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NOTE

Species composition and inshore migration of the tropical eels *Anguilla* spp. recruiting to the estuary of the Poigar River, Sulawesi Island

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ABSTRACT: In order to determine the species composition and inshore migration of the tropical eels *Anguilla* spp. migrating to an Indonesian river, we collected 21 633 glass eels at the mouth of the Poigar River, north Sulawesi Island, throughout 1997, and subjected these samples to both morphological examination and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Three species, *Anguilla celebesensis*, *A. marmorata* and *A. bicolor pacifica*, were found throughout the season in fluctuating abundance, while the previously recorded *A. borneensis* was not recognized in the study. *A. celebesensis* constituted a dominant 88.3% of all glass eels migrating to the Poigar River in 1997, and was seen throughout most of the year, with a peak in May and June. *A. marmorata* (11.3%) was also seen throughout most of the year, with peak occurrence in June. *A. bicolor pacifica* (0.4%) occurred only in January, March, April, October and December.

KEY WORDS: Eel · Glass eel · Tropical *Anguilla* spp. · Mitochondrial DNA · Species identification · Species composition · Inshore migration

Considerable knowledge has been accumulated on the inshore migration and early life history of juveniles of numerous temperate anguillid species, such as *Anguilla japonica* (Matsui 1952, 1972, Tzeng 1985, Tsukamoto 1992, Cheng & Tzeng 1996, Arai et al. 1997), *A. anguilla* (Deelder 1958, Tesch 1971, Gandolfi et al. 1984, Lecomte-Finiger 1992), *A. australis* and *A. dieffenbachi* (Jellyman 1977, 1979, Sloane 1984) and *A. rostrata* (Sorensen 1986, Tongiorgi et al. 1986, Martin 1995, Wang & Tzeng 1998). These reports have revealed the species composition and migration timing of juveniles, as well as environmental factors affecting the onset of juvenile migration, such as temperature, salinity, tidal cycles and moon phase. In addition, spawning area, birth date, larval age and growth, timing of metamorphosis and age at recruitment to the coastal waters have also been revealed.

However, knowledge of the inshore migration of tropical species is rudimentary compared to that on temperate anguillid species (Tabeta et al. 1976a, Budimawan 1997, Arai et al. 1999a,b). Furthermore, in tropical areas some anguillid species inhabit sympatrically, and thus their exact species identification is difficult because intra-species variation of the morphological key characters overlap considerably between species (Tabeta 1976b, Aoyama 1998). For this reason, species identification and composition have not been well established in the tropical species.

We collected glass eel samples over the course of 1 yr from the mouth of the Poigar River, north Sulawesi Island, Indonesia, where Ege (1939) once described *Anguilla ancestralis* as the ancestral eel for *Anguilla* spp., and Castle & Williamson (1974) established the synonym of *A. celebesensis* thereafter. In order to establish a basic foundation for ecological study in tropical eels, we examined the species composition of tropical anguillid eel juveniles by species identification based on their morphological characteristics and mitochondrial DNA (mtDNA).

Materials and methods. Glass eels were collected quantitatively in triangular scoop nets (mouth 0.3 m^2, mesh 1 mm) over a 10 m transect of beach at the mouth of the Poigar River, north Sulawesi Island, Indonesia, at 2 h intervals for a full day, at the new moon of each month of 1997 (Fig. 1). The glass eels sampled were fixed in 10% formalin immediately after collection, and the total number of specimens was counted. A

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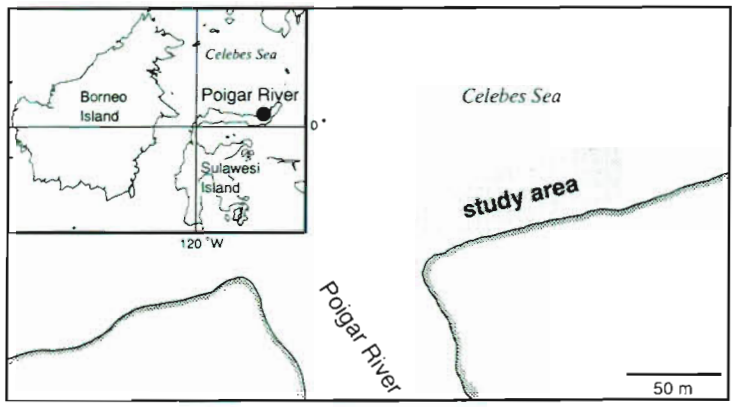

Fig. 1. Map showing the mouth of the Poigar River and the study area from which glass eels were collected

total of 21 633 specimens was collected during this year-long survey. Samples for genetic analysis (2 to 116 specimens each month) were also collected at night (20:00 to 02:00 h) just after each quantitative sampling, and were preserved in 99% ethanol immediately after collection.

