Yoshinaga et al. Zoological Studies 2014, 53:13
http://www.zoologicalstudies.com/content/53/1/13

Zoological Studies
a SpringerOpen Journal

RESEARCH Open Access

Occurrence and biological characteristics of glass eels of the Japanese eel *Anguilla japonica* at the Cagayan River of Luzon Island, Philippines in 2009

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Abstract

Background: The Japanese eel *Anguilla japonica* spawns in the North Equatorial Current in the Philippine Sea and their larvae are transported to their freshwater growth habitats in East Asia. Here we report the occurrence and biological characteristics of glass eels of *A. japonica* that were collected on northern Luzon Island of the Philippines, the southern limit of the distribution range of this species. Anguillid glass eels recruiting to the Cagayan River estuary in January and February 2009 were collected and identified using their morphological and genetic characteristics.

Results: Among the 767 specimens, 52 glass eels collected in January were found to be *A. japonica*, while the remaining were tropical anguillid species. Age estimation revealed that the glass eels of *A. japonica* from northern Luzon Island were 147.2 ± 21.3 days old (mean ± sd) with a range between 111 and 185. The hatch dates of these *A. japonica* suggested that they were derived from at least four spawning events in the 2008 spawning season.

Conclusions: Despite the increasing demand on the glass eels for aquaculture in the area where the five anguillid species simultaneously recruit, abundance of each species is yet unknown and investigations will be important for the protection of stock of the anguillids.

Keywords: *Anguilla japonica*; Cagayan River; Japanese eel; Philippines

Background

The Japanese eel *Anguilla japonica* has been one of the most valuable fishery resources in East Asian countries. The aquaculture of these eels solely depends on the glass eels harvested in estuaries because the artificial production techniques for anguillid eels have not yet been established at a commercial scale. The recruitment of the glass eels of *A. japonica*, however, has been decreasing in the last few decades, particularly since 2009 (Aoyama et al. 2012). In addition to the overexploitation of the glass eels, ocean-atmospheric changes have been proposed as one of the major reasons for the recent decline of *A. japonica* (Kim et al. 2007; Miller et al. 2009; Zenimoto et al. 2009).

The spawning site of *A. japonica* has recently been discovered in waters along the southern West Mariana Ridge (Tsukamoto 2006; Tsukamoto et al. 2011). The larvae of *A. japonica*, called leptocephali, are transferred by two connecting ocean currents in the western North Pacific, by firstly being transported westward from the spawning site by the North Equatorial Current and then by transferring to the Kuroshio near the eastern side of the Philippines that disperses them throughout their growth habitats in Taiwan, China, South Korea, and Japan (Zenimoto et al. 2009; Shinoda et al. 2011). The leptocephali that fail to transfer into the Kuroshio can be entrained into the Mindanao Current that transports them southward around southern Philippines and into the Celebes Sea of northern Indonesia and Malaysia where *A. japonica* has been rarely reported. It is known that a slight latitudinal shift of the spawning sites and the point of bifurcation of the two currents increases the amount of leptocephali transported southward and subsequently decreases their recruitment success in East Asia (Kimura et al. 2001; Kim et al. 2007; Zenimoto et al. 2009).

It is also possible that the recruitment patterns of *A. japonica* might have changed, at least, in the last recent

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years. A monthly monitoring for the recruitment of the glass eels of *A. japonica* since November 2009 observed that the recruitment peak at the Sagami River estuary in Japan occurred in early summer in both 2010 and 2011 (Aoyama et al. 2012). The recruitment peak of *A. japonica* has long been considered to be between late autumn and early spring, and the unusual recruitment in early summer implies that an ecological response to a global change of oceanic environment may have occurred (Aoyama et al. 2012). Ecological impacts of global climate changes have been reported to cause shifts of species distribution ranges and changes in the life history parameters of numerous terrestrial and aquatic organisms (Parmesan 2006).

