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Early life history of the Luzon mottled eel *Anguilla luzonensis* recruited to the Cagayan River, Luzon Island, the Philippines

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Abstract— The recently discovered freshwater eel species of the Luzon mottled eel, *Anguilla luzonensis*, has only been found in very limited area at the tip of the Northern Philippines. Here we investigated the early life history of *A. luzonensis* for the first time to help understand its larval dispersal pattern in the western North Pacific, and compared it to *A. celebesensis* and data from previous studies. Genetically-identified glass eels of *A. luzonensis* collected from the northern Luzon Island in January and February 2009 (n=3 and 8, respectively) were used to study the age of recruitment of this species using otolith microstructure. The leptocephalus stage was 129.6±10.7 days (mean±s. d.; range, 113 to 146), followed by 18.4±3.7 days (13 to 25) at the metamorphosing and 17.6±5.1 days (7 to 23) at the glass eel stages. These specimens were found to be hatched between August and September 2008. A previous study on a small number of the leptocephali of *A. luzonensis* collected offshore reported that the hatching dates were between February and March, and thus the spawning season of *A. luzonensis* is suggested to span at least a half of a year.

Key words: *Anguilla luzonensis*, Cagayan River, Luzon Island, Luzon mottled eel, the Philippines, glass eel, otolith microstructure

Introduction

Freshwater eels of the genus *Anguilla* have catadromous life history during which they migrate between their offshore spawning areas and their growth habitats in rivers, estuaries and coastal areas (Tesch 2003). Their transparent leaf-like larvae, so-called leptocephali, have unique morphology with large, laterally compressed bodies that are filled with gelatinous materials, and they have a long larval duration compared to other fish larvae (Pfeiler 1999, Miller 2009). These characteristics increase their buoyancy to remain them ocean surface layer and facilitate their long-distance passive transport by ocean currents (Tsukamoto et al. 2009). After being transported back near their recruitment areas, anguillid leptocephali undergo metamorphosis into glass eels after they reach a particular maximum larval size (greater than about 50 mm in total length, depending on the species; see Aoyama 2009) and then migrate across the continental shelf to the growth habitats.

It has been clearly shown that the location of spawning areas in relation to the oceanic currents transporting the leptocephali, which have specific larval duration periods, are critical factors to determine the geographic distribution of each species during their juvenile growth stage in freshwater and estuaries (Tsukamoto 1990, Cheng and Tzeng 1996, Wang and Tzeng 2000, Marui et al. 2001, Arai et al. 2001, Shiao et al. 2002). In cases of *A. japonica* and *A. marmorata* which share a sympatric spawning area in the North Equatorial Current (NEC) of the North Pacific Ocean (Miller et al. 2002, Kuroki et al. 2009), the former species grows slower and metamorphoses older than the latter during their larval migration (Kuroki et al. 2006, Leander et al. 2013). These differences in early life history traits may result in the different species ranges of *A. japonica* that is mostly only present in temperate areas in East Asia and of the tropical species *A. marmorata* mostly being present along the southwestern margin of the western North Pacific Ocean (Leander et al. 2013). This indicates that the oceanic larval period is one of the key ecological traits of anguillid eels.

The genus *Anguilla* had long been considered to consist of a total of 15 species in the world, three of which are further divided into two sub-species (Ege 1939, Castle and

Williamson 1974, Watanabe 2003). Recently, the Luzon mottled eel *A. luzonensis* has been described as the 16th species of the genus from the Cagayan River system, northern part of the Luzon Island of the Philippines (Watanabe et al. 2009). The previous studies on the anguillid glass eels recruiting to the Cagayan River reported the occurrence of four species, *A. marmorata*, *A. bicolor pacifica*, *A. japonica* and *A. celebesensis* based on the morphological characters (Tabeta et al. 1976, Arai et al. 2003). However, Watanabe et al. (2009) showed that there was a cryptic species of *A. luzonensis* present on northern Luzon Island that was morphologically indistinguishable with the Indonesian mottled eel *A. celebesensis* and this raised a question about the species identification of *A. celebesensis* glass eels carried out in the previous studies.

