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B. Budimawan, R Lecomte-Finiger. INSHORE MIGRATION OF THE TROPICAL GLASS EELS ANGUILLA MARMORATA RECRUITING TO POSO ESTUARY, SULAWESI ISLAND, INDONE-SIA. Vie et Milieu / Life & Environment, 2005, pp.7-14. hal-03218982

## HAL Id: hal-03218982 https://hal.sorbonne-universite.fr/hal-03218982v1

Submitted on 6 May 2021  $\,$ 

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## INSHORE MIGRATION OF THE TROPICAL GLASS EELS ANGUILLA MARMORATA RECRUITING TO POSO ESTUARY, SULAWESI ISLAND, INDONESIA

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ANGUILLA MARMORATA GLASS EELS RECRUITMENT OTOLITH MICROSTRUCTURE POSO ESTUARY

> ANGUILLA MARMORATA CIVELLE RECRUTEMENT OTOLITHE ESTUAIRE DE POSO INDONÉSIE

ABSTRACT. – In order to determine the inshore migration mechanisms of the tropical eel *Anguilla marmorata* (Quoy et Gaimard, 1824) entering the Poso estuary (Sulawesi, Indonesia), glass eels were collected throughout 1999. We subjected these samples to morphological examination and aged a subsample. Environmental factors were investigated. Glass eels were active at night, with high activity between 02.00 and 04.00 o'clock. Migration was initiated by increase in water flow. The peak of migration occurred in January-March. Pigmentation stages, length, weight and factor condition were analysed and showed variations. A subsample of glass eels was aged by examinations of sagittal otoliths. Duration of marine larval stage was estimated to be 97-102 days and discussed.

RÉSUMÉ. – La migration anadrome de l'Anguille marbrée Anguilla marmorata (Quoy et Gaimard, 1824) dans l'estuaire de Poso (Sulawesi, Indonésie) a été suivie en 1999. Des échantillons de civelles ont été collectés mensuellement et les facteurs environnementaux relevés. Les civelles ont été décrites morphologiquement et un sous-échantillon a été utilisé pour évaluer l'âge. Les civelles sont actives de nuit et leur pic d'activité se situe entre 02,00 et 04,00 heures. La migration débute avec la marée montante. Le pic de migration est observé entre janvier et mars. La taille, le poids, la pigmentation et l'indice de condition ont été analysés et présentent des variations. Les sagittae du sous-échantillon ont été extraits et la durée de vie marine estimée à 97-102 jours.

#### **INTRODUCTION**

The tropical eel Anguilla marmorata Quoy et Gaimard, 1824 is one of the most common eel species which has a widespread distribution in the world (Ege 1939, Jespersen 1942). It occurs throughout the Indo-Pacific region (Tesch 1977). The adults reach greater size than most temperate species. Like all anguillid species, A. marmorata spawns in the ocean and has leptocephalus larvae. After metamorphosis into glass eels, inshore migration starts. The wide distribution of A. marmorata throughout the Pacific and Indian oceans indicates that it differs from temperate species (e.g. A. anguilla and A. rostrata) and has multiple spawning areas. Compared with temperate eels little is known about the early life history and recruitment mechanisms. However, recent efforts have revealed the early life history and inshore migration of tropical eels (Budimawan 1997, Arai et al. 1999, Arai et al. 2001, Lecomte-Finiger et al.

2001, Sugeha *et al.* 2001a, 2001b, Miller *et al.* 2002, Robinet *et al.* 2003). The invasion of freshwater by juvenile eels (variously referred to as glass eels, elvers or "*civelles*" in French) of the genus *Anguilla* is a well known seasonal phenomenon in many countries. In temperate regions the peak of arrival time is from late winter to early spring (Tesch 1977, Sugeha *et al.* 2001). In tropical regions, however, this phenomenon has not been well documented (Arai *et al.* 1999, 2001, Sugeha *et al.* 2001). Knowledge of the inshore migration is rudimentary.

This paper deals with a yearly survey of the inshore migration of the Anguilla marmorata glass eels in the Poso estuary, Tomini Bay, Indonesia. The monthly and hourly recruitment patterns of A. marmorata were followed. The morphological characters, otolith microstructure, age of different pigmentation stages and growth rate were measured. The effects of environmental factors on glass eel movements during the inshore migration were examined.

