Early life history of the tropical eels Anguilla bicolor bicolor from cimandiri estuary , West Java (Indonesia), as revealed by otolith microstructures

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EARLY LIFE HISTORY OF THE TROPICAL EELS *ANGUILLA BICOLOR BICOLOR* FROM CIMANDIRI ESTUARY, WEST JAVA (INDONESIA), AS REVEALED BY OTOLITH MICROSTRUCTURES

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ANGUILLA BICOLOR BICOLOR
SAGITTAE
GROWTH INCREMENTS
LARVAL DURATION
OCEANIC MIGRATION

ABSTRACT. – Glass eels were collected during their inshore migration from the Indian Ocean to Cimandiri Estuary, West Java, Indonesia from December 1993 to July 1994. *Anguilla bicolor bicolor* was dominant (more than 97 %) among the three species present in this estuary, in all samples. The total length at arrival ranged from 52 to 65 mm and increased from December to July. Size at recruitment was negatively correlated with the pigmentation stage. The sagittal microstructure and the increment patterns were examined by scanning electron microscope. Based on the number of daily increments deposited on sagittae, the mean age at estuary arrival was estimated as (mean ± standard error respectively for sagittae): 106.4 ± 11.1 days. The spawning ground of these eels is presumably not far from the estuary. The location is discussed.

INTRODUCTION

Eels of the genus *Anguilla* Schrank, 1798 are widely distributed in the Indopacific and Atlantic regions. These fish spawn in the sea (Schmidt 1922, Tsukamoto 1992) and spend their long growing time to the maturity in the inland waters. Information about many aspects of the early life history is still lacking. The genus *Anguilla* comprises 15 species distributed around the world (Ege 1939). In the Indian Ocean, 4 species (1 sub-species) have been reported, namely *Anguilla marmorata* Quoy & Gaimard (1824), *A. mossambica* Peters (1868), *A. bicolor bicolor* McClelland (1844), *A. nebulosa* (*labiata* Peters 1852 and *nebulosa* McClelland 1844). The short finned eel *A. bicolor bicolor* occurs from the western part of the African continent and islands to eastern Asia (Malaysia, Sumatra, Java and Indian coast) and North West Australia (Ege 1939, Jespersen 1942, Frost 1957, Jubb 1961, Marquet *et al*. 1997, Arai *et al*. 1999a, 1999b, Sugeha *et al*. 2001). The long-finned eels *A. mossambica* and *A. nebulosa labiata* are exclusively reported on the East-African coast and the Mascarene Islands (Ege 1939, Jubb 1961), while the long-finned mottled *A. marmorata* is the most widely distributed eel species, from the South-East African coast to the Japanese archipelago and Polynesia (Ege 1939, Nishi & Imai 1969, Marquet & Lamarque 1986, Jellyman 1987, Marquet & Galzin 1991, Williamson & Boëtius 1993, Budimawan 1997, Marquet *et al*. 1997, Arai *et al*. 2002, Robinet *et al*. 2003, Budimawan & Lecomte-Finiger 2005). The long-finned *A. nebulosa nebulosa* occurred in Northern Indian Ocean (Ceylon, Indian hinterland, Burma, Andaman Islands) and the most southerly occurrence according to McClelland was Andaman Islands and west coast of North Sumatra (Ege 1939, Budimawan 1996). Substantial knowledge on the ecological aspects of these anguillid eels is limited. The biological data available cover taxonomic features, distribution area and ecology. Little is known about *Anguilla bicolor bicolor* glass eels ecology in Cimandiri estuary (Arai *et al*. 1999a, 1999b, Setawian *et al*. 2001), and in Reunion Island (Robinet *et al*. 2003).

