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Metamorphosis and inshore migration of tropical eels *Anguilla* spp. in the Indo-Pacific

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ABSTRACT: In order to determine the early life history and recruitment mechanisms of tropical eels *Anguilla* spp. in the Indo-Pacific, the timing of metamorphosis and age at recruitment to freshwater habitats were established from otolith microstructure and microchemistry analyses of juveniles of *Anguilla celebesensis*, *A. marmorata* and *A. bicolor bicolor*. Otolith increment width markedly increased from the age of 124 ± 12.0 d (mean ± SD) in *A. celebesensis*, 120 ± 13.0 d in *A. marmorata* (Philippines), 120 ± 15.6 d in *A. marmorata* (Indonesia) and 139 ± 15.9 d in *A. bicolor bicolor*. The timing of these increases was coincident with drastic decreases in otolith Sr:Ca ratios in each species, indicating the onset of metamorphosis from leptocephalus to glass eel. The mean duration of metamorphosis was 17 to 18 d in each species. Age at recruitment (mean ± SD) was 157 ± 13.7 d in *A. celebesensis*, 154 ± 13.5 d in *A. marmorata* (Philippines), 152 ± 15.2 d in *A. marmorata* (Indonesia) and 177 ± 16.4 d in *A. bicolor bicolor*. In all species examined, close linear relationships were found between ages at metamorphosis and recruitment, suggesting that individuals which metamorphosed earlier were recruited to freshwater habitats at a younger age.

KEY WORDS: Eel · Tropical *Anguilla* spp. · Otolith · Growth increments · Sr:Ca ratios · Metamorphosis · Long larval phase

INTRODUCTION

The freshwater eels of the genus *Anguilla*, being catadromous, migrate between freshwater growth habitats and offshore spawning areas. Among various life history events in *Anguilla* spp., metamorphosis from leptocephalus to glass eel is one of the more interesting phenomena. The timing of metamorphosis or the lengthy duration of the leptocephalus stage, seems to be an important biological key for determining the geographical distribution of the eel (Tsukamoto & Umezawa 1994). Long-term larval migration in the sea might be involved in the worldwide distribution of the genus and consequent speciation of *Anguilla* species (Tsukamoto 1994, Tsukamoto & Aoyama 1998). Worldwide, 18 species/subspecies of *Anguilla* have been reported (Ege 1939, Matsui 1972, Castle & Williamson 1974), 12 being known in tropical regions. Of the latter, 7 species/subspecies occur in the western Pacific around Indonesia, i.e. *A. celebesensis*, *A. interioris*, *A. nebulosa nebulosa*, *A. marmorata*, *A. borneensis*, *A. bicolor bicolor* and *A. bicolor pacifica* (Ege 1939, Matsui 1972, Castle & Williamson 1974). Recent mitochondrial DNA analysis revealed that *A. borneensis* from Borneo Island was closest to the ancestral form among the 18 present-day species/subspecies. Accordingly, freshwater eels have been suggested as having originated in the present-day Indonesian region during the Cretaceous (Aoyama 1998). Furthermore, the tropical species seem to be more closely related to the ancestral form than their temperate counterparts. Thus, studying larval migration and metamorphosis in tropical eels may provide some clues to understanding what is a primitive form of catadromous migration in freshwater eels and how large-scale migration of temperate species was established.

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Recent progress in otolith analytical techniques have revealed considerable details of early life history, including the timing and duration of metamorphosis of temperate *Anguilla* species, such as *A. japonica*, *A. anguilla* and *A. rostrata*. There have also been many reports describing otolith microstructure and growth patterns (Tabeta et al. 1987, Tsukamoto 1990, Tzeng 1990, Tsukamoto & Umezawa 1990, Umezawa & Tsukamoto 1990, Lecomte-Finiger 1992, Tzeng & Tsai 1992, Cheng & Tzeng 1996, Wang & Tzeng 1998, Arai et al. 1999), and patterns of Sr:Ca ratios (Otake et al. 1994, Tzeng 1994, Tzeng & Tsai 1994, Tzeng 1996, Arai et al. 1997). We validated the timing of metamorphosis in *A. japonica* by examining both otolith microstructure and microchemistry of fully-grown leptocephali (just before metamorphosis) and glass eels. A marked increase in otolith increment width, coincident with a drop in Sr:Ca ratio was found to herald the onset of metamorphosis (Arai et al. 1997). The latter appeared to be completed before the maximum peak of otolith increment width. However, such information has been obtained mainly from temperate species and little is known about the early life history of tropical species, including aspects such as spawning area and season, larval growth and metamorphosis, migration and even recruitment of juveniles to estuarine habitats.

In the present study, we examined otolith microstructure and microchemistry of the tropical species, *Anguilla bicolor bicolor* McClelland, *A. celebesensis* Kaup and *A. marmorata* Quoy & Gaimard, collected from tropical regions of the Indo-Pacific, and determined the timing and duration of metamorphosis, age at recruitment and hatching date. The results formed the basis of a discussion on the larval migration mechanisms in these species.