One place where changes in the distribution and recruitment patterns of *A. japonica* may be evident is at the edge of its range. Tabeta et al. (1976) investigated the anguillid glass eels that recruited to the Cagayan River at the northern tip of Luzon Island of the Philippines, the southern limit of the distribution range of *A. japonica*. They identified a very small number of *A. japonica* in January and February 1974 during the survey period of 1970 to 1974. Except for this record, however, there has been no scientific survey for the occurrence of *A. japonica* in the northern Philippines, and thus, the present status is unknown at the southern edge of the species range. In this study, accordingly, we collected glass eels at the same place where the previous study (Tabeta et al. 1976) had identified the recruitment of *A. japonica* in the 1970s and carried out species identification to verify the occurrence of this species on northern Luzon Island.

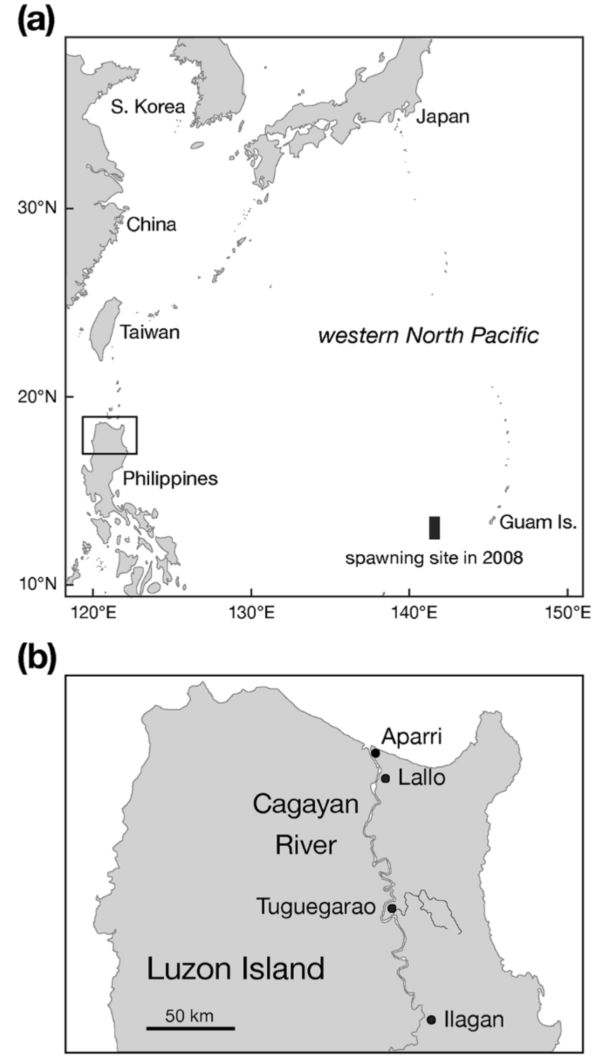

Figure 1 Map showing the distribution range of *Anguilla japonica* (a) and details of the study site (b). The spawning location of the Japanese eel *A. japonica* in 2008 was found to be at 12.5° to 13.5° N, 141.5° to 142° E in June and July 2008 (Tsukamoto et al. 2011), as shown by the closed box in **(a)**.

Methods

Glass eels

Anguillid glass eels were collected by local fishermen using a set net of about 5 m long at the estuary of the Cagayan River in Aparri, on northern Luzon Island of the Philippines on 26 January and 19 February in 2009 ($n = 309$ and 458, respectively; Figure 1). January and February were chosen for the collection of specimens because *A. japonica* had been found during those 2 months in the previous study (Tabeta et al. 1976). The specimens were immediately preserved in 99.5% (*v*/*v*) ethanol and transferred to the laboratory at ambient temperature.

