INSHORE MIGRATION OF THE TROPICAL ANGUILLID GLASS EELS RECRUITED IN THE ESTUARY OF DUMOGA RIVER (NORTH SULAWESI, INDONESIA)

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ABSTRACT

Species composition, abundance, and timing of migration of the tropical anguillid glass eels were observed in order to understand their inshore migration mechanism in the estuary of Dumoga River (North Sulawesi Island, Indonesia). A quantitative sampling method was conducted during 12 hours observation (18.00 to 05.00) at 3 nights of new moon in 2004 and 5 nights of new moon in 2005 and in 2006. Based on morphology and genetic analysis, at least four species of the tropical anguillid eels was recognized to enter the estuary, *eq. Anguilla marmorata, A. bicolor pacifica, A. celebesensis* and *A. interioris.* For ecological study, the last two species was grouped as *Anguilla* spp since they were overlapped in morphological characters. *Anguilla marmorata* was the most dominant species (2004 = 64.55%; 2005 = 90.02%; 2006 = 60.17%) recruited in the estuary while *Anguilla* spp in the second position (2004 = 28.61%; 2005 = 9.88%; 2006 = 34.40%) and *A. bicolor pacifica* in the third position (2004 = 10.99%; 2005 = 0.19%; 2006 = 1.05%). In general, *Anguilla bicolor pacifica* was appeared in the estuary just after sunset (19.00-20.00) following first ebb tide in the afternoon. *Anguilla marmorata* was appeared along the night of sampling time (19.00-05.00) when both flood and ebb tides was occurred. *Anguilla* spp was arrived in the estuary in the middle of night (22.00-03.00) when the second ebb tide gradually increased. These studies suggested that inshore migration mechanism in the estuary of Dumoga River was influenced by circadian rhythmic that regulated by environmental conditions such as new moon period and tidal cycle that typically occurred in the region of Indonesian Waters. Inter annual variation in timing of inshore migration resulting differences inshore migratory behavior between tropical anguillid eel species recruited on that area.

Keywords: species composition, abundance, timing of inshore migration, circardian rhythmic, new moon, tidal cycle

INTRODUCTION

The term of glass eels refers to a development stage of juvenile anguillid eels that lasts for relatively short period of time from the end of metamorphosis to the fully pigmented elver or small yellow eel stage. Glass eel is the important stage in the life history of anguillid eels. As its name infers, most early phase glass eels are entirely transparent like glass without any pigmentation on the body except for the eye and on the tip of the tail. The whole glass eel stage is further divided into several phases by the degree of pigmentation that develops on the tail and eventually the head and this pigmentation is a useful means to define the different phase (Bertin, 1956; Tabeta *et al.*, 1976a,b; Tesch, 1977; Tzeng and Tabeta, 1983; Haro and Krueger, 1988).

Knowledge about the basic biological information on tropical glass eels, such as their body size and pigmentation at the time of recruitment to estuaries, and inshore migratory behavior is limited compared to that of temperate species. There has been considerable research over the years on the recruitment pattern of the glass eels of the temperate species including Japanese eel (Tsukamoto, 1990; Shinoda *et al.*, 2001); Atlantic eels (Haro and Krueker, 1988; Wang and Tzeng, 2000; Powles and Warlen, 2002), and Australian and New Zealand eels (Sloane 1984; Jellyman *et al.*, 1999, 2002). But only few study on the glass eels of the tropical anguillid species, and the studies have mostly been conducted in the Indonesian region (Budimawan, 1997; Arai *et al.*, 1999a, b; Sugeha *et al.*, 2001a Setiawan *et al.*, 2001).

The first study on the early life history of the tropical anguillid glass eels was conducted in Poso River estuary (Budimawan, 1997) based on otolith microstructure analyses. However first study to examine the recruitment season and inshore migration pattern of the tropical anguillid glass eels based on morphology, genetic, and otolith microstructure and microchemistry analyses was conducted by Arai *et al* (1999a, b) and Sugeha *et al* (2001) in the Poigar River on the north of Sulawesi Island (Indonesia), in multi years observation. Another study on the seasonal inshore migration of tropical glass eels species was done in the Cimandiri River estuary, on the west of Jawa Island (Setiawan *et al*, 2001), based on otolith microstructure alone.

The most recent study on the biodiversity, distribution, and abundance of the tropical anguillid eels in the Indonesian Waters suggested that inshore migration mechanism of the glass eels into their growth habitat in Indonesian archipelago was different between western, central, and eastern region of Indonesian Waters and was regulated by different monsoon and

oceanographic condition between the regions (Sugeha *et al*, 2008). It is widely known that the Indonesian Waters have a great marine biodiversity and a wide range of environmental conditions due to its complex physical and oceanographic conditions (Godfrey, 1996; Hatayama *et al.*, 1996; Creswell, 2000). This suggest that inshore migration patterns of the freshwater eels in the tropic may vary spatially and temporally and may be influenced by atmospheric conditions such as the seasonal monsoons or the El Nino Southern Oscillation (ENSO). Therefore, in order to understand the inshore migration mechanism of the tropical anguillid eel species in the Indonesian region, more data must be accumulated for a variety of species and locations.

In the present study, species composition, seasonal abundance, and timing of migration of the tropical anguillid glass eels into the mouth of the Dumoga River were observed during drying season of 2004, 2005, and 2006. The objective of this study is to describe and analyze the basic biological data on the early life history and inshore migration mechanism of the tropical anguillid glass eels recruited to the north part of Sulawesi Island, Indonesia.