MATERIALS AND METHODS

Fish and otolith preparation. Juveniles of *Anguilla celebesensis* and *A. marmorata* were collected with a scoop net at the mouth of the Cagayan River, Philippines, on September 24, 1994 (Fig. 1). The juveniles sampled were kept in freshwater for 10 d, transported to Japan and thereafter frozen. Juveniles of *A. marmorata* were collected with a dip net at the mouth of the Dumoga River, North Sulawesi Island, Indonesia, on June 5, 1996. Juveniles of *A. bicolor bicolor* were collected with a scoop net at the mouth of the Cimandiri River, Java Island, Indonesia, on May 30, 1996. Juveniles of the latter 2 species were preserved in 99% ethanol immediately after sampling. A total of 65 specimens (17 *A. celebesensis*, 13 *A. marmorata* from the Philippines, 20 *A. marmorata* from Indonesia and 15 *A. bicolor bicolor*) was used for the present study (Table 1). Total length (TL), external morphology, and predorsal, ano-dorsal and preanal length of the specimens were measured to the nearest 0.1 mm. The pigmentation stage was also examined, following Bertin (1956). After otolith extraction, all of the specimens were refixed (in 10% formalin), stained with alizarin, and predorsal, ano-dorsal, preanal and total vertebrae counted. All

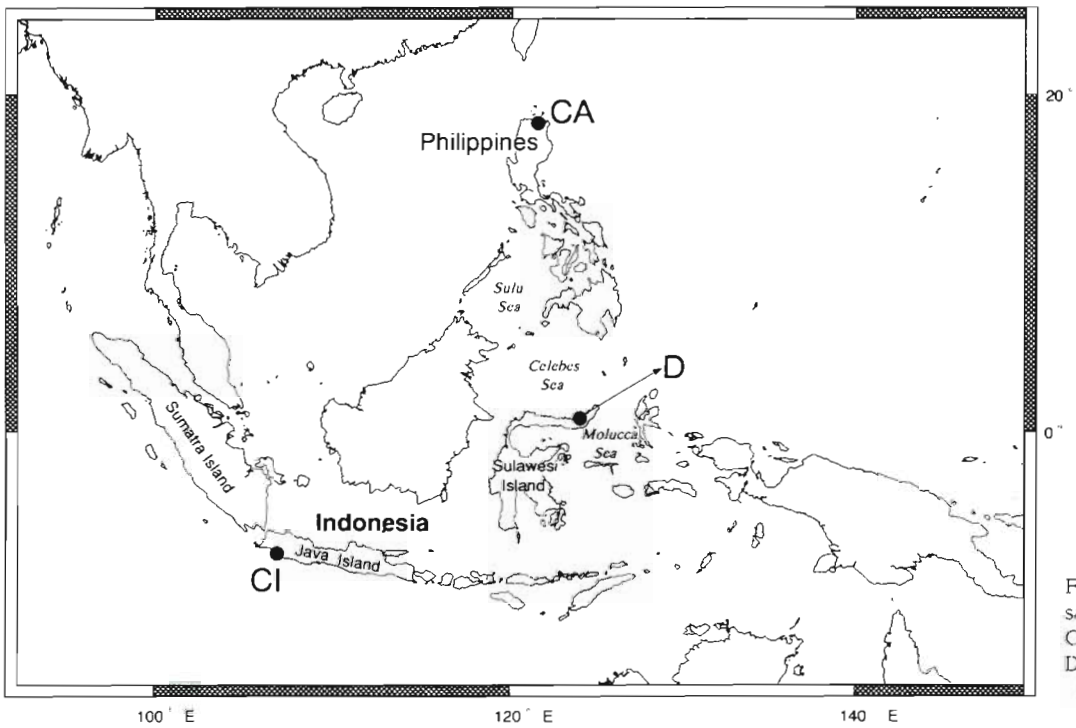

Fig. 1 Map showing sampling locations (●). CI: Cimandiri River; D: Dumoga River; and CA: Cagayan River

Table 1. Tropical *Anguilla* spp. TL: total length; OR: otolith radius; DL: duration of leptocephalus stage; DM: duration of metamorphosis stage; AR: age at recruitment; and HD: hatching date (Mean ± SD, range) of juveniles

Sampling location:	*A. celebesensis* Cagayan River, Philippines	*A. marmorata* Cagayan River, Philippines	*A. marmorata* Dumoga River, Indonesia	*A. bicolor bicolor* Cimandiri River, Indonesia
No. of specimens examined	17	13	20	15
No. of specimens age determined	13	10	18	12
TL (mm)	51.2 ± 1.7, 48.4 to 54.6	49.9 ± 1.4, 47.2 to 51.6	50.9 ± 2.0, 47.9 to 54.8	49.4 ± 2.4, 45.5 to 52.3
OR (µm)	163 ± 7.4, 156 to 182	160 ± 10.2, 144 to 174	146 ± 5.3, 134 to 156	164 ± 6.3, 155 to 176
DL (d)	124 ± 12.0, 104 to 147	120 ± 13.0, 105 to 140	120 ± 15.6, 96 to 147	139 ± 15.9, 119 to 171
DM (d)	17 ± 3.2, 12 to 24	17 ± 4.3, 13 to 26	17 ± 3.3, 13 to 24	18 ± 4.2, 13 to 27
AR (d)	157 ± 13.7, 130 to 177	154 ± 13.5, 136 to 178	152 ± 15.2, 129 to 177	177 ± 16.4, 148 to 202
HD	Apr 20 1994 ± 13.7 Mar 31 1994 to May 17 1994	Apr 23 1994 ± 13.5 Mar 30 1994 to May 11 1994	Jan 4 1996 ± 15.2 Dec 11 1995 to Jan 28 1996	Dec 5 1995 ± 16.4 Nov 10 1995 to Jan 3 1996

specimens were identified from their external morphology and sectional vertebral counts according to Ege (1939) and Tabeta et al. (1976).

Sagittal otoliths were extracted from each fish, embedded in epoxy resin (Struers, Epofix) after measurement of the radius and mounted on glass slides. The otoliths were then ground to expose the core, using a grinding machine equipped with a diamond cup-wheel (Struers, Discoplan-TS), and further polished with 6 µm and 1 µm diamond paste on an automated polishing wheel (Struers, Planopol-V). Finally, they were cleaned in an ultrasonic bath and rinsed with deionized water, prior to examinations.