In those months when only a few specimens (less than 30) were collected for mtDNA analysis (February, March, September and December), all specimens were examined (2 to 10 specimens), while during the other months (January, April to August, October and November), 30 specimens were randomly selected from each sample bottle, for a total of 272 glass eels examined genetically. The total length (TL) and predorsal, ano-dorsal and preanal lengths of the specimens were measured to the nearest 0.1 mm, and the pigmentation stage was determined after Bertin (1956). Based on the ano-dorsal length as a percentage of TL (Ege 1939, Castle & Williamson 1974, Tabeta et al. 1976b), the glass eels were first classified as either long-finned (6.0 to 18.9%; 253 specimens) or short-finned eels (–6.0 to 3.9%; 19 specimens). According to Ege (1939) and Castle & Williamson (1974), 1 short-finned eel, *Anguilla bicolor pacifica*, and 3 long-finned eels, *A. celebesensis*, *A. marmorata* and *A. borneensis*, are known to be distributed in the vicinity of north Sulawesi Island. We initially excluded *A. bicolor pacifica* by morphological examination. However, *A. celebesensis* and *A. borneensis* have been reported as being difficult to distinguish by their vertebral characteristics alone (Tabeta 1976b). We therefore carried out polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, which has recently been established as a convenient tool for identifying members of the genus *Anguilla* (Aoyama 1998).

For long-finned eels (253 specimens), total genomic DNA was extracted once using phenol/chloroform/isoamyl alcohol (25:24:1 vol/vol) and twice using diethyl ether, then concentrated by ethanol precipitation. A portion of mitochondrial 16S ribosomal RNA was amplified via polymerase chain reaction (PCR) using oligonucleotide primers, L1854 (Aoyama 1998) and H3058 (Miya & Nishida 1996). Amplification parameters were 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s. PCR products were processed by 2 restriction enzymes, Bsp1286I (Takara Shuzo Co., Ltd) and BbrPI (Toyobo Co., Ltd). Restriction procedures were carried out in a solution containing 5 µl PCR product, 10 units restriction enzyme and 2 µl restriction enzyme buffer (supplied by manufactures) for a final volume of 20 µl, which was incubated at 37°C overnight. Restricted fragments were detected by electrophoresis on 1% agarose gel, and by ethidium bromide staining. The fragment patterns obtained from all long-finned specimens were compared with previously established species-specific genotypes (Aoyama 1998).

We obtained the species composition in % of specimens morphologically and genetically analyzed (272) and estimated the species composition of the main sample collected quantitatively every month.

Results. Glass eels were present in the Poigar Estuary throughout the course of the study (Fig. 2). The peak season of inshore migration was from May (4832 specimens) to June (13 497 specimens). A few specimens were collected in February (29 specimens), March (22 specimens), September (19 specimens) and December (2 specimens) (Fig. 2). All specimens showed undeveloped pigmentation with only a few melanophores in the caudal region, or at the skull, caudal and rostral regions of the body (VA or VB).

The PCR-RFLP analysis carried out with Bsp1286I exhibited a single genotype (fragments of 920 and 370

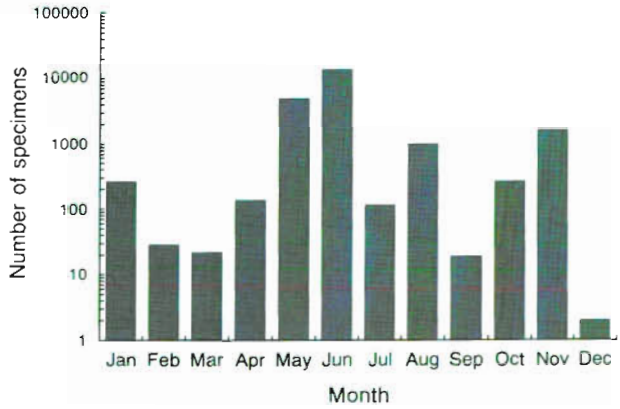

Fig. 2. Monthly catch of *Anguilla* spp. glass eels at the mouth of the Poigar River throughout 1997