One way to reveal the early life history of the eel is by the examination of the otolith microstructure. This allows an estimation of the time required to migrate from the spawning ground to coastal waters. Since Pannella's first description (1971) of daily increments in otoliths, many investigations have been published. Recent progress in otolith techniques has revealed considerable data on the early life histories of various eel species, European eel *A. anguilla* (Lecomte-Finiger & Yahyaoui 1989, Lecomte-Finiger 1992), American eel *A. rostrata* (Castonguay 1987, Martin 1995, Cieri & McCleave 2001), Japanese eel *A. japonica* (Tabeta *et al*. 1987, Tsukamoto 1989, Umezawa *et al*. 1989, Arai *et al*. 1999a) and tropical eels such as *A. marmorata* (Budimawan 1997, Arai *et al*. 1999a, 1999b, Sugeha *et al*. 2001, Robinet *et al*. 2003, Budimawan & Lecomte-Finiger 2005). Since Ege (1939) and Jespersen (1942), however, bio-ecological studies of tropical eel species such as *A. bicolor bicolor* have been rare compared to the abundant literature on "temperate eels" *A. anguilla*, *A. rostrata* and *A. japonica* (Setiawan *et al*. 2001, Arai *et al*. 1999b, 2000, Robinet *et al*. 2003).

This study presents an original data set on glass eels recruiting in Cimandiri estuary (Java-Indonesia) i) to describe the changes in species composition and morphometric patterns of glass eels that recruit in Cimandiri estuary from December 1993 to July 1994 and, ii) to bring more information, based on otolith microstructure analysis, on whether one of the spawning area of this species is close to Java or not.

MATERIALS AND METHODS

Sampling

Four samples of glass eels from December 1993, January 1994, June 1994 and July 1994 (C12-93, C01-94, C06-94 and C07-94 respectively), were collected from Cimandiri estuary, West Java, Indonesia, (Fig. 1) using a typical glass eel trap made of bamboo, and preserved in 70 % ethanol. They were then identified to species by examining their ano-dorsal to total length ratio (A-D/TL) and caudal pigmentation (Ege 1939). Fifteen glass eels of each species were randomly sampled for vertebrae counting through X-Ray investigations (Sigma 2060). *Anguilla bicolor bicolor* glass eels were sorted.

Morphometry and pigmentation stage

All *A. bicolor bicolor* glass eels and elvers were measured and weighed: total length (TL) to the nearest 1.0 mm and weight (W) to the nearest 0.1 mg. The condition factor (Cc) was then calculated ($Cc = 1000 \times W_{(g)} \times TL_{(cm)}^{-3}$). The pigmentation stage was defined according to Tabeta *et al.* (1976) and Elie *et al.* (1982) classification. Glass eels in transparent stages ranked from VA (= 5A), VB, VIAo, (6Ao), to VIA with the appearance of pigmentation. Elvers in pigmented stages ranked from VIA_2, VIA_3, VIA_4 to VIB.

Otoliths

Fifty glass eels from sample C06-94 (the peak season of migration) were randomly taken for otolith examination, and the left sagittae were used. Otoliths were embedded in metacrylate resin, ground with 1000 and 2400 grit paper until the nucleus was visible, then etched with 5% ethylenediaminetetraacetate solution (EDTA) and coated with gold (10 nm) before examination with scanning electron microscope (SEM, Hitachi S-520) at various magnifications. Using SEM photographs of otolith sections (Fig. 2), different patterns were identified in accordance with conventional characteristics established for other eel species (core or nucleus (n) with primordium (p), hatch check (1) and first feeding-check (2), crystalline band, leptocephalus zone (L), metamorphosis zone (M: Tabeta *et al.* 1987, Lecomte-Finiger 1992, Budimawan 1997, Robinet *et al.* 2003). The maximum diameter was measured along the longest axis from edge to edge through the primordium. To separate the metamorphosis zone to the leptocephalus zone, occurrence of wider increments was used. The number of increments was counted from the first feeding check to the transition ring (considered as the end of the marine life) when present. Increments were considered as daily as established by previous authors (Umezawa *et al.* 1989, Arai *et al.* 2000, Sugeha *et al.* 2001). The resulting number of increments was interpreted as the marine larval life or early life. The duration of each larval stage (Leptocephalus and Metamorphosis), age at recruitment and hatching date were deduced.