Otolith x-ray microprobe analysis. For electron microprobe analyses, 30 otoliths (5 *Anguilla celebesensis*, 5 *A. marmorata* from the Philippines, 10 *A. marmorata* from Indonesia and 10 *A. bicolor bicolor*) were carbon coated by high vacuum evaporator. Sr and Ca concentrations were measured along the longest axis of each otolith using a wavelength dispersive x-ray electron microprobe (JEOL JXA-733), as described in Arai et al. (1997, 1999) and Arai & Tsukamoto (1998). Accelerating voltage and beam current were 15 kV and 7 nA, respectively. The electron beam was focused on a point approximately 1 µm in diameter, with intervals of 1 µm between spacing measurements. Each datum represents the average of 3 measurements (each counting time: 4.0 s). Microprobe measurement points, which were seen as burn depressions (Fig. 2A), were assigned to otolith growth increments, which were examined as described below. Averages of Sr and Ca concentration data, pooled after every 10 successive growth increments, were used for the life-history transect analyses.

Otolith increment analysis. Following electron microprobe analysis, the otoliths were repolished to remove the coating, etched with 0.05 M HCl and vacuum coated with Pt-Pd in an ion-sputterer for scanning elec-

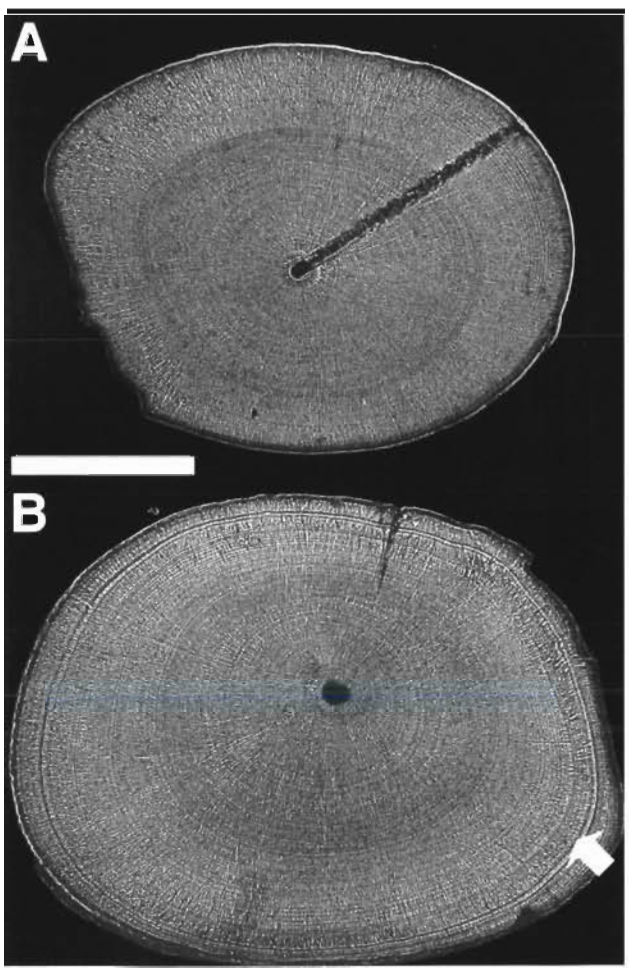

Fig. 2. *Anguilla celebesensis* and *A. marmorata*. SEM photographs showing microstructure of etched otolith. (A) *A. marmorata* glass eel (TL 47.9 mm) collected at mouth of Dumoga River. (B) *A. celebesensis* elver (TL 54.6 mm) collected at mouth of Cagayan River. (Arrow) distinct check. Scale bar: 100 µm

tron microscope (SEM, Hitachi S-4500) observations. The ground surfaces of otoliths of 35 specimens (12 *Anguilla celebesensis*, 8 *A. marmorata* from the Philippines, 10 *A. marmorata* from Indonesia and 5 *A. bicolor bicolor*), which were not used for electron microprobe analysis, were also etched and coated using the above procedure for SEM observation. SEM photographs at various magnifications (150, 180, 1000, and 1500×) were used for counting the number of growth increments and measuring their widths. The 'radius', from the core to the edge along the longest axis of the ground otolith surface, was regarded as the otolith radius along which increment widths were measured. The averages of every 10 successive ring widths between the hatch check (Umezawa et al. 1989) (about 10 µm in diameter) and the edge or check (see 'Results': Otolith microstructure) were used for the otolith growth analyses. Since Umezawa et al. (1989), Tsukamoto (1989) and Umezawa & Tsukamoto (1991) showed clearly that otolith increments in *A. japonica* were deposited daily, we considered the number of increments to represent the daily age of each specimen, although daily deposition had not been validated in these species.

Statistical analyses. Differences among data were tested by an analysis of variance (ANOVA) and afterwards Scheffe's multiple range test for the combination of 2 data. Significance of the correlation coefficient and regression slope were tested by Fisher's Z-transformation and an analysis of covariance (ANCOVA), respectively (Sokal & Rohlf 1969).

RESULTS

Size and pigmentation at recruitment

The TLs of juvenile *Anguilla celebesensis* and *A. marmorata* from the Philippines were 51.2 ± 1.7 mm (mean ± SD) and 49.9 ± 1.4 mm, respectively, and those of *A. marmorata* from Indonesia and *A. bicolor bicolor* 50.9 ± 2.0 mm and 49.4 ± 2.4 mm, respectively (Table 1). Although a significant difference in TL occurred between *A. marmorata* from Indonesia and *A. bicolor bicolor* (ANOVA, $p < 0.05$), a combination between *A. celebesensis* and *A. marmorata* from the Philippines was not significant.

The pigmentation in both *Anguilla marmorata* from Indonesia and *A. bicolor bicolor* was scarcely developed, being only on the caudal or skull, caudal and rostral regions of the body and was thus apparently classified at the glass eel stage (VA or VB). Pigmentation in juvenile *A. celebesensis* and *A. marmorata* from the Philippines was advanced along the entire dorsal region of the body, resulting in classification as stage VIA_{II} or VIA_{IV}, i.e. elvers.