Morphological screening

Glass eels of four anguillid species of the Pacific subspecies - the Indonesian short-finned eel *Anguilla bicolor pacifica*, the Indonesian mottled eel *Anguilla celebesensis*, the Japanese eel *A. japonica*, and the giant mottled eel *Anguilla marmorata* - had been reported to recruit to the Cagayan River (Tabeta et al. 1976), but the Luzon mottled eel *Anguilla luzonensis* has recently been described from a branch of this river system (Watanabe et al. 2009). The *A. celebesensis* specimens that were previously reported from the Cagayan River were likely erroneously identified as that species due to their morphological similarity with *A. luzonensis* that was not known to exist at that time (Tabeta et al. 1976; Watanabe et al. 2009), but the total number of anguillid species recruiting to the northern Luzon Island has not yet been investigated enough. Accordingly, in this study, we assumed that five species of anguillid glass eels could occur at the Cagayan River estuary.

The glass eels that were collected were first divided into two groups by measuring their total length (TL) and the length between the verticals through the anus and origin of the dorsal fin (ano-dorsal length, ADL) to classify the short-fin (*A. bicolor*) and long-fin species

(*A. celebesensis*, *A. japonica*, *A. luzonensis*, *A. marmorata*) according to previous studies (Ege 1939; Tabeta et al. 1976; Watanabe 2003; Leander et al. 2012). The long-fin species were further classified by the presence/absence of pigmentation on the tail tip: well-developed pigment species (tropical species of *A. celebesensis*, *A. luzonensis*, *A. marmorata*) and pigment-absent species (temperate species of *A. japonica*) according to Tabeta et al. (1976).

Genetic species identification

The genetic species identification was conducted to verify the morphological screening for all the glass eels classified to be *A. japonica* ($n = 52$) according to Yoshinaga et al. (2011). A subsample of the other long-fin specimens ($n = 32$) was also genetically identified. Briefly, a small portion of tissue was dissected from the abdomen and used for the DNA extraction by a DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands). A partial fragment of the mitochondrial DNA 16S ribosome gene was amplified by polymerase chain reaction (PCR), and the DNA nucleotide sequences were determined for the 3′-end. The species identification was then carried out by a BLAST search.

Age determination

To estimate the ages at recruitment for the glass eels of *A. japonica*, sagittal otoliths were extracted and embedded in an epoxy resin (Epofix, Struers, Westlake, OH, USA) ($n = 12$). The otoliths 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 (LaboPol-35 equipped with LaboForce-Mi, Struers). Subsequently, the otoliths were etched with 50 mM hydrochloric acid and coated with platinum-palladium for the observation by a scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan). The SEM photographs were taken at various magnifications of × 300 to × 2,500 and were used to count the number of daily increment rings. The widths of each ring were also measured to clarify the early life stages according to previous studies (Otake et al. 1994; Arai et al. 1997). Linear regression analysis was performed by a StatPlus:mac version 2009 (AnalystSoft Inc., Vancouver, Canada).

Results

Species identifications

The glass eels collected in January 2009 consisted of both short-fin and long-fin species ($n = 31$ and 278, respectively). The short-fin specimens were considered to be *A. bicolor pacifica* because it is the only short-finned eel ever observed in the Cagayan River (Tabeta et al. 1976) or that is present in the western North Pacific (Ege 1939; Watanabe 2003). Subsequently, based on the development of the pigmentation on the tail tip, the long-fin species were divided into the pigment-present and pigment-absent groups ($n = 226$ and 52, respectively). The subsample of the pigmented group ($n = 32$) was genetically identified and found to include *A. luzonensis* ($n = 4$), *A. marmorata* ($n = 27$), and *A. celebesensis* ($n = 1$). The long-fin species without pigmentation were genetically verified to be *A. japonica* ($n = 52$). In contrast, in February 2009, there were no *A. japonica* in the sample and the specimens consisted of the short-fin and long-fin species with the pigmentation ($n = 261$ and 197, respectively).

The percentile ratio of the ADL to the TL (% ADL/TL) of the short-fin species was 0.0 ± 1.0 (mean ± sd; range, −1.5 to 3.3; $n = 50$; Figure 2a). Those of the long-fin species with and without pigmentation on the tail tip were 14.5 ± 2.5 (10.1 to 18.9; $n = 50$; Figure 2b) and 8.0 ± 1.3 (5.1 to 10.6; $n = 50$; Figure 2c), respectively.