MATERIAL AND METHOD

Study area and specimen collection: Glass eel sampling was carried out in the estuary of Dumoga River (north of Sulawesi Island, Indonesia), at 0.2141°N and 124.08592°E. The mouth of the river was directly facing the relative calm of the tropical Sulawesi Sea **(Appendix 1).** They were caught along the beach using 2 triangular scoop nets (mouth 0.3 m^2, 1 mm mesh) following the sampling technique by Sugeha *et al.* (2001). Environmental factors including water temperature and salinity were measured at the beginning of each sampling hour using thermometer and refractometer, respectively. Tidal data was obtained from the Tide Tables Prediction (Dinas Hidro-Oseanografi) in Manado (North Sulawesi) in 2004, 2005, and 2006. The glass eels were fixed separately in 10% formalin and ethanol absolute just after captured then labeled and transported to the laboratory for future analysis. Subsamples of the specimens from each sampling time were used for morphology analysis while 30 specimens among them were genetically analysis for adjustment the species identification.

Morphology and genetic analysis: A total of 12508 glass eel specimens that consist of 1000 specimens collected in 2004, 2882 specimens in 2005, and 8626 specimens in 2006 were morphological examined in the present study. External morphology analyses in term of body length measurement including total length (TL) pre-dorsal length (PDL), pre-anal length (PAL), and ano-dorsal length (ADL) were done to the nearest 0.1 mm. Pigmentation observation was determined according to Bertin (1956) in order to adjust the developmental stage of the specimens. Internal morphology analyses in term of vertebrae counts including total vertebrae (TV), pre-dorsal vertebrae (PDV), pre-anal vertebrae (PAV), and ano-dorsal vertebrae (ADV) also conducted after bone staining analyses the formalin specimens with alizarine-red solution.

For the ethanol specimen, after external morphology analyses as the first step of species identification procedures, a series of genetic analyses was conducted. Total genomic DNA (deoxyribonucleic acid) extraction from 30 specimens of glass eel was carried out following a standard protocol (Aoyama and Tsukamoto, 1997). DNA was isolated and purified using phenol-chloroform-isoamyl alcohol (25:24:1, v/v) twice with diethyl ether, then concentrated by ethanol precipitation before finally suspended in the TE solution and stored in the freezer. A portion of the mitochondrial 16S ribosomal RNA gene was amplified via polymerase chain reaction (PCR) using the oligo-nucleotide primers that were nested in the 16SrRNA: L1854: 5'-AAA-CCT-CGT-ACC-TTT-TGC-AT- 3' (Aoyama, 1998) and H3058: 5'- TCC-GGT-CTG-AAC-TCA-GAT-CAC-GTA- 3' (Miya and Nishida, 1996).

The PCR was carried out with the Gen Amp PCR system 7200 (Applied biosystem), with a 25 ul reaction volume containing 13.8 ul sterile distilled water, 2.5 ul 10XPCR buffer (Perkin Elmer-Cetus), 2.5 ul dNTP (deoxynucleotide triphospate) of 2 mM, 2.5 ul each primer of 5 uM, 0.4 ul of Taq DNA polymerase (AmpliTaq, Perkin Elmer Cetus), and 50 to 1,000 ng of template DNA. Amplification parameters were 30 cycles of denaturation at 94 °C for 15 sec, annealing at 55 °C for 15 sec, and extension at 72 °C for 30 sec.

A longer double-stranded mitochondria DNA product from PCR was examined using Restriction Fragment Length Polymorphism (RFLP) analysis with the six type of restriction enzymes *Alu* I, *Hha* I, and *Bsp* 1286I (Promega); *Eco*T14I and Mva I (Takara Shuzo Co., Ltd); and BbrP I (Toyobo Co., Ltd) which made it possible to identify the species of tropical anguillid eels as described in Aoyama *et al* (2000), Watanabe (2000), and Sugeha (2003), Sugeha *et al.* (2008). Restriction procedures were carried out in a 15 ul final volume containing 5 ul PCR product, 1 ul restriction enzyme, 1.6 ul restriction enzyme buffer supplied by manufacturers and 7.5 ul sterile distilled water, and incubated at 37 °C overnight. Restriction fragment length

polymorphism (haplotype) was detected by electrophoresis on 1 % agarose gel with ethidium staining.

RESULT

Morphology characters

A total of 56864 glass eels was collected from the estuary of Dumoga River, at the new moon phase in August 2004 (3013 individuals), June to September 2005 (143254 individuals), and May to October 2006 (39604 individuals). From the total number of catch, most of them were released back to the sea in life condition while about 12508 individuals of the total catch were used for future analysis.

From both external **(Appendix 1)** and internal **(Appendix 2)** morphology analysis it was found that the tropical anguillid glass eels entering the estuary of Dumoga River could only separated in 3 species, e.q. *Anguilla bicolor pacifica, A. marmorata*, and *Anguilla* spp. *Anguilla bicolor pacifica* is a tropical short-finned eel while *A. marmorata* and *Anguilla* spp is the tropical long-finned eels. Internal key characters of number of ano-dorsal vertebrae (ADV) completely separate the three species were *A. bicolor pacifica* was -3 to 3 in range of ADV, while *Anguilla* spp and *A. marmorata* was 6 to 13 and 14 to 17 in range of ADV, respectively. However, external key character of anodorsal length in percentage of total length (ADL/%TL) was only could separate the short-finned eel species (-2 to 0) from those two long-finned eels species, while the two long-finned eels species was overlapped (9 to 11) in range of ADL/%TL.