Otolith microstructure

The mean radii of otoliths of *Anguilla celebesensis* and *A. marmorata* from the Philippines were 163 (±7.4 SD) µm and 160 (±10.2 SD) µm, respectively. Those of *A. marmorata* and *A. bicolor bicolor* from Indonesia were 146 (±5.3 SD) µm and 164 (±6.3 SD) µm, respectively (Table 1). A highly significant difference in otolith radius was obtained between *A. marmorata* from Indonesia and *A. bicolor bicolor* at the same developmental stage (ANOVA, $p < 0.0001$), while *A. celebesensis* and *A. marmorata* from the Philippines did not differ significantly. An otolith core was observed as a deep hole in each etched otolith, a hatch check being visible as a deep circular groove surrounding the hole (Fig. 3). The diameter of the hatch check was 10.4 ± 0.9 µm (mean ± SD) in *A. celebesensis*, 9.8 ± 0.8 µm (mean ± SD) in *A. marmorata* from the Philippines, 9.6 ± 1.1 µm (mean ± SD) in *A. marmorata* from Indonesia and 9.9 ± 0.9 µm (mean ± SD) in *A. bicolor bicolor*. A significant difference occurred between *A. celebesensis* and *A. marmorata* from Indonesia (ANOVA, $p < 0.05$), but other combinations were not significant. Distinct concentric growth increments, comprising a series, were

Fig. 3. *Anguilla marmorata*. SEM photograph showing otolith growth increments of a glass eel (total length 51.2 mm) collected at mouth of Dumoga River. Scale bar: 10 µm

observed around the core in each species (Fig. 3). Those otolith structures were similar to those reported in *A. japonica* (Tabeta et al. 1987, Tsukamoto 1990, Tzeng 1990, Umezawa & Tsukamoto 1990, Tzeng & Tsai 1992, Cheng & Tzeng 1996), *A. anguilla* (Lecomte-Finiger 1992), *A. marmorata* (Budimawan 1997), *A. rostrata* (Wang & Tzeng 1998) and *A. bicolor pacifica* (Arai et al. 1999).

A distinct check was observed some 15 µm from the otolith edge in all otoliths of *Anguilla celebesensis* (mean ± SD: 15.3 ± 2.0 µm, range: 11.3 to 17.3 µm) and *A. marmorata* from the Philippines (mean ± SD: 14.4 ± 2.1 µm, range: 11.4 to 17.0 µm) (Fig. 2B), growth increments being unclear or irregular in the region outside the check. Campana & Neilson (1985) suggested that the formation of such checks and discontinuities in otoliths appear during periods of perturbation and stress to the fish. The distinct check in all of the otoliths from the Philippines samples might have formed while the latter were maintained in freshwater after sampling. Therefore, growth increments outside the check were omitted from the age determination of Philippines samples, and the number of rings inside the check regarded as the age at recruitment. Such a check was not formed in otoliths of *A. bicolor bicolor* and *A. marmorata* from Indonesia (Fig. 2A).

Age and hatching date

Age at recruitment of *Anguilla celebesensis*, *A. marmorata* from the Philippines, *A. marmorata* from Indonesia and *A. bicolor bicolor* were 157 ± 13.7 d (mean ± SD), 154 ± 13.5 d (mean ± SD), 152 ± 15.2 d (mean ± SD) and 177 ± 16.4 d (mean ± SD), respectively (Table 1). Significant differences occurred between *A. marmorata* from Indonesia and *A. bicolor bicolor* (ANOVA, $p < 0.001$), between *A. bicolor bicolor* and *A. celebesensis* (ANOVA, $p < 0.05$), and between *A. bicolor bicolor* and *A. marmorata* from the Philippines (ANOVA, $p < 0.005$). No significant difference occurred between the 2 samples of *A. marmorata* (ANOVA, $p > 0.1$).

The estimated hatch dates, back-calculated from the sampling date and ages, were from late March 1994 to mid-May 1994 for *Anguilla celebesensis*, from late March to early May 1994 for *A. marmorata* from the Philippines, from mid-December 1995 to late January 1996 for *A. marmorata* from Indonesia and from mid-November 1995 to early January 1996 for *A. bicolor bicolor* (Table 1). Significant differences occurred between *A. celebesensis* and *A. marmorata* from the Philippines (ANOVA, $p < 0.0001$), between *A. celebesensis* and *A. marmorata* from Indonesia (ANOVA, $p < 0.0001$), between *A. celebesensis* and *A. bicolor bicolor* (ANOVA, $p < 0.0001$), between the 2 samples of *A. marmorata* (ANOVA, $p < 0.0001$), between *A. marmorata* from the Philippines and *A. bicolor bicolor* (ANOVA, $p < 0.0001$) and between *A. marmorata* from Indonesia and *A. bicolor bicolor* (ANOVA, $p < 0.0001$). No significant difference was apparent between *A. celebesensis* and *A. marmorata* from the Philippines (ANOVA, $p > 0.1$).