#### **MATERIALS AND METHODS**

Sampling and morphological characters: The samples were collected (Fig 1) in the Poso Estuary (0° 15' S; 120º 10' E), Tomini Bay, Center of Sulawesi (Indonesia) for one year, from January to December 1999 using a typical glass eel trap made of bamboo. A monthly and hourly sampling of A. marmorata in the Poso Estuary was conducted to explain the recruitment mechanism and chronology. The monthly period of sampling was fixed on 3<sup>rd</sup> – 4<sup>th</sup> week of lunar cycle. This period is assumed, based on other tropical species (Sugeha et al. 2001), as the season of upstream migration of juvenile eels in the Poso Estuary. The environmental parameters such as salinity, temperature, turbidity (with the Secchi disk), current velocity (through floating object) were investigated. At the peak season, hourly sampling was conducted to determine migration rhythm. At that moment, glass eels were scooped out three times during one minute with a long handled dip net. The mean number of glass eels by minute (cpue) was obtained.

All specimens were preserved in 70% ethanol and then identified using morphomeristic criteria: caudal pigmentation, ano-dorsal distance to total length and total number of vertebrae (Ege 1939, Tabeta *et al.* 1976, Tesch 1977). *Anguilla marmorata* specimens were weighed to the nearest 0.01 g and measured to the nearest 1 mm and then classified through their pigmentation stage according to Elie *et al.* 1982. The condition factor (Cc) was calculated as following:

$$CC = \frac{W}{L^3} \times 10^3$$

where: W = individual weight in g; L = individual total length in cm

Otolith preparation: Thirty Anguilla marmorata specimens of January and February'99 were used for ageing. The sagittae were removed, embedded in epoxy resin, ground (600 and 1000 grit paper) and polished until the core was exposed. To reveal the marks for age determination, the polished otoliths were etched with 5% EDTA and coated with gold before examination on Scanning Electron Microscope (SEM Hitachi S-520) at different magnifications. SEM-photographs were used for counting the number of growth increments and measuring their widths. To estimate the age of the fish beyond hatching otolith increments were counted, as suggested in the previous studies (Lecomte-Finiger 1992, Budimawan 1997). An optical imaging system was used to count increments and to measure increment widths, and the distance from core to the first exogenous feeding ring. Incremental counts were discriminated between leptocephalus and metamorphic zones. Total ages of the fish were estimated as the age from hatching ring to the transion mark or to the edge of the otolith. The growth pattern was determined. Otolith increments of A. marmorata have been validated to be deposited daily (Sugeha et al. 2001a).



Fig. 1. - Poso Estuary in Sulawesi (Indonesia).

#### RESULTS

#### Length, weight and condition

Total body length (TL) ranged from  $4.66 \pm 0.20$  to  $5.06 \pm 0.23$  cm, weight (W) from  $0.10 \pm 0.01$  to  $0.15 \pm 0.03$  g and condition (Cc) from  $0.89 \pm 0.11$  to  $1.32 \pm 0.16$  g/cm<sup>3</sup> (Table I).

Table I. – Anguilla marmorata. Size and weight of glass eels (Poso Estuary, 1999).

Sample	Ν	Total length	Weight	Condition
	fish	$(Mean \pm S.D)$	$(Mean \pm S.D)$	$(Mean \pm S.D)$
		cm	g	
January	200	$5.02 \pm 0.23$	$0.13 \pm 0.05$	$0.97 \pm 0.49$
February	200	$4.95 \pm 0.23$	$0.14 \pm 0.03$	$1.19 \pm 0.22$
March	200	$5.06 \pm 0.23$	$0.14 \pm 0.04$	$1.07 \pm 0.24$
April	168	$4.66 \pm 0.20$	$0.12 \pm 0.04$	$1.24 \pm 0.64$
May	270	$5.04 \pm 0.24$	$0.15 \pm 0.03$	$1.15 \pm 0.11$
June	269	$4.85 \pm 0.26$	$0.11 \pm 0.02$	$0.92 \pm 0.14$
July	281	$4.93 \pm 0.24$	$0.11 \pm 0.02$	$0.90 \pm 0.13$
August	266	$4.95 \pm 0.19$	$0.13 \pm 0.02$	$1.07 \pm 0.16$
September	278	$4.98 \pm 0.18$	$0.14 \pm 0.02$	$1.12 \pm 0.12$
October	118	$4.73 \pm 0.15$	$0.13 \pm 0.02$	$1.26 \pm 0.16$
November	118	$4.84 \pm 0.18$	$0.10 \pm 0.01$	$0.89 \pm 0.11$
December	121	$4.84 \pm 0.22$	$0.15 \pm 0.03$	$1.32 \pm 0.16$

May '99 and June '99 showed elvers and could be considered as samples of the end of the migrating season.