Genetic characters

A total of 30 specimens fixed in 100% ethanol was randomly separated from whole specimen collected, and was extracted for collecting their DNA. The genetic species identification was made based on recent report on the morphology and genetic character of the tropical anguillid eels inhabit in Indonesian Waters (Sugeha *et al.*, 2006; 2008). The complete results of the genetic species identification are shown in **Appendix 1**.

The PCR analysis of the 16SrRNA for the all specimens showed several different restricted fragment patterns (haplotype). The restriction enzyme *Alu* I showed two different haplotypes which were composed of fragment of mainly 600 and 390 base pairs and 400 and 300 base pairs. The enzymes *Bbr*P I exhibited two different haplotype, which were composed of and unrestricted fragment, and 780 and 540 base pair fragments. The *Bsp*1286 I showed two different haplotype, the first one was similar with haplotype reported by Aoyama *et al* (2000) and the other one was new haplotype.The *Eco*T14 I also showed two different haplotypes which composed of un-restricted fragment, and 700 and 450 base pair long fragment. The *Hha* I and the *Mva* I only showed one haplotype that similar with haplotype reported by Aoyama *et al.* (2000). Thus the 16SrRNA processed by the six restriction enzymes of *Alu* I, *Bbr*P I, *Bsp*1286 I, *Eco*T14 I, *Hha* I and *Mva* I were clearly exhibit unambiguous fragment pattern whose haplotypes were designated alphabetically.

Among the haplotypes for the different species shown in **Appendix 1**, there were two new types of fragment pattern or haplotypes that were never described previously by Aoyama *et al.* (2000) and categories as *Anguilla* spp. The *Anguilla* spp consist of 16 specimens or more than 50 % of the total 30 specimens that genetically identified in the present study. From those haplotypes, the specimen analyzed in the present study could be identified as *A. bicolor pacifica* (A,A,F,A,A,B), *A. marmorata* (A,A,B,A,AB and A,A,F,A,A,B), *A. celebesensis* (B,B,F,A,A,B), *A. interioris* (A,B,F,A,A,B), and *Anguilla* spp (A,A,F,B,A,B and B,B,F,B,A,B).

Species composition

Based on morphology and genetic character, species composition of the tropical anguillid glass eel recruited in the estuary of Dumoga River consist of *A. bicolor pacifica*, *A. marmorata*, *A. celebesensis*, and *A. interioris*. For the ecological study on the abundance and timing of migration in association with tidal cycle, the specimen used on the study was derivate from formalin specimen so that could be analyzed for morphological only. Therefore, the species composition was consisting of *A. marmorata*, *A. bicolor pacifica*, and *Anguilla* spp. The last species may consist of *A. celebesensis*, *A. interioris*, and might be the new haplotype that found in the present study. *Anguilla marmorata* is the most dominat species collected in the present study, and followed by *Anguilla* spp in the second rank, and *A. bicolor pacifica* in the third rank.

Based on yearly species composition **(Appendix 2)**, it was found that *A. marmorata* also dominant from year to year and followed by *Anguilla* spp and *A. bicolor pacifica*, respectively.

However, the biggest catch of *A. marmorata* was observed in 2005, while the biggest catch of *Anguilla* spp was observed in 2006, and *A. bicolor pacifica* was observed in 2004. Based on monthly species composition **(Appendix 3)**, it was found that *A. marmorata* is the most dominant species in each month of investigation, and followed by *Anguilla* spp and *A. bicolor pacifica*, respectively. Interestingly that the biggest catch of *A. marmorata* was fall in August, *Anguilla* spp was fall in June, and *A. bicolor pacifica* was fall in October. Based on hourly species composition **(Appendix 2)** it was found that *A. marmorata* were dominant in every hour of observation along the night. *Anguilla celebesensis* appeared almost through the night but have tendency to migrate inshore after midnight. *Anguilla bicolor pacifica*, only appear in the early night with very small in number of migrant.

Abundance

Based on quantitative sampling field, the abundance of the tropical anguillid glass eels recruited in the estuary of Dumoga River could be described and compared between the years of observation. Hourly fluctuation in abundance **(Appendix 4)** occurred in each sampling hour from 18.00 to 05.00 in 2006, except in 2004 and in 2005 that no glass eels collected at 18.00. However, fluctuation in abundance varied between years of observation. In 2004, number of glass eels fluctuated from hour to hour of sampling. First invasion initiated with relative small number of catch (6 individuals) at 19.00 or just after peak of tide (1.6 m) in the afternoon (18.00), and number of catch gradually increase until reach the first peak of abundance (97 individuals) just before mid night (22.00) when the tide gradually decreased till reach the lowest level (0.5 m) at 24.00. Second invasion in 2004 were observed after mid night (01.00) when number of catch gradually increase following the increasing of tidal level. The second peak of abundance occurred at 03.00 with 354 individual catch and about 1.4 m of tidal level. Highest level of tidal observed at 05.00 but at that time the number of catch reach the lowest catch (4 individuals). In 2005, two peak of hourly abundance also occurred with similar tidal pattern observed during the night of sampling. However, the first peak in early night (21.00) was higher than second peak after mid night (03.00). Total number of catch in the first and second peaks was 770 and 316 individuals, respectively. First peak occurred when the tide level about 1.2 m and the second peak occurred when the tide level about 1.3 m. In 2006, the hourly fluctuation in abundance was similar with the pattern observed in 2004. Two peak of hourly abundance was occurred along the night, but the first peak in early night (23.00) was lower (1165 individuals) than the second peak after midnight (01.00) when number of catch reach up to 2058 individuals. First peak was occurred at 1.2 m of tidal level after gradually decrease from highest level in the afternoon (1.8 m) at 18.00, while second peak was occurred at 0.5 m of tidal level when the tidal just start to increase from the lowest level (0.4 m) at 24.00.