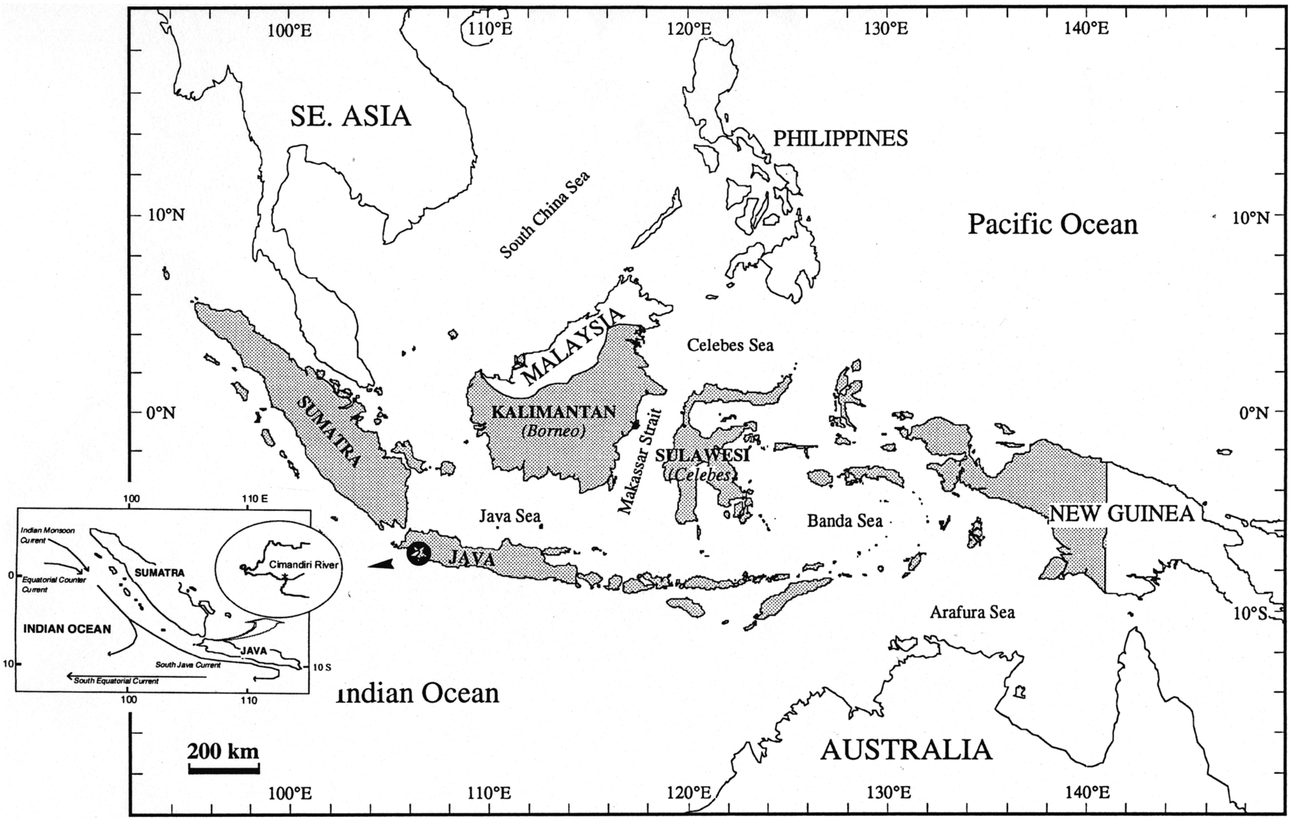

Fig 1. – Sampling area in Cimandiri estuary, West Java, Indonesia

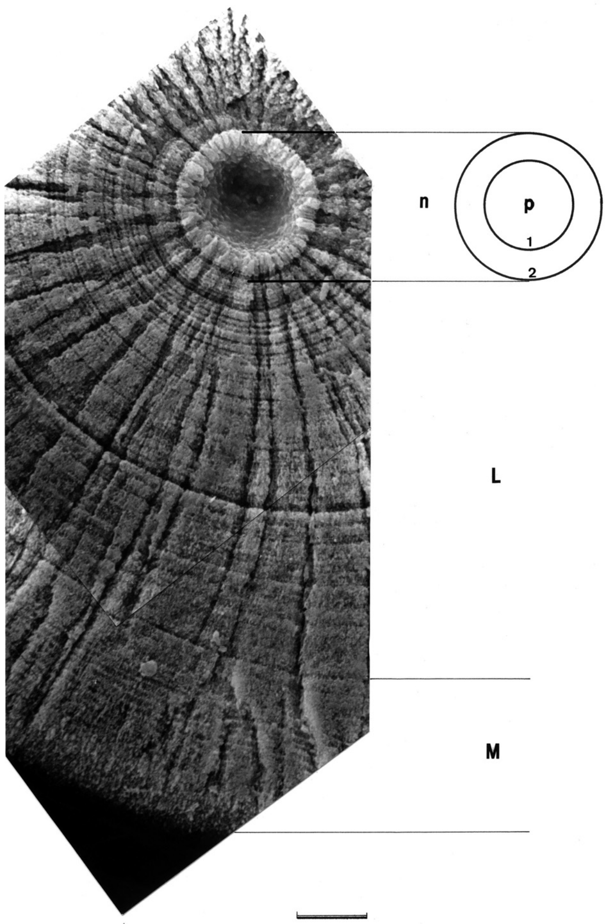

Fig 2. – Microstructural pattern in the sagitta of *Anguilla bicolor bicolor* core or nucleus (n) with primordium (p), hatch check (1) and first feeding-check (2), crystalline band, leptocephalus zone (L), metamorphosis zone (M). Scale: 10m m

RESULTS

Among the collected glass eels, the tail pigmentation and the morphology (ano-dorsal ratio and number of vertebrae) allowed to separate doubtlessly 3 glass eels by species: *Anguilla bicolor bicolor* McClelland (1844), *A. marmorata* Quoy & Gaimard (1824), *A. nebulosa nebulosa* McClelland (1844).

Anguilla bicolor bicolor is the dominant species in Cimandiri estuary, with more than 97 % of individuals and it is found year round (Table I). *A. bicolor bicolor* presented a caudal pigmentation characterized by little spots, concentrated in the caudal fin edge, an ano-dorsal length characteristic at one per cent of total length, and a mean number of vertebrae of 110 (110.1-110.8).