Table II. – Anguilla marmorata. Frequency (%) of glass eels (upper value) and elvers (lower value) in Poso Estuary (1999).

Sample	Ν	VB	$VIA_1$	$VIA_2$	VIA <sub>3</sub>	$VIA_4$	VIB
January	200	100.0					
February	200	80.5	19.5				
March	200	100.0					
April	168	18.5	26.8	28.0			
					25.5	1.2	
May	270	29.0	20.5	15.5	20.0	5.0	
					30.0	5.0	
June	269	17.5	7.5	17.5	51.0	( )	0.5
					51.0	0.0	0.5
July	281	87.8	1.0				
August	266	76.0	23.0				
September	278	80.5	19.5				
October	118	85.0	15.0				
November	118	91.0	9.0				
December	121	97.0	3.0				

#### Time of arrival

#### Pigmentation

For the purpose of the present study, the term of glass eels refers to transparent stages VB, VIA<sub>1</sub> and VIA<sub>2</sub>, while pigmented stages VIA<sub>3</sub>, VIA<sub>4</sub> and VIB are elvers. Glass eels recruited to the estuary thoughout the year (Table II). Samples of April '99,

From the morphological data (Table I) and from the pigmentation-stage data, the beginning and the peak season of the inshore migration of *Anguilla marmorata* glass eels in the Poso Estuary were in January and March respectively, while the end of the inshore season was in June. The beginning and the peak of the glass-eel migration was characterized by big and heavy glass eels, while the end of the migration was characterized by small and light elvers (Table III top).

Table III. – Anguilla marmorata. Top, Evolution of Weight, Length, and Condition according to the pigmentation stage (sample of June 1999): \*Mean  $\pm$  S.D. Middle, measurements (mean  $\pm$  std Deviation, in (m) of the microstructures of Sagittae (n = 19). Bottom, number of increments (in days) in each zone on otoliths.

	Pigmentation stage								
Data	VB	$VIA_1$	VIA <sub>2</sub>	VIA <sub>3</sub>	VIA <sub>4</sub>	VIB			
Weight *	$0.16 \pm 0.02$	$0.16 \pm 0.02$	$2  0.16 \pm 0.02$	$0.15 \pm 0.02$	$0.14 \pm 0.02$	0.16			
Length *	$4.77 \pm 0.18$	$4.76 \pm 0.10$	$6   4.81 \pm 0.21$	$4.70 \pm 0.18$	$4.65 \pm 0.22$	5.10			
Condition	$1.51 \pm 0.15$	$1.47 \pm 0.17$	7 1.44 $\pm$ 0.22	$1.45 \pm 0.18$	$1.38 \pm 0.25$	1.21			
N Fish	35	15	35	102	12	1			
	Core Diameter	Yolk sac period	Diameter of the first feeding check intake	Leptocephalus Growth	Metamorphosis Growth	Otolith Diameter			
January	9.5 ± 1.4	$5.2 \pm 0.7$	19.9 ± 1.9	71.8 ± 8.3	$35.5 \pm 7.7$	$220.1 \pm 15.8$			
February	9.7 ± 1.6	$5.1 \pm 0.7$	$19.8 \pm 2.4$	$60.7 \pm 7.5$	$37.4 \pm 10.3$	221.2 ± 19.1			
		N	Leptocephalus growth zone	Metamorpl Growth z	nosis one	Total			
January		23	79.1 ± 11.2	19.2 ± 2	2.1 97	'.3 ± 12.3			
February		19	$82.8 \pm 6.5$	19.4 ±1	.7 102	$2.3 \pm 7.6$			

#### **Environmental** factors

At the peak recruitment in March (Fig. 2A) an average catch of 20 glass eels per minute occurred (Table II). Three of the four environmental factors, turbidity (Fig. 2B), relative depth (Fig. 2C) and velocity of current (Fig 2E) did not show a clear correlation with the recruitment; only salinity (Fig. 2D) showed a correlation. There was a recruitment peak when salinity decreased. Decreasing of salinity coincided to the freshwater discharge from river induced an increase in turbidity.

Glass eels migrating in the Poso Estuary were collected only at night, 18.00-06.00 h with two peaks: the main peak between 02.00 and 04.00 h and another between 18.30 and 20.00 h (Fig 3). This result is different to those reported in other results where the main peak lies between 18.00-20.00 (Sugeha *et al.* 2001). It is important to emphasize that this peak coincided with a simultaneous occurrence of a spring high tide from the sea and the freshwater discharge from the Poso River.