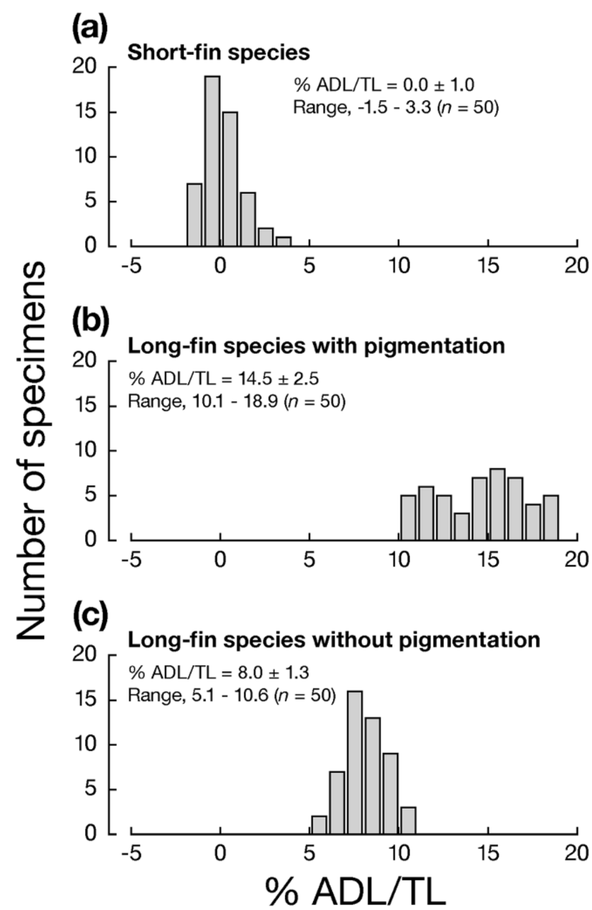

Figure 2 Percentile ratio of length between verticals through the ADL to TL for anguillid glass eels. The eels were collected at the estuary of the Cagayan River, Luzon Island, Philippines in January and February 2009 (% ADL/TL; mean ± sd). The specimens were divided into three groups by the morphological characteristics: short-fin species (*A. bicolor pacifica* **(a)**), long-fin species with developed pigmentation on the tail tip (*A. celebesensis*, *A. luzonensis*, and *A. marmorata* **(b)**) and long-fin species without pigmentation (*A. japonica* **(c)**).

Biological characteristics of *A. japonica* glass eels at Cagayan River estuary

The TL of the glass eels of *A. japonica* collected in January 2009 at the Cagayan River was 51.9 ± 2.2 mm (47.2 to 56.1; $n = 50$). Two specimens were omitted from the morphological measurements due to their damaged body. All the specimens were at the pigmentation stage of V_A, corresponding to the newly metamorphosed glass eel (Fukuda et al. 2013).

The microstructural analyses of otoliths were carried out for representative specimens covering the TL range of the *A. japonica* glass eels identified in this study (51.7 ± 3.0 mm; 48.0 to 56.1; $n = 12$). The ages of *A. japonica* collected at the river mouth of the Cagayan River in January 2009 varied between 111 and 185 days with a mean of 147.2 ± 21.3 days (Table 1). Their early oceanic life stages were further divided into three substages according to the widths of each ring: leptocephalus, metamorphosing, and glass eel stages. The duration of the leptocephalus stage was 110.4 ± 20.8 days (77 to 146), followed by 19.3 ± 4.5 days (13 to 28) at the metamorphosing and 18.3 ± 6.4 days (7 to 32) at the glass eel stages (Figure 3a). The difference in the age at recruitment was mostly due to a large variation in the duration of the leptocephalus stage (Table 1, Figure 3a), and the individuals that began to metamorphose earlier were found to recruit into the estuary at younger ages ($y = 0.93x - 26.0$, sem of slope = 0.09, $r^2 = 0.90$, $p < 0.01$, $n = 12$; Figure 3b). The ages of *A. japonica* recruited into the Cagayan River were found to be relatively younger than those that arrived at the northern distribution range of Taiwan (range, 122 to 171), Japan (153 to 222), China (138 to 173), and South Korea (186 to 191) (Han 2011).