Otolith growth pattern

Patterns of changing otolith increment widths along life-history transects from the core to the edge in each species are shown in Fig. 4. The common pattern observed in all specimens examined was divided into 4 phases, with drastic changes occurring in the latter 2. A typical conceptual model for the changes in increment width is illustrated in Fig. 5. Otolith increment widths increased between the hatch check and age 20 to 40 d in each species (1st phase), thereafter becoming constant or gradually decreasing (average widths 0.38 to 0.44 µm) (2nd phase). Beyond age 124 ± 12.0 d (mean ± SD) in *Anguilla celebesensis*, 120 ± 13.0 d in *A. marmorata* from the Philippines, 120 ± 15.6 d in *A. marmorata* from Indonesia and 139 ± 15.9 d in *A. bicolor bicolor*, increment widths increased sharply to a maximum (average 2.51 to 2.76 µm) (3rd phase), followed by a rapid drop (4th phase). The latter ages differed significantly between *A. celebesensis* and *A. bicolor bicolor* (ANOVA, $p < 0.05$), between *A. marmorata* from Indonesia and *A. bicolor bicolor* (ANOVA, $p < 0.01$), and *A. marmorata* from the Philippines and *A. bicolor bicolor* (ANOVA, $p < 0.01$). No significant difference was apparent between the 2 samples of *A. marmorata* (ANOVA, $p > 0.1$). The mean duration of the 3rd phase, 17 to 18 d (Table 1), did not differ among the species (ANOVA, $p > 0.1$).

Otolith Sr:Ca ratios

Otolith Sr:Ca ratios changed dramatically along the life-history transect in all species (Fig. 6), a typical conceptual model for the changes in Sr:Ca ratios is illustrated in Fig. 5. This pattern of microchemical changes was also commonly observed in the juveniles examined. Sr:Ca ratios, averaging 9.5 to 10.8×10^{-3} at the core, dropped slightly at approximately the first phase of otolith growth. Subsequently, the ratios increased to a maximum level (average 13.2 to 16.3×10^{-3}) around the second phase and a markedly decreased thereafter toward the edge (coincident with the onset of the third phase of otolith growth). In *Anguilla marmorata* from Indonesia and *A. bicolor bicolor*, minimum values of 6.7 to 7.5×10^{-3} were recorded in the

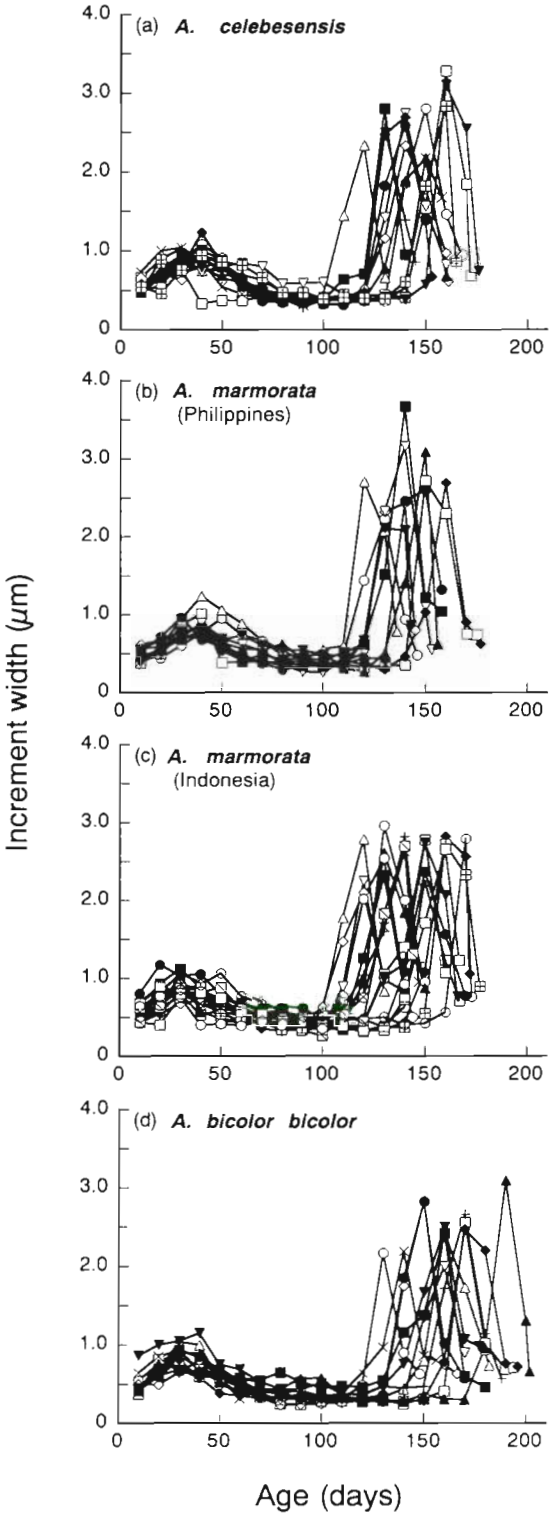

Fig. 4. Tropical *Anguilla* spp. Profiles of otolith incremental width from the core to the edge. Each point represents average of data for 10 d. (a) *A. celebesensis* collected at mouth of Cagayan River, (b) *A. marmorata* collected at mouth of Cagayan River, (c) *A. marmorata* collected at mouth of Dumoga River, and (d) *A. bicolor bicolor* collected at mouth of Cimandiri River

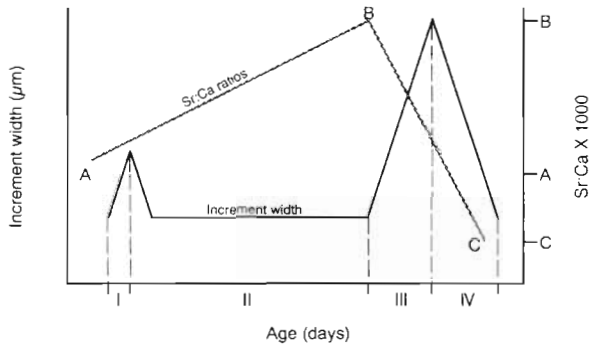

Fig. 5. Tropical *Anguilla* spp.. Conceptual model showing changes in otolith incremental widths and Sr:Ca ratios during the early life history of tropical *Anguilla* spp.. I, II, III and IV indicate phases of width change; A, B and C indicate points of changing Sr:Ca ratios