#### **Otolith microstructure**

All sagittae revealed the similar microstructural patterns (Fig 4) as previously described by Budimawan (1997). The primordium was characterized by a deep dark hole in the central area (Fig 4A). Its form varied slightly from round to subelliptical and triangular. It was surrounded by a white band called the core. The primordium and core corresponded to the nucleus. The hatch-check (Fig 4B) was characterized by a heavy dark ring lying between the core and the embryonic-life band, which showed a crystalline structure oriented perpendicularly to the increments. Up to this band, no increment could be identified. The increments formed regularly after being initiated by a discontinuous zone. This check is associated with a critical period lasting from the end of yolk resorption to the first exogenous feeding. The leptocephalus growth zone was characterized by the clear increments in this area. This zone includes from the first increment until the first metamorphosis ring. The metamorphosis growth zone was formed after the leptocephalus growth zone. The principal characteristics of microstructure on sagittal otoliths are listed in Table III, middle.

#### Ageing and growth rate

The mean age (Table III bottom) of these samples were estimated to be about 3 months (97 - 102 days). Our analysis shows that there was a variation of increment width, with a remarkable peak just before metamorphosis in sagittae (Fig 5). The mean growth rate in the metamorphosis zone (M-increments)was approximately 2.20 ( $\pm$  0.13) µm per day



Fig. 2. – Environmental factors and number of recruits. (A): Turbidity (B), Relative depth (C), Salinity (D) and Velocity of current (E).



Fig. 3. - Hourly catch of Anguilla marmorata glass eels in the Poso Estuary (a night-catch on March 1999).

and statistically higher than that of leptocephalus zone (L-increments), 0.93 ( $\pm$  0.06)  $\mu$ m per day.

#### DISCUSSION

Three species of tropical eels (Arai *et al.* 1999) entered the Poso estuary: *Anguilla celebesensis* was the most dominant species (88.3%) throughout 1997 followed by *A. marmorata* (11.3%) and *A. bicolor pacifica* (0.4%). Sugeha *et al.* (2001b) made a three year survey and determined that the three species were found each year in fluctuating abundances. In the present one-year study of the *A. marmorata* composition of glass eels and elvers using external and internal morphological analysis, a new understanding of the biological and behavioural factors were reported about the inshore migration.

Anguilla marmorata glass eels enter the Poso estuary throughout the year with a peak in March indicating that probably this species spawns all months of the year. The high proportion of glass eels (transparent and considered as early larvae) throughout the year and the 20-day duration of the metamorphosis growth band in otoliths indicate also that they probably recruit relatively soon after metamorphosis. Sugeha *et al.* (2001a) reported the same results for other eel species in the estuary of the Poigar River in northern Sulawesi.

Morphological data confirmed that tropical glass eels like *Anguilla marmorata* are shorter and lighter than both Atlantic eels, European eel *A. anguilla* (TL =  $7.2\pm 0.2$  cm; W= $0.42\pm0.08$  g; Cc= $1.2\pm0.2$ ) and American eel, *A. rostrata* (TL= $6.2\pm 0.5$  cm; W= $0.32\pm0.08$  g; Cc= $1.1\pm0.2$ ) (Yahyaoui 1983, Cantrelle 1984, Guerault *et al.* 1992, Desaunay *et al.* 1996, Budimawan 1997). The advance in pigmentation stage recorded late in the season reflects two factors: first, a possible accumulation of glass eels at the mouth of the estuary, the larvae becoming progressively more pigmented; second, the arrival of glass eels later in the season means an extended post metamorphic marine life. Length, weight and condition declined from stages VB to VIB. The growth increased again at VIA3 stage during which the elvers began exogenous feeding (Lecomte-Finiger 1983).

Glass eels arrive only at night and the water flow played an important role. This result is well known for temperate eels species and glass eels are active at night and rest during daytime (Tesch 1977, Sloane 1984, Martin 1995). This is consistant with the glass-eel behavior including *Anguilla anguilla* (Lecomte-Finiger & Prodon 1979), *A. japonica* (Tsukamoto 1990), *A. rostrata* (Martin 1995), *A. australis* and *A. dieffenbachii* (Jellyman 1977). Outside this period, in the diurnal period (06.00 – 18.00 o'clock), swimming activity of glass eels decreases. During this period, glass eels rest in the sediment (Lecomte-Finiger & Prodon 1979).