The hatching dates of *A. japonica* were back-calculated using their collection dates and ages at the recruitment (Table 1). The earliest and latest were on 25 July and 7 October 2008, respectively. The 12 specimens hatched in July ($n = 1$), August ($n = 6$), September ($n = 4$), and October ($n = 1$) in 2008, respectively.

Discussion

A. japonica in northern Luzon Island

During 1970 to 1974, only six individuals of *A. japonica* had been identified in 1974 among the 5,404 anguillid glass eels (also called elvers) and young eels observed at the Cagayan River on northern Luzon Island in a previous study (Tabeta et al. 1976). In this study, we identified 52 specimens of *A. japonica* among the 767 of anguillid glass eels that recruited to the same place in 2009 (Figure 4). Further, we determined the age of *A. japonica* collected at the southern limit of their distribution range for the first time and found that they hatched between July and October 2008 with a peak in August (Table 1). The glass eels of *A. japonica* were found to have a relatively shorter leptocephalus stage when they entered the Cagayan River in comparison with those in the northern regions (Han 2011). Because *A. japonica* has been found to spawn every month prior to new moon from spring to winter (Tsukamoto 2006; Tsukamoto et al. 2011; Aoyama et al. 2012), it is likely that the *A. japonica* recruited to the Cagayan River in 2009 had originated from at least four different new moon spawning events in 2008 rather than being derived from a single cohort in a particular month (Table 1).

A. japonica distributes widely in the northern temperate or subtropical regions along the margins of the western North Pacific, such as in Japan, Taiwan, China, Korea, and as far south as the northern tip of the Luzon Island of the Philippines (Figure 1a). However, one genetically identified leptocephalus of *A. japonica* has been collected in the

Table 1 Age and estimated hatch date of glass eels of *Anguilla japonica* in January 2009

TL (mm)	Early life stages (days)			Age at the recruitment (days)	Collection date	Hatch date
	Leptocephalus	Metamorphosing	Glass eel			
51.8	146	18	21	185	26 Jan 2009	25 Jul 2008
48.5	137	15	21	173	26 Jan 2009	6 Aug 2008
48.0	128	13	18	159	26 Jan 2009	20 Aug 2008
52.1	119	17	21	157	26 Jan 2009	22 Aug 2008
55.0	102	20	32	154	26 Jan 2009	25 Aug 2008
55.1	106	28	18	152	26 Jan 2009	27 Aug 2008
47.8	117	13	18	148	26 Jan 2009	31 Aug 2008
56.1	108	25	9	142	26 Jan 2009	6 Sep 2008
51.2	108	21	7	136	26 Jan 2009	12 Sep 2008
51.8	98	21	12	131	26 Jan 2009	17 Sep 2008
54.8	79	22	17	118	26 Jan 2009	30 Sep 2008
48.5	77	19	15	111	26 Jan 2009	7 Oct 2008