outermost region of the otolith. In *A. celebesensis* and *A. marmorata* from the Philippines, values of 6.1 to 6.4 $\times 10^{-3}$ were recorded in the outermost region at about 15 µm inside the otolith edge. Furthermore, in *A. celebesensis* and *A. marmorata* from the Philippines, Sr:Ca ratios in the region outside the distinct concentric check at about 15 µm from the otolith edge (growth increment analysis omitted), decreased to even lower levels, averaging 3.4×10^{-3} (range: 3.0 to 4.3×10^{-3}) in the former and 2.8×10^{-3} (range: 2.6 to 3.0×10^{-3}) in the latter. The additional drop of otolith Sr:Ca ratios in the region outside the check may have resulted from the specimens being maintained in freshwater, as well as the formation of the distinct check in that region.

Timing of metamorphosis

Based on previous data on Sr:Ca ratios in *Anguilla japonica* and *A. bicolor pacifica* (Otake et al. 1994, Arai et al. 1997, 1999), age at Point B (Fig. 5) where Sr:Ca ratios showed a drastic decrease was regarded as the onset of metamorphosis in each species examined here. Close linear relationships were apparent between age at metamorphosis and age at recruitment in all groups (Fisher's Z-transformation, $p < 0.0005$). No significant difference in regression slopes occurred between the 2 *A. marmorata* samples (ANCOVA, $p > 0.1$) (Fig. 7).

DISCUSSION

Metamorphosis

All otoliths of the juveniles examined in the present study displayed a similar growth pattern which was divided into 4 phases (Fig. 6), the last 2 phases over-

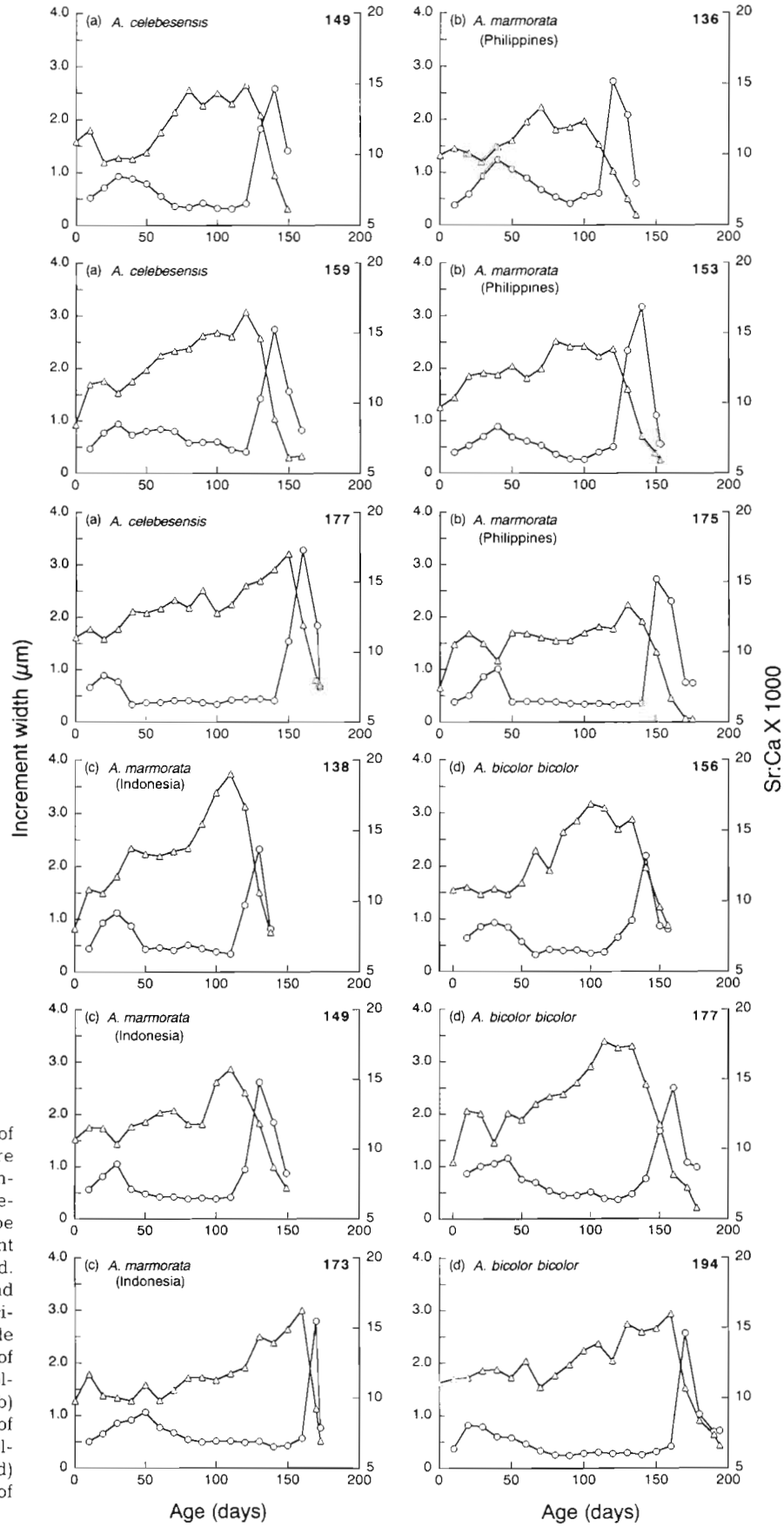

Fig. 6. Tropical *Anguilla* spp.. Profiles of otolith incremental width from the core to the edge (○) and otolith Sr:Ca concentration ratios measured with a wavelength dispersive electron microprobe from the core to the edge (△). Each point represents average of data for 10 d. Numbers in **bold** in upper right-hand corner suggest age (d). Profiles of specimens of least (top rows), average (middle rows) and greatest age (bottom rows) of each species. (a) *A. celebesensis* collected at mouth of Cagayan River, (b) *A. marmorata* collected in the mouth of Cagayan River, (c) *A. marmorata* collected at mouth of Dumoga River, and (d) *A. bicolor bicolor* collected at mouth of Cimandiri River