Kuroki et al. (2012) reported the first collection of five leptocephali of *A. luzonensis* ranging from 29.2 to 51.2 mm of the TL in the NEC region of the western North Pacific along and estimated their ages to be 103 to 138 days using otolith microstructure analysis. In addition to this information, they re-evaluated the age at recruitment of the glass eels identified as *A. celebesensis* from the northern Luzon in the previous studies (Arai et al. 2003) because these samples appeared to have been *A. luzonensis*. Based on that available information it was concluded that this species probably spawns offshore in the NEC, possibly between about February to May (Kuroki et al. 2012). However, so few larval specimens collected offshore do not provide any clear information about the early life history of *A. luzonensis*, so a better understanding is needed, especially since the species has already been harvested for aquaculture purposes (T. Yoshinaga, unpublished data). Accordingly, the aim of this study is to clearly determine the age at recruitment of genetically-identified *A. luzonensis* glass eels that recruited to the type locality of the species, the Cagayan River, to contribute to and increased understanding their early life histories. The genetically-identified glass eels of *A. celebesensis* collected in this study were also examined to evaluate the previous studies.

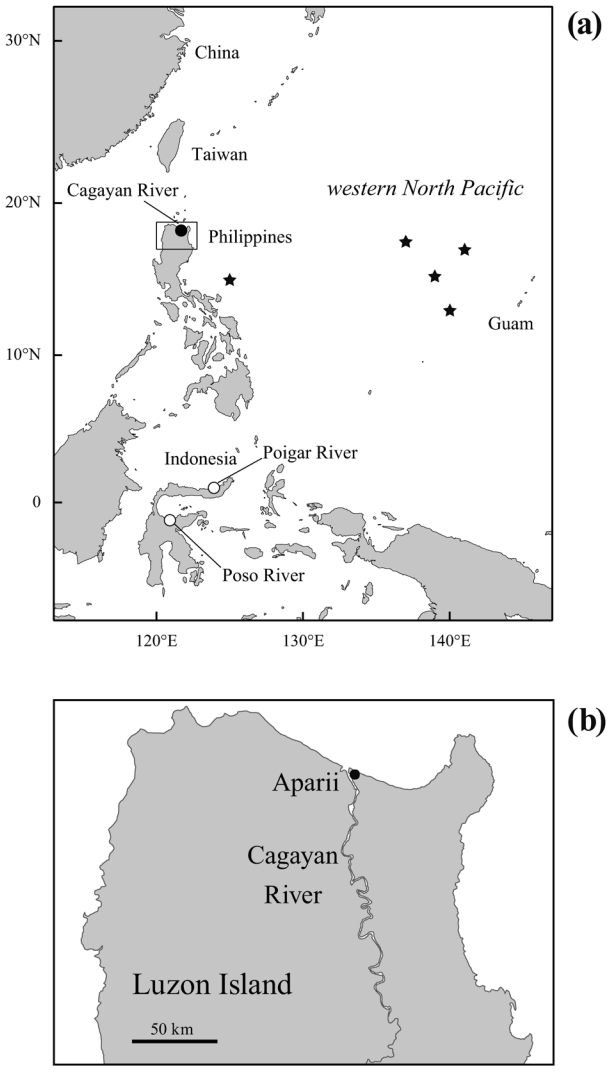

Fig. 1. Map showing the oceanic distribution of the *Anguilla luzonensis* (a) and location of the study site (b). The larvae of *A. luzonensis* were collected between 2002 and 2009 (Kuroki et al. 2012), at the locations shown by the stars in the panel (a). The black circles indicate study site of present study (a, b), and white circles show locations where *Anguilla celebesensis* glass eels have been used in previous studies (a).

Materials and methods

Specimens

Anguillid glass eels were collected by local fishermen using an approximately 5-m long set net used in the estuary of the Cagayan River in Aparri, on northern Luzon Island of the Philippines on 26 January and 19 February in 2009 (Fig. 1). The specimens were immediately preserved in 99.5% (v/v) ethanol and transferred to the laboratory in Japan. Total length (TL) of the specimens were measured to the nearest 0.1 mm and pigmentation stages were categorized following Fukuda et al. (2013). The category defined by Fukuda et al. (2013) was specifically modified version for *A. japonica* from the original method for the glass eels of *A. anguilla* (Strubberg 1913, Bertin 1956, Elie et al. 1982, Tesch 2003), but these methods may be used for the tropical anguillid species as have been employed (Arai et al. 1999, 2001, 2003, Marui et al. 2001, Leander et al. 2013).