Size and Pigmentation Stage

Results are summarized in Table II. TL ranged from 52.00 to 65.00 mm and W from 0.10 to 0.15 g. Comparing samples at stage VB, TL (mean ± standard dev.) of C06-94 (55.58 ± 1.77) mm and C07-94 (57.55 ± 0.55) mm were statistically higher (t-test; $p < 0.05$) than those of C12-93 (52.86 ± 2.08) mm and C01-94 (54.00 ± 1.00) mm. The weight of C06-94 (0.140 ± 0.015) g and C07-94 (0.140 ± 0.016) g were statistically higher (t-test; $p < 0.05$) than those of C12-93 (0.110 ± 0.015) g and C01-94 (0.098 ± 0.011) g. The condition of C06-94 (0.082 ± 0.007) and C07-94 (0.073 ± 0.006) were higher than those of C12-93 (0.076 ± 0.0.010) and C01-94 (0.062 ± 0.006). Specimens of C06-94 and C07-94 were statistically heavier and longer (t-test; $p < 0.05$) than those of C12-93 and C01-94.

The pigmentation stage examination showed glass eels in all samples, but the frequency varied significantly among samples. The C06-94 (70.45 %) had more glass eels than the C12-93 (33.00 %), C01-94 (8.46 %), and C07-94 (10.77 %). Elvers were abundant in C01-94.