Monthly fluctuation in abundance **(Appendix 5)** was observed during drying season from May to June in 2006. Initiation of monthly recruitment was observed in June 2006 even the sampling was started from May 2006 and the peak of monthly fluctuation was fall in August. Inter-annual variation in number of peak abundance was differ between years observation where the biggest catch was occurred in August 2006 (19802 individuals) after a peak of abundance in August 2005 (13899 individuals) and in August 2004 (3004 individuals). Peak of abundance in 2004 and in 2005 was occurred when the tidal level gradually decreased at 1.14 m and 1.16 m, respectively. In contrast, peak of abundance in 2006 was fall in August when the tidal level reached the second peak (1.22 m) not in the first peak in April (1.24 m). If associated with monthly fluctuation of tidal level during the year of investigation, it was found that period of recruitment in drying season from May to October was initiated with one modus of period of highly tidal level that occurred from January to June in 2004, in 2005, and in 2006. Peak of tidal level was fall in April 2004 (1.24 m), May 2005 (1.23 m), and April 2006 (1.24 m).

Timing of inshore migration

Timing of inshore migration of *A. bicolor pacifica* was differ between years of observation. In 2004, the short-finned eel species of *A. bicolor pacifica* appeared in the mouth of Dumoga River three period of invasion: 19.00-21.00 (3 hour); 23.00-24.00 (2 hours); and 03.00-05.00 (3 hours). However, in 2005 the species only appeared in one period of invasion: 20.00 (1 hour), while in 2006 the species appeared in three period of invasion: 19.00 (1 hour); 24.00 (1 hour); 04.00 (1 hour). Timing of inshore migration of *Anguilla* spp was differs in each year of observation. In 2004 two period of invasion: 20.00-23.00 (4 hours); 24.00-05.00 (6 hours). In 2005, three period of invasion was observed: 19.00 (1 hour); 22.00-03.00 (6 hours); 05.00 (1 hour). In 2006, two period of invasion: 21.00 (1 hour); 23.00-04.00 (6 hours). Timing of inshore

migration of *A. marmorata* was almost similar in each year of investigation. In 2004 and 2005 the species appears in the mouth of Dumoga River from 19.00-05.00 (11 hours), but in 2006 the period of invasion was from 19.00 to 04.00 or about 10 hours. It was found that the range and the level of hourly tidal in 2004 (1.9-0.3 m =1.6 m) almost similar with in 2005 (1.7-0.5 m=1.2 m) and in 2006 (1.9 m-0.3 m=1.6 m).

DISCUSSION

Inshore migration mechanism

Inshore migration mechanism of the tropical anguillid glass eels recruited in the estuary of Dumoga River has been carried out based on species composition, fluctuation in abundance, and timing of inshore migration. About 4 species and sub species of tropical anguillid eels recognized in the present study; e.g. *A. bicolor pacifica*, *A. marmorata*, *A. celebesensis*, and *A. interioris*. The first three species as similar as the species composition of the tropical anguillid eels recruited in the estuary of Poigar River (north of Sulawesi Island, Indonesia) as reported by Arai *et al.* (1999 a,b) and Sugeha *et al.* (2001, 2006, 2008). However, the occurrence of *A. interioris* in the present study is a new record since the species reported to inhabit in the eastern of Indonesian region or around Papua New Guinea (Ege, 1939; Jespersen, 1942; Watanabe, 2000). Some aspect could be considered as the possible reason to explain this fact likes different in sampling time, different in method for species identification between the present study and the most previous studies. Change in distribution pattern that might be regulated by change in oceanographic condition where the species comes from, would be the next reason to explain why the *A. interioris* expanded their distribution until reach the study area. Change in oceanographic condition would be reasonable since the phenomena of global climate change recently reported to affect the existence and the activities of the marine living organisms in the ocean.

Anguilla marmorata is the most dominant species reported in the present study and then followed by *Anguilla* spp (*A. celebesensis*, *A. interioris*, the new haplotypes), and *A. bicolor pacifica*. The dominancy of *A. marmorata* in the estuary of Dumoga River also similar with study in the estuary of Poigar River from 1998 to 2002 as reported by Sugeha *et al* (2001) even Arai *et al* (1999a,b) reported the dominancy of *A. celebesensis* in the Poigar River in 1997. The exchange on the species dominancy was possible influenced by the EL NINO phenomenon that happened in 1998 ago. The phenomena of EL NINO probably influence some species of tropical eels to exchange their migratory behavior as a kind of environmental adaptation for survivorship. If this idea is true than it would be possible that tropical eel have to exchange their migratory route and might resulting on the exchange of species dominancy. A time series data on the inshore migration of tropical eel recruited in the Indonesian Waters and that covered at least two cycles of EL NINO event should be conducted in the future in order to validate those ideas.