The mean otolith radius of  $125.07 \pm 3.16 \,\mu\text{m}$ (with 92 increments i.e. average width increment of 1.36 µm) of Anguilla marmorata glass eels was slightly higher (133 increments in 137 µm, i.e average width increment =  $1.03 \ \mu m$ ) than reported by Tabeta et al. (1987) for A. japonica. Two zones may be distinguished on the basis of increment growth-rate as demonstrated in other species: Solea solea (Lagardère 1989), A. japonica (Umezawa & Tsukamoto 1987, Tzeng & Tsai 1992). The leptocephalus growth zone and the metamorphosis growth zone are separated by a diffuse zone characterized by irregular-width increments which make counting difficult, and in consequence an interpolation value should be used. Antunes & Tesch (1997) designated this zone as "zone without ring". The precise position of the transitions from the leptocephalus growth zone to the diffuse zone and from the diffuse zone to the metamorphosis growth



Fig. 4. – Microstructural pattern in sagittae of *Anguilla marmorata* (Scale bar 20 µm). A: Sagittal section; L: Leptocephalus zone; M: Metamorphosis zone; T: Transition; 1: nucleus; B: Details; C: Core; 2: First exogenous; feeding; L: Leptocephalus zone.



Fig. 5. – Daily growth of Sagitta of *A. marmorata*. MS: Metamorphosis zone on sagitta.

zone is difficult to define. A comparison of the leptocephalus growth zone and the metamorphosis growth zone shows that the mean width of (M)-increments is higher than that of (L)-increments.

The evidence of daily increment (Pannella 1971) suggests a presence of daily deposition in the eel species: Anguilla anguilla (Lecomte-Finiger & Yahyaoui 1989), A. rostrata (Castonguay 1987), A. marmorata (Tabeta et al. 1987). It has been validated in the Japanese eel, A. japonica (Tsukamoto et al. 1989, Umezawa et al. 1989, Lee & Lee 1989) in the American eel A. rostrata (Martin 1995) and recently in A. marmorata (Sugeha et al. 2001a). This age could be associated with marine larval life during which the larvae required a migration from spawning ground to coastal waters. This estimated age is largely shorter than in other eel species: A. japonica, age at recruitment in Taiwan 112.8 to 156.5 days (Tzeng 1990, Tzeng & Tsai 1992, Cheng & Tzeng 1996), in Japan 218 ± 29 days (Schmidt 1922, Tsukamoto 1990), A. anguilla 9 to 11 months or more than one year in the European estuaries (Lecomte-Finiger 1992, Wang & Tzeng 2000), A. rostrata, 8 to 12 months one year (Tsukamoto et al. 1991, Wang & Tzeng 1998).

As all samples have the same age, their distance from spawning site to recruitment site (Poso Estuary) is the same. The presence of these transparent glass eels in great proportion could indicate the existence of a spawning area not far from this bay. Budimawan (1996) suggested that the spawning area could be in Malacca Sea and/or in Tomini Bay. It is well known that this species has a very extensive distribution, West to East, from Africa to the Marquesas Islands (Ege 1939). In contrast to temperate eels Anguilla anguilla, A. rostrata and A. *japonica*, each species spawns in one breeding area, the tropical eels may utilize many spawning grounds. For example, Marquet (1992) presumed that A. marmorata from French Polynesia spawned near French Polynesia waters (130°-135°W;15-20°S)

and, Miller *et al.* 2002 reported a spawning area in the western north Pacific in the North Equatorial Current (NEC). Another breeding ground in the Indian Ocean lies east of Madagascar (Jespersen 1942). Future studies on tropical eels are needed to provide information about their marine adult life.

In conclusion, recruitment of *Anguilla marmorata* in Poso Estuary occurs throughout the year with beginning, peak and end of season lying respectively in January, March and June. Upstream migration of *A. marmorata* glass eels in Poso Estuary occurs at night, with two peaks, one at 02.00-04.00 o'clock the most abundant, another at 18.30 - 20.00 o'clock local time. The low salinity due to freshwater discharge from river and tidal flow from the sea should be considered as an important factor affecting the recruitment of tropical eel *A. marmorata*. The information here was collected at only one site and during one year, so it is not possible to generalize these results. Additional research is needed.

ACKNOWLEDGEMENTS. – The authors thank the Yaoung Academic Project of URGE (Higher Directorate, Departement of National Education of Indonesia) for financial support, Pr E Taylor and the referees for their critical comments.

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Reçu le 15 janvier 2004; received January 15, 2004 Accepté le 12 mai 2004; accepted May 12, 2004