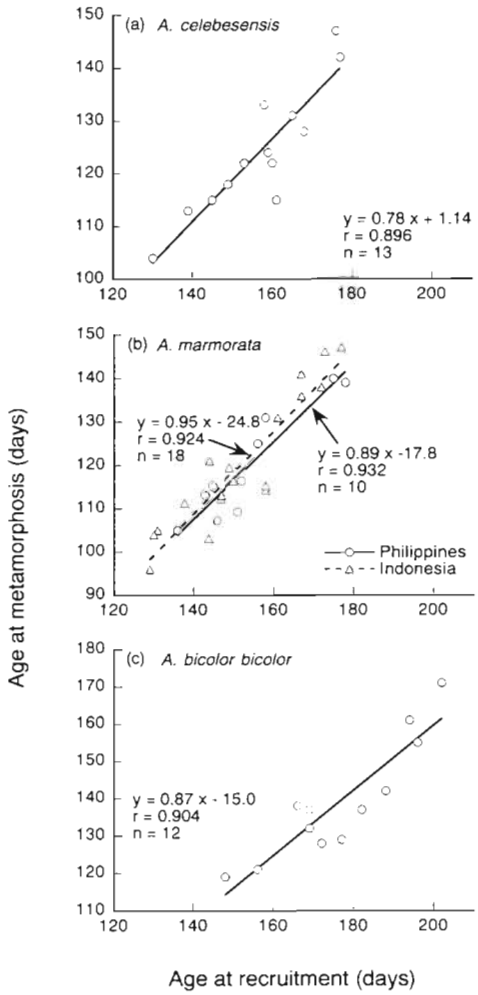

Fig. 7 Tropical *Anguilla* spp. Relationship between age at recruitment and age at metamorphosis. (a) *A. celebesensis* collected at mouth of Cagayan River, (b) *A. marmorata* collected at mouth of Cagayan River and Dumoga River, and (c) *A. bicolor bicolor* collected at mouth of Cimandiri River

lapping exactly with a drop in otolith Sr:Ca ratios. Such a fluctuation pattern, associated with the Sr:Ca ratios, appeared to be quite similar to those in *Anguilla japonica* (Otake et al. 1994, Arai et al. 1997) and *A. bicolor pacifica* (Arai et al. 1999). Otake et al. (1997) found a similar phenomenon in *Conger myriaster*. The coincidence in timing of the rapid increase in increment width and sharp decrease in Sr:Ca ratios in the otolith seems to be a common pattern in anguillid fishes. A comparison of microstructure and microchemistry analyses in fully grown leptocephali and glass eels led to the conclusion that the timing of the rapid increase in otolith increment widths and sharp decrease in Sr:Ca ratios heralded the onset of metamorphosis in *A. japonica* (Arai et al. 1997). Therefore, early life-history transects of otoliths of the tropical *Anguilla* species can be interpreted as follows: Phases I and II—preleptocephalus and leptocephalus, Phase III—metamorphosis and Phase IV—glass eel. Accordingly, the onset of metamorphosis in *A. celebesensis*, *A. marmorata* from the Philippines, *A. marmorata* from Indonesia and *A. bicolor bicolor* seemed to occur at mean ages of 124 d (±12.0 d SD), 120 d (±13.0 d SD), 120 d (±15.6 d SD) and 139 d (±15.9 d SD), respectively. Arai et al. (1997, 1999) suggested that metamorphosis is completed before the maximum peak of otolith increment width, because the increment width did not decrease following its maximum level in several specimens recruited to a coast. Therefore, the maximum duration of metamorphosis is likely to be at most 17 or 18 d for each species. It is noteworthy that the duration of the metamorphosis stage is constant, even in different species.

Recruitment to freshwater

The relationship between the timing of metamorphosis and age at recruitment clearly showed that juveniles undergoing the former at an earlier age tended also to migrate to a coastal region at a younger age, indicating that early metamorphosing larvae are recruited earlier (Fig. 7). Tsukamoto & Umezawa (1994) and Arai et al. (1997, 1999) found the same phenomenon in *Anguilla japonica* and *A. bicolor pacifica*, respectively.

In the present study, the mean duration of the leptocephalus stage across the species ranged from 120 to 139 d, the ages at recruitment being determined as averaging between 152 and 177 d (Table 1). The early life history parameters were almost the same between the 2 groups of *Anguilla marmorata*, the duration of the leptocephalus stage in both being 120 d and the ages at recruitment 154 d (Philippines) and 152 d (Indonesia). Similar results have also been found between subspecies of *A. bicolor*, *A. bicolor bicolor* beginning to metamorphosis at 139 d and undergoing recruitment to western Java Island at 177 d (present study), and *A. bicolor pacifica* from the mouth of Dumoga River, Indonesia, metamorphosing at 135 d and being recruited at 173 d (Arai et al. 1999). These results suggested that early life history parameters such as duration of the leptocephalus stages and ages at recruitment were almost equal in *A. marmorata* and *A. bicolor*, in spite of their different geographical distribution, ocean migration routes and growth histories. Furthermore, the early life history parameters found in all tropical species overlapped with the range of those in the temperate species *A. japonica* although the former were a little shorter than those of *A. anguilla* and *A. rostrata* (Table 2).