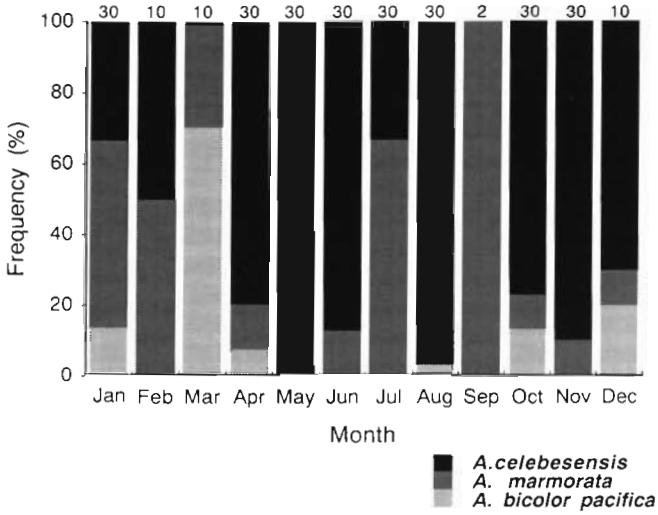

Fig. 3. Frequency distribution of *Anguilla celebesensis*, *A. marmorata* and *A. bicolor pacifica*, as revealed by both morphological characteristics and mtDNA analysis of 272 glass eels from monthly samples collected at the mouth of the Poigar River in 1997. Numbers at the top of each column indicate the number of specimens examined

base pairs in length were observed) that corresponded to an *Anguilla celebesensis*/*A. marmorata* species group-specific fragment pattern, while the genotype corresponding to the fragment pattern of *A. borneensis* (650, 370 and 300 base pairs long) was not found among the specimens examined here. The analysis by BbrPI clearly showed 2 genotypes (one with no restriction site and the other being 780 and 540 base pairs long). The former fragment pattern was reported as *A. marmorata* specific, and the latter as *A. celebesensis* (Aoyama 1998), and thus the examined specimens could be precisely distinguished as follows: 190 *A. celebesensis*, 63 *A. marmorata*, and 19 *A. bicolor pacifica*.

Seasonal changes in species composition of the glass eels revealed by mtDNA analysis are shown in Fig. 3. *Anguilla celebesensis* was the most dominant species (69.9%), occurring almost throughout the year, except in March and September. *A. marmorata* was the second most dominant (23.1%), and was seen throughout the year. A few *A. bicolor pacifica* (7.0%) occurred in January, March, April, October and December.

Based on glass eel abundance shown in Fig. 2 and species composition shown in Fig. 3, the number of inshore migrants was estimated for each species in each month (Fig. 4). *Anguilla celebesensis* represented a dominant 88.3% of all eel species seen, with a peak number of more than 10000 individuals as catch per unit effort (CPUE) in June. The number of *A. marmorata* (11.3%) also peaked in June, with more than 1000 individuals as CPUE. In the case of *A. bicolor pacifica*, although the total number collected over the whole migration period did not exceed 100 individuals, peaks were observed in January and October.

Discussion. The species composition and seasonal occurrence of anguillid glass eels in the Poigar River, Sulawesi Island, were precisely determined in this study. Ege (1939) recognized 4 anguillid species in the Poigar River, i.e. *Anguilla ancestralis*, *A. celebesensis*, *A. marmorata* and *A. bicolor pacifica*. However, after