Sagittae

The hatch check and the first exogenous feeding check diameters were respectively 9.5 ± 1.9 and 20.0 ± 2.6 μm. The mean width of the leptocephalus zone, the metamorphosis zone and the transition ring averaged respectively 77.8 ± 6.7, 46.0 ± 8.3, and 9.7 ± 4.4 μm.

The duration of the leptocephalus phase, of the metamorphosis and of the transition phases were estimated to 85.2 ± 12.8, 21.9 ± 2.1, and 3.8 ± 1.1 days respectively. Therefore, the mean duration of marine early life was estimated to 106.4 ± 11.1 days.

The estimated birthdates (for the June sample) are the following in 1994: between January 26th and February 18th.

Growth rate

The increment width expressed in growth rate was shown in Fig. 3. Around the centre near the first feeding check, the width of increment measured about 0.8 μm. A slight peak of 1.1 μm (at N increments = 20) and a

Sample	N glass eels	Species	Frequency
C12-93	105	*A. bicolor bicolor*	97.14
		A. sp	2.86
C01-94	1 068	*A. bicolor bicolor*	96.91
		A. sp	3.09
C06-94	1 018	*A. bicolor bicolor*	100
C07-94	1 305	*A. bicolor bicolor*	97.17
		A. sp	2.83

Table I. – Composition of eel species from Cimandiri estuary, West Java, Indonesia.

Table II. – Morphometry (% of glass eels, N number of vertebrae, size, weight, condition) of glass eels of *Anguilla bicolor bicolor*.

Sample	%	N	Total length (mm)	Weight (g)	Condition
C12-93	33.00	110.8 ± 0.7	52.86 ± 2.08	0.110 ± 0.015	0.076 ± 0.010
C01-94	8.46	110.6 ± 1.2	54.00 ± 1.00	0.098 ± 0.011	0.062 ± 0.006
C06-94	70.45	110.1 ± 1.4	55.58 ± 1.77	0.140 ± 0.015	0.082 ± 0.007
C07-94	10.77	110.3 ± 1.2	57.55 ± 0.55	0.140 ± 0.016	0.073 ± 0.006

Table III. – *Anguilla bicolor bicolor* in literature. Total length, otolith radius, duration of leptocephalus stage, duration of metamorphosis, age at recruitment (first column: minimum value-maximum value; second column: mean and SD range).

	Cimandiri 1995 (Arai *et al.* 1999)		Cimandiri 1998-1999 (Setawian *et al.* 2001)		Reunion 2001 (Robinet *et al.* 2003)		Present study 1993-1994	
Total length (mm)	45.5-52.3	49.4 ± 2.4	47-58.5	52.7 ± 2.1	46-60	54.0 ± 2.1	50-58	
Otolith radius (μm)	155-176	164 ± 6.3						
Duration Lepto Stage (days)	119-171	139 ± 15.9		148.4 ± 26.6	39-57	46.2 ± 5.8		85.2 ± 12.8
Duration Met Stage (days)	13-27	18 ± 4.2		26.4 ± 5.7	24-48	33.6 ± 7.5		21.9 ± 2.1
Age at recruitment (days)	148-202	177 ± 16.4	118-258	182.8 ± 28.0	68-96	79.8 ± 7.7		106.4 ± 11.1

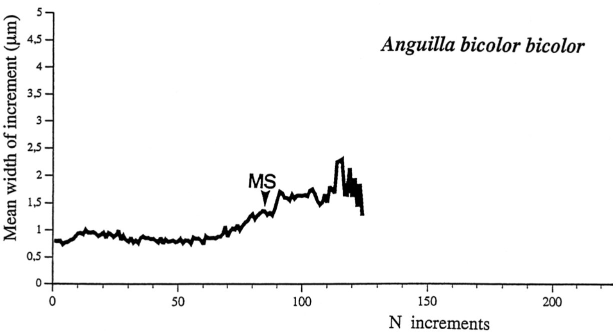

Fig 3. – *Anguilla bicolor bicolor* - sagittae - growth increments - larval duration - oceanic migration. Mean Increment growth rate on sagittae. n = 29 sagittae. MS: beginning of metamorphosis zone

decrease to 0.8 μm before the metamorphosis were then observed. The width of increments during metamorphosis increased up to 2.0 - 2.5 μm. In the leptocephalus stage, growth rate was ca. 1.0 μm/d, with a slight peak (> 1.0 μm/d). In metamorphosis stage, growth rate increased gradually from 1.0 to 2.0 μm/d.