It was reported before that tropical anguillid glass eels have to recruit to Indonesian Waters almost through out the year (Budimawan, 1997; Arai *et al.*, 1999a, b; Sugeha *et al.*, 2001; Setiawan *et al.*, 2001; Lecomte *et al.*, 2005) except for some species in some area that has to recruit almost through out the year (Sugeha *et al.*, 2003). In the present study, recruitment event of the tropical glass eel observed only during drying season. and not covered recruitment during rainy season. However, compared to the previous report from estuary of Poigar River, it was proven that the period of inshore migration mostly occurred during drying season and the peak of seasonal abundance was fall around June to August in Poigar's studies (Arai *et al.*, 1999 and Sugeha *et al.*, 2001) and was fall in August for Dumoga's study. Peak of abundance in the estuary of Dumoga River is more constant than in the estuary of Poigar River. This is probably has a conjunction with spawning distance and stability of source of recruitment into the stock. Different spawning distance could be resulting on the different in timing of arrival in the estuary, so might be the spawning distance of the glass eels recruited in the estuary of Dumoga River was longer than the spawning distance of the glass eel recruited in the estuary of Poigar River. In contrast, the similarity in spawning distance could be affecting the stability of source of recruitment into the stock. In the other word, the glass eels recruited in the estuary of Dumoga River may be comes from one source or one spawning ground and resulting on the stagnancy of peak of seasonal abundance. But, the glass eels in the recruited in the estuary of Poigar River were comes from different source or different spawning ground, and resulting on the variety in the peak of seasonal abundance from year to year of investigation. Future study on population structure and age at recruitment of the specimen from estuary of Dumoga River were needed to conduct in order to proof all the possibilities.

Temperate glass eels reported to migrate inshore without consider the lunar phase, so they would appear in the many estuary of temperate coastal region in full moon, new moon, or even waxing and waning moons. But for tropical eels, they never migrate to estuary in full moon and mostly appeared in new moon with few migrant in waxing moon and waning moon. The phenomena also found in the tropical glass eel that recruited in the estuary of Dumoga River. During the 12 new moon of investigation at night (18.00-05.00), the tropical glass eels always appeared in the estuary of Dumoga River. Similar with recruitment in the estuary of Poigar River, the recruitment in the estuary of Dumoga River was occurred only in night time. This suggested that tropical anguillid eels were very sensitive for light intensity as well as the high contrast of day and night in the tropic.

Tidal rhythmic was specific in the Indonesian region and resulting on variety of tidal cycle from western, central, and eastern part of Indonesian Coastal Waters (Tomascik *et al.*, 1997). Semi diurnal tidal cycle occurred in the central Indonesian Waters, including in the northern coastal of Sulawesi Island where the estuary of Dumoga River located. The estuary has to face two time flood tide and two time ebb tide during 24 hours of day and night. The tropical eel were used the type of tidal cycle as a "transportation" to enter the estuary especially the tidal cycle that occurred at night. This is a kind of adaptation for getting a successful migration that usually called "selective tidal stream transport" behavior or STST behavior (McCleave and Kleckner, 1982; McCleave and Wippelhauser, 1987). Such kind of behavior also found in the recruitment mechanism of temperate glass eels. However, in the present study, all the species of tropical eels has to recruit in the estuary of Dumoga River without consider the tidal cycle because they enter the estuary weather the condition in ebb or flood tides. Species specific behavior regulated by specific environmental condition could be explained this anomaly. If this idea is true than it would be possible that the population of glass eel that entering the estuary of Dumoga River was different from the population of glass eels that entering estuary of Poigar River.

Inter annual variation in both hourly and monthly fluctuation in abundance suggested that estuary of Dumoga River also influenced by the complex of oceanographic condition in the central of Indonesian Waters as the way for Indonesian throughflow that bring seawater mass of the Pacific Ocean to the Indian Ocean and also affecting by a specific current of Eddy that occurred in the great basin of Sulawesi Sea (Tomascik *et al.*, 1997, Creswell, 2000; Godfrey, 1996; Hatayama *et al.*, 1996). Such condition should be considered as one reason why the inshore migration mechanism of the glass eel into the estuary of Dumoga River was varied not only in hourly and monthly but also in yearly. Therefore, inshore migration tropical anguillid glass eels always be dynamic from time to time following the complex oceanographic condition in the region, and not like the temperate eel that have tendency to perform one pattern of inshore migration from time to time because relative stagnant in environmental condition.

Spawning Ecology

From the result showed in the present study it was appear a new perspective that the tropical anguillid glass eel recruited in the estuary of Dumoga River was come from specific source or specific population. It is also means that the glass eel entering Dumoga River was come from specific spawning ground. Arai *et al.* (1999) suggested that the glass eels recruited in the estuary of Poigar River may come from Pacific Ocean (*A. bicolor pacifica* and *A. marmorata*), and Sulawesi Sea (*A. celebesensis*). How about the glass eel recruited in the estuary of Dumoga River? Is this the same? First we should look for the location were both study has been conducted. Estuary of Dumoga River and Poigar River were located in the northern coast of Sulawesi Island and directly facing the great basin of Sulawesi Sea. If the spawning ground of the tropical eels recruited in both estuaries come from Sulawesi Sea it would be reasonable explain why they have similar stage (glass eel stage VA and VB) when collected, which means they just finished metamorphosis and just enter the brackish water area. But why the species composition was different between estuaries? Actually estuary of Poigar River is closer to Pacific Ocean compared with estuary of Dumoga River. It means the probability of *A. interioris* to enter the estuary of Poigar River is higher than to enter the estuary of Dumoga River, if we asumpted that the species was come from the waters of Papua New Guinea. But, no *A. interioris* reported to enter the estuary of Poigar River. There was two important cues to explain this fact, first that species identification method to identify the specimen from estuary of Poigar River was not appropriate, so *A. interioris* might be included in the specimen collected but could not be recognized. Second that population of *A. interioris* recruited in the estuary of Dumoga River was separated from the population of the same species from Papua New Guinea. It means they may not be spawn in the Pacific Ocean that closer to Papua New Guinea. It is possible that *A.*

interioris and the others species of tropical eel that recruited in the estuary of Dumoga River has to come from Sulawesi Sea, or some where around central of Indonesian Waters, and not far from their recruitment area. This hypothesis supported by the recent study on the distribution of leptocephalus in the central Indonesian seas (Aoyama *et al*, 2003). The authors suggested that tropical anguillid eels have a short distance spawning migration, so they would be spawn not far from their growth habitat. Future study on the species validation and population structure of the tropical anguillid eels based on genetic approach strongly required to answer the questions as well as to do sampling of ell larvae (leptocephalus) in the Sulawesi Sea in order to discover the spawning ground of the tropical anguillid eel recruited in the north of Sulawesi Island.