To identify *A. luzonensis* and *A. celebesensis* from the glass eel specimens that consisted of five species, they were first morphologically classified into two groups of the long-fin (*A. celebesensis*, *A. japonica*, *A. luzonensis*, *A. marmorata*) and short-fin (*A. bicolor*) species (Yoshinaga et al. 2014). The long-fin species with the pigmentation on the tail tip, a typical character for tropical anguillid glass eel species, were then subjected to the genetic species identification using their mitochondrial DNA 16S ribosomal RNA gene sequences according to Yoshinaga et al. (2011). The geneti-

Table 1. Age and estimated hatch date of *Anguilla luzonensis* and *A. celebesensis* glass eels collected at the Cagayan River estuary on Luzon Island of the Philippines in January and February 2009.

Species	TL (mm)	Early life stages (days)			Age at the recruitment (days)	Collection date	Hatch date
		Leptocephalus	Metamorphosing	Glass eel			
A. luzonensis	47.3	146	22	16	184	19-Feb-09	19-Aug-08
	47.9	142	21	19	182	19-Feb-09	21-Aug-08
	49.0	130	25	16	171	26-Jan-09	8-Aug-08
	50.0	141	13	16	170	19-Feb-09	2-Sep-08
	50.1	130	15	22	167	19-Feb-09	5-Sep-08
	43.1	126	15	23	164	19-Feb-09	8-Sep-08
	49.0	120	22	22	164	19-Feb-09	8-Sep-08
	47.8	131	18	11	160	26-Jan-09	4-Sep-08
	48.4	132	16	7	155	26-Jan-09	24-Aug-08
	49.5	113	19	22	154	19-Feb-09	18-Sep-08
	46.1	115	16	20	151	19-Feb-09	21-Sep-08
A. celebesensis	45.4	87	10	24	121	26-Jan-09	27-Sep-08
	46.9	100	28	18	146	26-Jan-09	2-Sep-08

cally-identified *A. luzonensis* collected in January (*n*=3), February (*n*=8) and *A. celebesensis* collected in January (*n*=2) were then used for the following age determination.

Otolith analyses

Sagittal otoliths were extracted from the glass eels of *A. luzonensis* (*n*=11) and *A. celebesensis* (*n*=2) under binocular microscope (SMZ-1500, Nikon). The otoliths were embedded in an epoxy resin (Epofix, Struers), and were ground to expose the core with a diamond cup-wheel (Discoplan-TS, Struers), and then polished with colloidal silica suspension (OP-S suspension, Struers) on a polishing wheel (Labo-Pol-35 equipped with LaboForce-Mi, Struers). The otoliths were etched with 50 mM hydrochloric acid for 30 to 60 seconds, and coated with platinum-palladium (Hitachi, E-1045) to be observed by a scanning electron microscope (SEM, Keyence VE-9800). The SEM photographs were taken at various magnifications of 300 to 2,500 to count the number of daily increment rings. The widths of 10 continuous rings each were also measured to clarify the early life stages according to previous studies (Otake et al. 1994, Arai et al. 1997).

Results

Biological characteristics of *A. luzonensis* and *A. celebesensis* glass eels at Cagayan River estuary

The TL of the glass eels of *A. luzonensis* collected in January and February 2009 at the Cagayan River were 48.0±2.0 mm (mean±s.d.; range, 43.1 to 50.1; *n*=11), and *A. celebesensis* collected in January were 45.4 and 46.9 mm (*n*=2), respectively (Table 1). Pigmentation stages of *A. luzonensis* were varied at V_A (*n*=6), V_{B1} (*n*=3), V_{B2} (*n*=1) and