The estimated hatching periods of *Anguilla marmorata* collected in the Philippines and Indonesia

Table 2. *Anguilla* spp. DL: duration of leptocephalus stage, and AR: age at recruitment reported in literature for otolith SEM studies of various *Anguilla* species

Species	DL (d)	AR (d)	Source
Temperate species			
A. japonica	110 to 140	120 to 173	Tabeta et al. (1987)
		113 to 157	Tzeng (1990)
	116 to 138	155 to 182	Cheng and Tzeng (1996)
	80 to 160	143 to 206	Arai et al. (1997)
A. anguilla	176 to 196	216 to 276	Lecomte-Finiger (1992)
A. rostrata	189 to 214	220 to 284	Wang & Tzeng (1998)
Tropical species			
A. marmorata		143 to 165	Tabeta et al. (1987)
	81 to 86	96 to 131	Budimawan (1997)
	105 to 140	136 to 178	Present study (Philippines)
	96 to 147	129 to 177	Present study (Indonesia)
A. bicolor pacifica	101 to 172	124 to 202	Arai et al. (1999)
A. bicolor bicolor	119 to 171	148 to 202	Present study
A. celebesensis	104 to 147	130 to 177	Present study

differed from each other by 2 mo. The recruitment sites were different, as were the ocean current systems around each area. However, the age at recruitment was almost equal at the different sites, suggesting the existence of at least 2 reproductively isolated populations of *A. marmorata* in the Indo-Pacific region. Nevertheless, not all of the recruitment period was covered. In order to discriminate between populations of *A. marmorata*, time series samples need to be examined or a molecular analysis of the species undertaken.

Size at recruitment and early growth

Conspicuous early life history discrepancies between the tropical and temperate species were apparent in the total lengths of juveniles. The TLs of *Anguilla marmorata* from Indonesia and *A. bicolor bicolor* at recruitment (50.9 ± 2.0 mm in the former, 49.4 ± 2.4 mm in the latter; mean ± SD) were 10 to 20 mm less than those of temperate *Anguilla* species, such as *A. anguilla* (68 mm) (Lecomte-Finiger 1992) and *A. japonica* (57 mm) (Umezawa 1991, Cheng & Tzeng 1996, Arai et al. 1997), even though all 4 species conform to the same pigmentation stage, i.e. stage VA or VB. Similarly, juvenile TLs at recruitment of *A. celebesensis* (51.2 ± 1.7 mm; mean ± SD) and *A. marmorata* (49.9 ± 1.4 mm; mean ± SD) from the Philippines were 10 to 20 mm less than those of temperate *Anguilla* species, such as *A. australis* (59 to 73 mm) (Jellyman 1979), *A. rostrata* (55.8 to 60.9 mm) (Haro & Krueger 1988) and *A. anguilla* (65 mm) (Lecomte-Finiger 1992). Budimawan (1997) and Arai et al. (1999) also reported small sizes in tropical eel juveniles (47.0 to 51.8 mm in *A. marmorata* and 48.9 mm in *A. bicolor pacifica*), similar to those species used in the present study, when they arrived at river mouths in tropical areas. Furthermore, differences in the total lengths of fully grown leptocephali were also found between tropical and temperate species. The TLs of fully grown leptocephali of the temperate eels, *A. anguilla*, *A. rostrata* and *A. japonica*, were estimated as 75 mm (Jespersen 1942, Tesch 1977), 70 mm (Kleckner & McCleave 1985) and 60 mm (Tabeta & Konishi 1986), respectively, while those of tropical species collected in the Indo-pacific region including *A. celebesensis*, *A. marmorata* and *A. bicolor bicolor*, have been reported as being around 50 mm (Jespersen 1942). According to our age determinations, the growth rates of leptocephali of the latter 3 species were estimated to range from 0.36 to 0.42 mm d^{-1}, that is, less than those reported for the temperate eel, *A. japonica* (0.56 to 0.59 mm d^{-1}) (Umezawa & Tsukamoto 1990, Tsukamoto et al. 1992), which has a similar length larval phase. The lower growth rate during the leptocephalus stage of tropical eels, including *A. celebesensis*, *A. marmorata* and *A. bicolor bicolor*, seems to result in the smaller size of the fully grown leptocephali and smaller size at recruitment in those species.

Ocean migration and distribution

Anguilla celebesensis, *A. marmorata* and *A. bicolor bicolor* leptocephali were determined as taking about 5 to 6 mo to migrate from their spawning area to estuarine habitats, and *A. bicolor pacifica* requiring a similar period, according to a previous study (Arai et al. 1999). In temperate eels, which migrate via oceanic current systems (Schmidt 1922, 1925, Tsukamoto 1992), the duration of oceanic migration seems to be related to the distance and complexity of the current systems between the spawning areas and the freshwater habitat destinations. According to Jespersen (1942), spawning areas of tropical eels, including *A. bicolor bicolor* and *A. marmorata* from Indonesia, distributed off Java and the North Sulawesi Islands, respectively, are possibly situated off the southwestern coast of Sumatra for the former and the Celebes, Sulu and Molucca Seas for the latter, i.e. close to their distribution area (Fig. 1). Therefore, the rather long migration periods of those species relative to the short distances

between their growth habitat and spawning area may be due to complicated local current systems around Sumatra and Java, and the North Sulawesi Islands. The occurrence of leptocephali of various sizes, including preleptocephalus to metamorphosing stages, in waters off Sumatra (Jespersen 1942) support that supposition. This situation is quite different to that of temperate eels, suggesting that the migration mechanisms of tropical eel larvae are not as simple as those of temperate eels, which can be interpreted as simple transportation by steady currents. These considerations suggest that primitive eels would initiate diadromous migration from a local short-distance movement in complex currents in tropical coastal waters rather than from a long-distance migration of temperate eels well established in subtropical gyres in both hemispheres.

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