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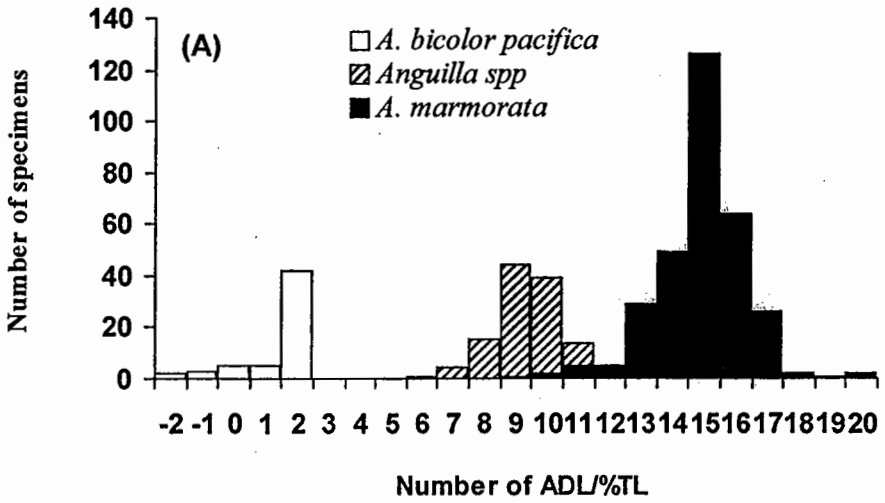
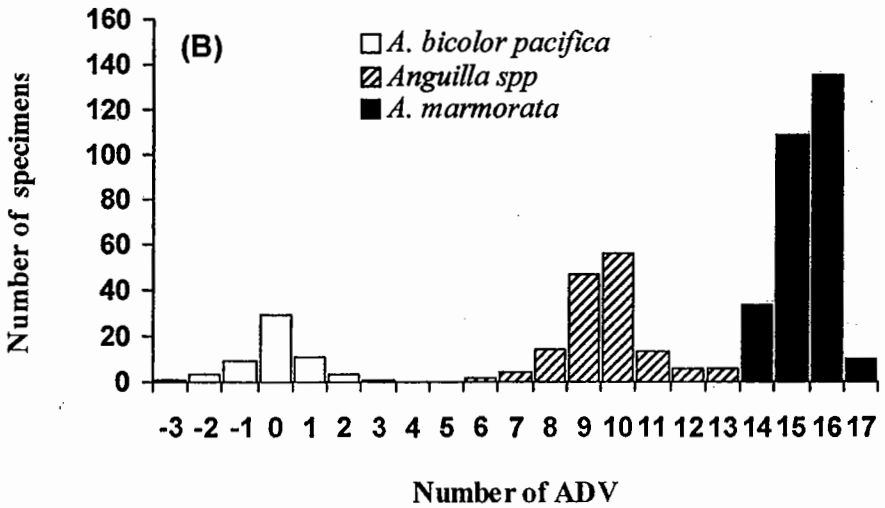
Appendix 1.

Table 01. Genetic character of the tropical anguillid glass eels species, collected in the estuary of Dumoga River (North Sulawesi, Indonesia), that revealed by PCR-RFLP analysis of the 16S ribosomal RNA gene of the DNA mitochondrial