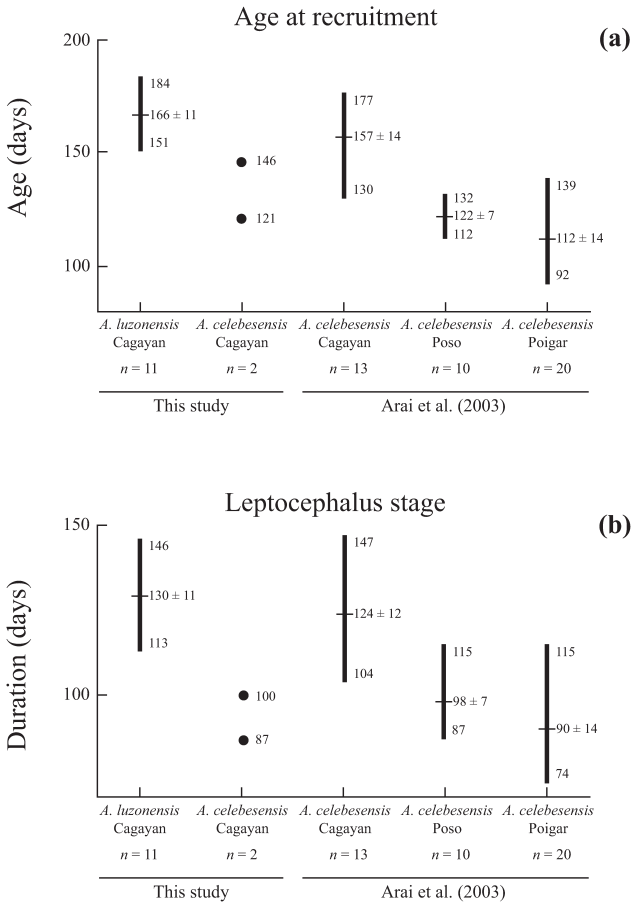

Fig. 2. Ranges and means of age at recruitment of *Anguilla luzonensis* and *A. celebesensis* (a) and the duration of their leptocephalus stage (b). The glass eels were collected from the estuaries of the Cagayan River, Poso River and Poigar River. Data are adapted from Arai et al. (2003) for *A. celebesensis* from Cagayan River in Luzon Island, and the Poso and Poigar rivers on Sulawesi Island.

VI_{A0} (*n*=1), and *A. celebesensis* were V_A (*n*=2) stages; all of which correspond to newly metamorphosed glass eel stages (Fukuda et al. 2013). The TLs of glass eels of *A. celebesensis* were overlapped with those of *A. luzonensis* (Table 1).

Ages and early life histories of *A. luzonensis* and *A. celebesensis* glass eels at Cagayan River estuary

The ages of *A. luzonensis* collected at the river mouth of the Cagayan River in January and February 2009 were 165.6±10.7 days (151 to 184) (Table 1, Fig. 2). Each duration of early oceanic life stages of *A. luzonensis* were as follows: the leptocephalus stage was 129.6±10.7 days (113 to 146), followed by 18.4±3.7 days (13 to 25) at the metamorphosing, and 17.6±5.1 days (7 to 23) days at the glass eel stages (Table 1, Figure 2). The two glass eels of *A. celebesensis* had the ages at the recruitment for 121 and 146 days, respectively (Table 1, Fig. 2). The leptocephalus stage was 87 and 100 days, 10 and 28 days at the metamorphosing, and 24 and 18 days at the glass eel stages.

The ages and durations of the leptocephalus stage of *A. celebesensis* were either shorter than those of *A. luzonensis* (statistical test was not available due to a small number of *A. celebesensis* examined). The hatching dates of *A. luzonensis* were back-calculated using their collection dates and ages at the recruitment (Table 1). The earliest and latest were on 8 August and 21 September 2008, respectively, and the 11 specimens were found to be hatched in August (*n*=4) and September (*n*=7) in 2008, respectively. The hatching dates of *A. celebesensis* were 2 and 27 September in 2008 as well (Table 1).

Discussion

This study succeeded for the first time to directly examine some aspects of the early life history of *A. luzonensis* glass eels that were precisely identified using their mitochondrial DNA 16S ribosomal RNA gene sequences. Although the number of specimens examined in this study is not a large enough to comprehensively understand their early life history, the data from otolith analysis with their biological characters provided new insights that can help to guide future research, because our data showed some significant inconsistencies with the previous reports (Arai et al. 2003, Kuroki et al. 2012).

