, 9, 13, and 16 in Utrecht and Holleboon.[13)] In their report, the width of the first growth zone was greatly different between leptocephali otoliths (18 μm) from the mid North Atrantic and the Portuguese coasts, and the glass eel otoliths (50 μm) from Ijmuiden, Bordeaux and Nantes. The values must be originally the same in both leptocephali and glass eels.

*3 Tsukamoto *et al.* unpublished.

*4 Tsukamoto, unpublished. Age: 217±28 days (Mean±SD), ranging 5–9 months, Birthdate: July 22±42 days, N: 149.

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