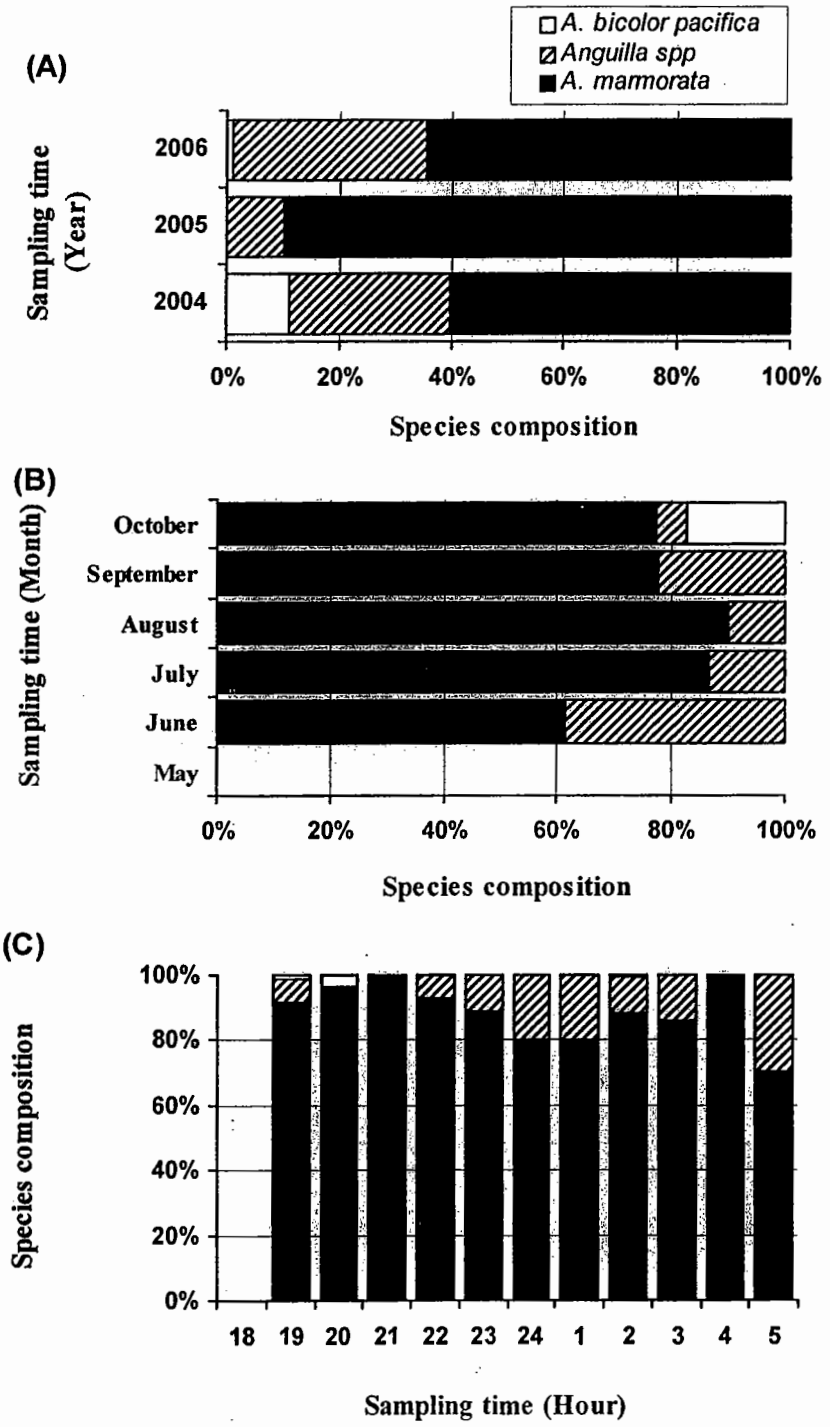
No.	Code	Location	Restriction Enzymes						PCR-RFLP	Character ADL in % TL
			Alu I	*Bbr* P I	*Bsp* 1286 I	*Eco* T14I	*Hha* I	*Mva* I		
1	DM39	Estuary of Dumoga River	A	A	F	A	A	B	*A. bicolor pacifica*	Shortfinned eel
2	DM4	Estuary of Dumoga River	B	B	F	A	A	B	*A. celebesensis*	Longfinned eel
3	DM6	Estuary of Dumoga River	A	B	F	A	A	B	*A. interioris*	Longfinned eel
4	DM75	Estuary of Dumoga River	A	A	B	A	A	B	*A. marmorata*	Longfinned eel
5	DM7	Estuary of Dumoga River	A	A	F	B	A	B	*Anguilla* spp	Longfinned eel
6	DM31	Estuary of Dumoga River	B	A	F	B	A	B	*Anguilla* spp	Longfinned eel

Appendix 2. Frequency distribution of (A) ano-dorsal length in percentage of total length (ADL/%TL) and (B) anodorsal vertebrae (ADV) of the tropical anguillid glass eels recruited in the estuary of Dumoga River (North Sulawesi, Indonesia).