The TLs of glass eels of *A. luzonensis* examined here ranging from 43.1 to 50.1 mm, supported the possible maximum larval size of this species estimated to be about 51 mm by Kuroki et al. (2012) as the anguillid larvae were known to shrink about 10% in the TL during their metamorphosis (Tsukamoto and Umezawa 1994, Tesch 2003, Kuroki et al. 2010). The duration of leptocephalus stage of *A. luzonensis* obtained in the present study (113 to 146 days) was almost similar with the ages of three large leptocephali collected in the NEC (103 to 138 days; Kuroki et al. 2012). The hatching dates of *A. luzonensis* obtained in the present study, however, were from August to September, considerably different from February to May that was reported by Kuroki et al. (2012). This suggested that the spawning of this species likely occurs at least over a half year. These facts supported the offshore spawning of this species as has been suggested by Kuroki et al. (2012), but the spawning season was clearly showed much longer than the previous estimation of February to May.

Two previous reports have been described the apparent occurrence of *A. celebesensis* glass eels from the Cagayan River estuary (Tabeta et al. 1976, Arai et al. 2003). The morphological identifications in these studies, however, are now questionable after the discovery that *A. luzonensis* is present in this area and is morphologically indistinguishable from *A. celebesensis*. Kuroki et al. (2012) concluded that *A. celebesensis* collected from the Cagayan River in the previous studies were actually *A. luzonensis*, because the northern Philippines is farther north than the known distribution range of *A. celebesensis* (Ege 1939), and no *A. celebesensis* have been identified using the DNA analysis from this area. However the latest study confirmed the occurrence of at least a few glass eels of *A. celebesensis* from the Cagayan River, the northern Philippines (Yoshinaga et al. 2014), and this study succeeded to compare their ages with precisely identified *A. luzonensis*.

Arai et al. (2001) examined otoliths of a total of 189 *A. celebesensis* glass eels collected in the Poigar River, Sulawesi, Indonesia (Fig. 1), that were genetically identified in Arai et al. (1999) and showed their ages at recruitment (109±10.9 days, 89 to 139 days) and the duration of leptocephalus stage (88±9.8 days, 70 to 117 days). Based on these data, the authors characterized *A. celebesensis* to have oceanic larval duration considerably shorter than the other anguillid species ever reported (Arai et al. 2001). A similar result has been obtained from the study on the genetically identified leptocephali of this species by Kuroki et al. (2006) because their larval growth rate was estimated to be faster and their larval migration period was shorter (maximum 110 days, *n*=40; Kuroki et al. 2006). Our data on the two *A. celebesensis* glass eels were consistent with these previous studies (Table 1).

Arai et al. (2003) later compared the early life history of *A. celebesensis* using the glass eels collected at Poso and Poigar Rivers of Sulawesi Island and from the Cagayan River in northern Luzon Island (Fig. 1), but only morphologically identified the specimens. The study showed that the ages at recruitment and durations of the leptocephalus stage of the specimens from Sulawesi Island (Poso River, 112 to 132 and 87 to 115 days; Poigar River, 92 to 139 and 74 to 115 days) were similar to those precisely identified as *A. ce-*

lebesensis (89 to 139, 70 to 117; Arai et al. 2001). However, the specimens collected at the Cagayan River estuary (130 to 177, 104 to 147) showed much longer larval durations than those from Sulawesi Island (Arai et al. 2003). The present study showed the age at recruitment and duration of leptocephalus stage of genetically-identified *A. luzonensis* to be 151 to 184 days and 113 to 146 days, respectively, those were almost longer than those in the specimens collected at Cagayan River estuary in Arai et al. (2003), but the ranges between the previous and present studies were largely overlapped. It is interesting that a part of the specimens collected from the Cagayan River in Arai et al. (2003) showed much shorter larval durations that were entirely out of the range of *A. luzonensis* revealed in the present study. This suggested that *A. celebesensis* from the Cagayan River examined in Arai et al. (2003) were all, or almost all, *A. luzonensis*.

The results of this study can stimulate future research to reveal the early life history of *A. luzonensis* in detail larger sample sizes and different sampling seasons. Studies on the other four species that recruit to the Cagayan River estuary are also needed to understand the specific characteristics of the early life history of this newly described anguillid species.

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