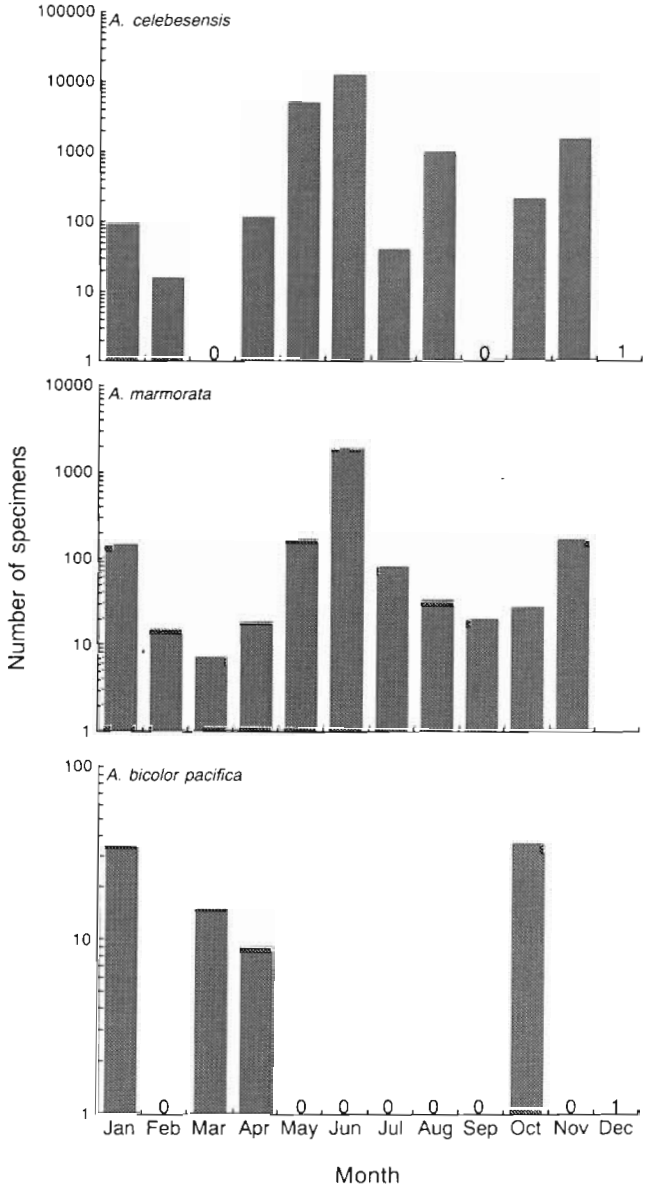

Fig. 4. Seasonal variations in the inshore migration of *Anguilla celebesensis*, *A. marmorata* and *A. bicolor pacifica*, as estimated by quantitative monthly collection of glass eels at the mouth of the Poigar River in 1997

re-examination of Ege's sample, Castle & Williamson (1974) proved that *A. ancestralis* should be regarded as *A. celebesensis*, because there was no valid means of distinguishing between the two. They also recognized *A. borneensis* based on 2 adults and 4 elver specimens, and thus reported a total of 4 *Anguilla* spp. in the Poigar River, i.e. *A. celebesensis, A. marmorata, A. bicolor pacifica* and *A. borneensis*. Tabeta et al. (1976b) suggested that it was difficult to distinguish between *A. borneensis* and *A. celebesensis* based on only the vertebral characteristics in the juvenile stage. We found no *A. borneensis* in the Poigar River, based on mtDNA analysis of our monthly samples for the year. Therefore, continuous investigation on species composition in the Poigar River will be needed to examine more specimens in different years, together with a re-examination of the *A. borneensis* specimens described by Castle & Williamson (1974).

In this study, glass eels were found to occur throughout the year. Tabeta et al. (1976a) also observed a similar occurrence of tropical anguillid elvers in the Cagayan River, Philippines. In the temperate species, however, inshore migration of juveniles occurs over a limited period, i.e. from winter to spring for *Anguilla japonica* (Matsui 1972), *A. australis* and *A. dieffenbachi* (Jellyman 1979) and *A. rostrata* (Martin 1995). The differences in inshore migration periods between tropical species and temperate species might be due to differences in their spawning periods, their migration route and ocean current systems, and early life history parameters such as timing of metamorphosis and age at recruitment. In temperate eels, it has been revealed that *A. japonica* spawns from April to November (Tsukamoto 1990), and migrates via steady oceanic currents (Tsukamoto 1992), taking about 5 to 6 mo to travel from the spawning area to estuarine habitats (Cheng & Tzeng 1996, Arai et al. 1997). In tropical species, it has been suggested based on otolith microstructure analysis that *A. celebesensis* and *A. marmorata* spawn throughout the year (Arai unpubl.). Otolith Sr:Ca ratio analyses using an X-ray electron microprobe have also suggested that metamorphosis likely begins 3 to 4 mo after hatching, and recruitment to the estuary at 4 to 5 mo of age (Arai et al. 1999a,b, Arai unpubl.). Furthermore, these 2 otolith life history parameters were found to be almost constant throughout the year (Arai unpubl.). Therefore, the inshore migration period in tropical species would extend throughout the year due to year-round spawning and stable larval transport, while that in the temperate species might occur over only a limited period due to a limited spawning season. However, leptocephalus collection and oceanic observation will be needed to obtain direct information on the spawning season and larval transport mechanisms in tropical anguillid eels.

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