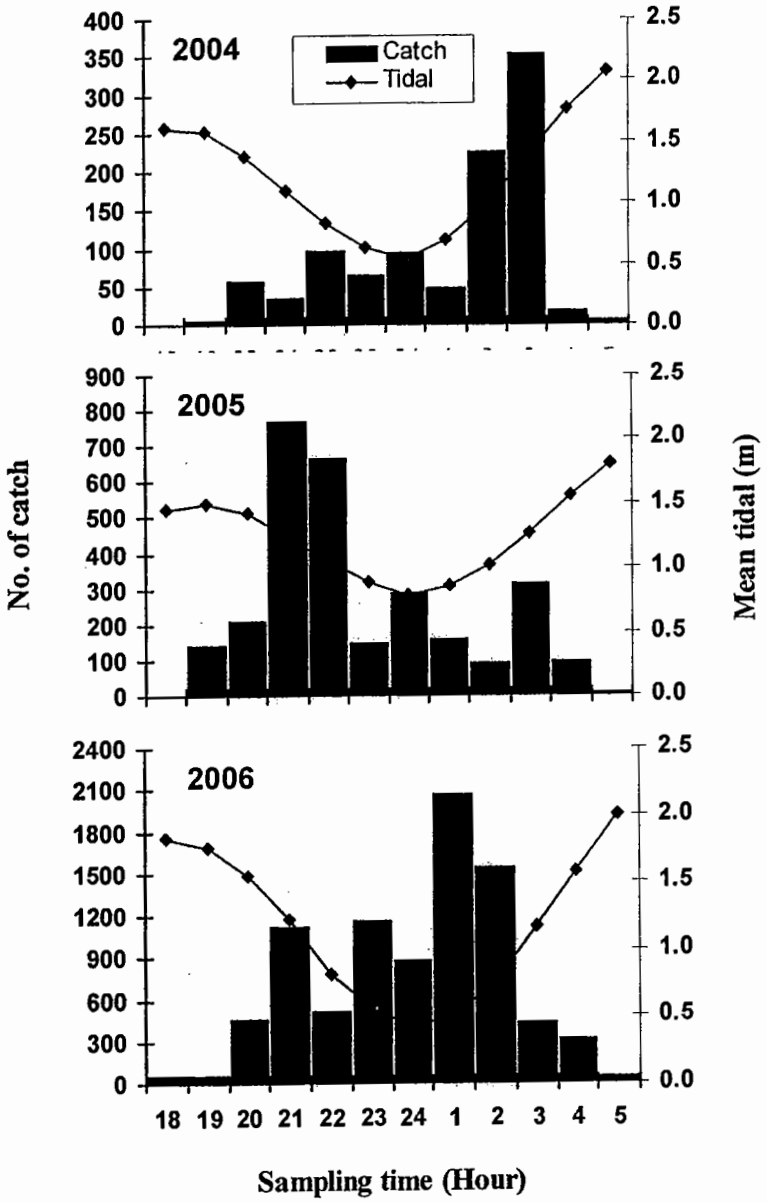




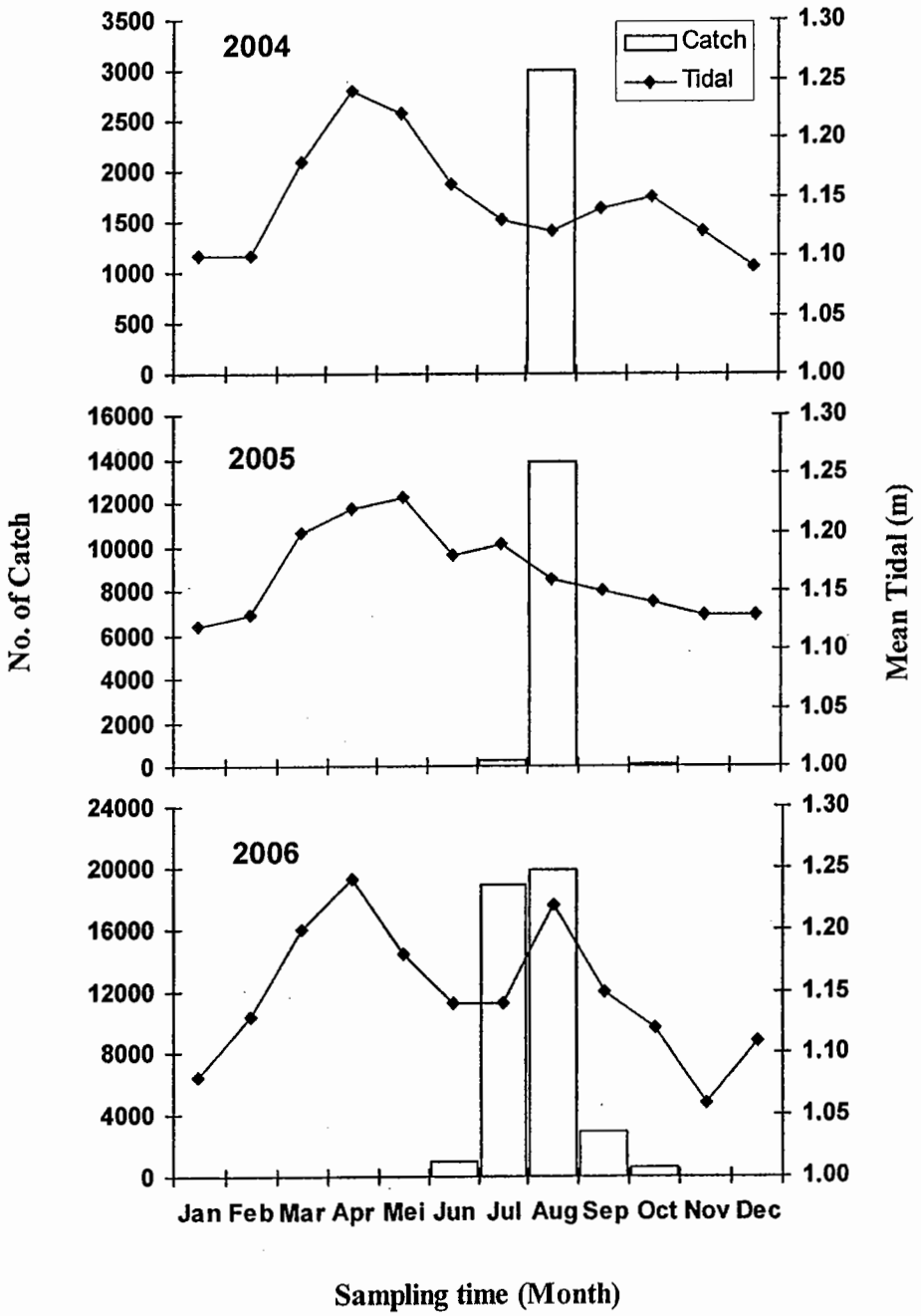
Appendix 3. Species composition in yearly (A), monthly (B), and hourly (C), of the tropical anguillid glass eels recruited in the estuary of Dumoga River (north of Sulawesi Island , Indonesia)



Appendix 4. Inter annual variation on the peak of hourly fluctuation in abundance of the tropical anguillid glass eels recruited in the estuary of Dumoga River (north of Sulawesi Island, Indonesia), and its association with semi diurnal tidal cycle in the region in 2004, 2005, and 2006



Appendix 5. Peak and monthly fluctuation in abundance of the tropical anguillid glass eels recruited in the estuary of Dumoga River (north of Sulawesi Island, Indonesia), and its association with monthly fluctuation of tidal cycle in the region in 2004, 2005, and 2006



Appendix 6. Timing of inshore migration of each species of the tropical anguillid glass eels recruited in the estuary of Dumoga River (north of Sulawesi Island, Indonesia), and its association with tidal cycle in August of 2004, 2005, and 2006