DISCUSSION

Three eel species (Budimawan 1996) were found to be recruited in the Cimandiri estuary. We also pointed out the constant proportion (97 %) of *Anguilla bicolor bicolor* as shown by Setawian *et al*. (2001). *Anguilla marmorata* is the species with a very large extensive distribution and for that, it is not surprising to find it in Cimandiri. *Anguilla nebulosa nebulosa* embraced generally the Indian continent. But, according to McClelland (in Ege 1939) it occurred on the Andamans and west coast of North Sumatra.

The correlation between size parameters (TL and W) and pigmentation stage was clearly established in *Anguilla anguilla*. In fact, at the beginning of metamorphosis, leptocephali stop to feed until reaching the stage VIA3 (Lecomte-Finiger 1983). There is clearly a negative correlation between size (TL and W) and pigmentation stage, exclusively from VA to VIA3. It is well known that recruited glass eels with the greatest size and dominantly earliest stage of pigmentation characterize a seasonal peak of new glass eels. In this way, our sample of *Anguilla bicolor bicolor* of June (70.45 % transparent glass eels, TL: 55.58 ± 1.73 mm, W: 0.140 ± 0.015 g) and July (10.77 %, TL: 57.55 ± 0.71 mm, W: 0.140 ± 0.016 g) are close to the peak season. By contrast, the C12-93 and C01-94 respectively December and January represent the end of the migration. Thus, the seasonal peak of *Anguilla bicolor bicolor* in Cimandiri estuary was estimated to be from May to July and the end of the inshore migration from January to March. Setiawan *et al*. (2001) reported that their samples consisted of stage of VA and VB with a length of 52.7 ± 2.1 mm (TL). They estimated that there were two peaks of spawning season: May (dry season) and December (rainy season). Accordingly, their prediction of the arrival peak season of *Anguilla bicolor bicolor* into Cimandiri estuary was November and June (Table III). Nevertheless, our sampling composed of four samples could be considered as representative because it covered both dry and rainy seasons (C12-93 and C01-94 from the rainy season, and C06-94 and C07-94 from the dry season). It is interesting to note that total length of the glass eels from Cimandiri (eastern part of Indian Ocean) ranged from 45.5 to 58.5 mm and did not differ between years. The same range of values was found in Reunion Island situated in South-West Indian Ocean (Robinet *et al*. 2003).

In the present study, the mean duration of the leptocephalus stage (through sagitta examination) was 85.2 ± 12.8 days, the age at recruitment was 106.4 ± 11.1 days.

Different results of age at recruitment and age at metamorphosis (or duration of leptocephalus stage) have been found by Setawian *et al*. (2001) and Arai *et al*. (1999) in the same estuary, and by Robinet *et al*. (2003) in Reunion Island. The variations in the time of metamorphosis between sites could arise from different environmental conditions that influence growth during the leptocephalus stage. These results may suggest the existence of one or two spawning sites in the Indian Ocean.

In conclusion, one of the complexities of the *Anguilla*'s biological cycle is to locate the spawning site. Several indications such as newly recruited glass eels, leptocephali and data on ocean currents can be useful to locate a spawning area. A spawning site for *Anguilla bicolor bicolor* was reported by Jespersen (1942) in the waters of Eastern Indian Ocean, along Sumatra Island. Robinet *et al*. (2003) reported that due to the short migration length (79.9 days) to recruit in the Reunion Island, *A. bicolor bicolor* must have a second spawning area in the Indian Ocean close to the Mascarene Archipelago.

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