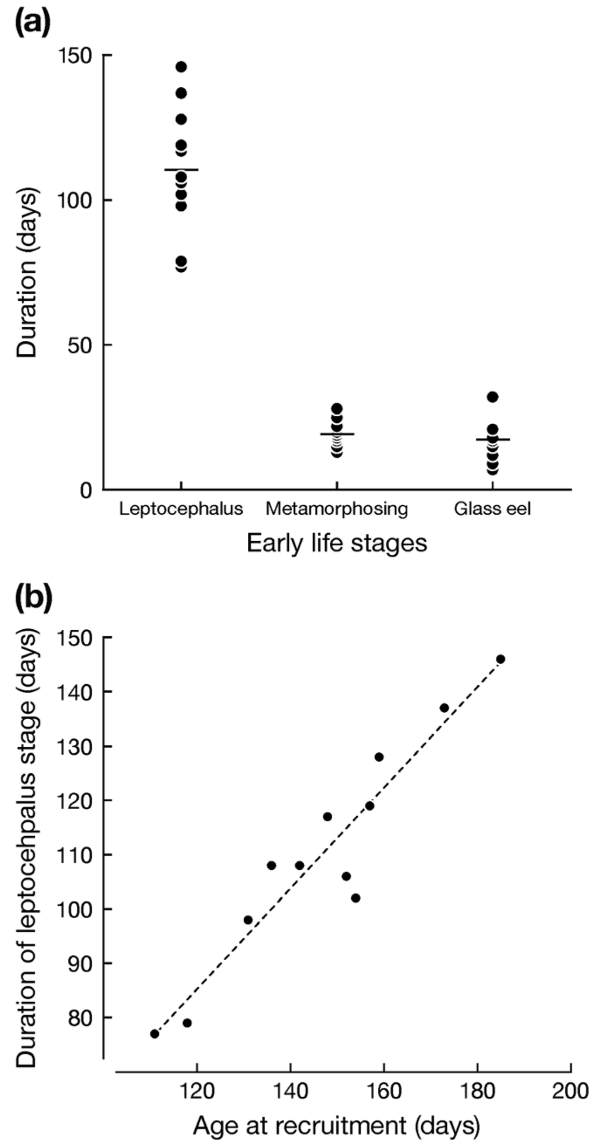

Figure 3 Duration of early oceanic life stage (a) and relationship between age at recruitment and onset of metamorphosis (b). Bars in **(a)** denote mean values ($n = 12$).

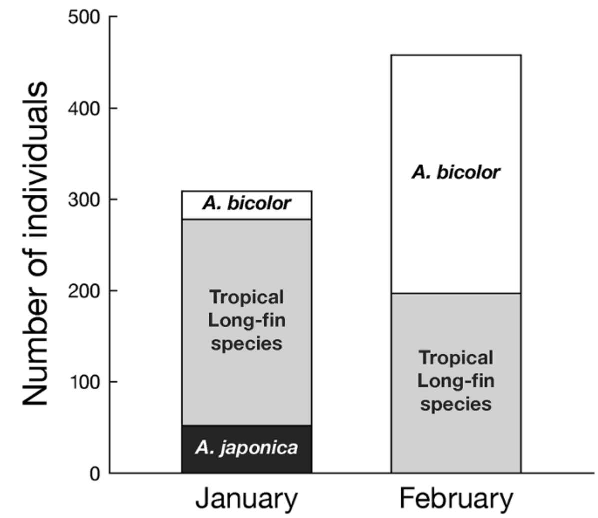

Figure 4 Number of anguillid glass eels collected at Cagayan River estuary, Luzon Island, Philippines in 2009. The species consisted of *A. japonica* (black), tropical long-fin species (gray), and *A. bicolor pacifica* (white).

Celebes Sea (5.5° N, 121.5° E) in November 2002, which had been transported there by the Mindanao Current (Miller et al. 2009; Shinoda et al. 2011). Tsukamoto et al. (2011) suggested that the spawning sites of *A. japonica* have frequently shifted southward from around 15° N in the 1990s to 12° to 13° N in the 2000s (Figure 1a). Numerical simulations predict that the southward shift of the spawning sites precludes the leptocephali of *A. japonica* from transferring to the Kuroshio to disperse northward (Zenimoto et al. 2009). It can therefore be proposed that the recent climatic change might have caused the spawning sites of *A. japonica* to be frequently shifted southward, and subsequently, the glass eel recruitment along the Kuroshio decreased, while the numbers entering southern areas such as northern Luzon Island may have increased. Global atmospheric changes that have caused frequent *El Niño* events reduced precipitation in the tropical region (Chou et al. 2009). The salinity front that is a landmark to determine the spawning site of Japanese eels in the North Equatorial Current (Aoyama et al. 2014) then moved southward due to a reduction of the low-salinity water supply by rainfall to the ocean surface. More information from both oceanographic and ecological approaches is required to understand the fluctuations in the recruitment success in the western North Pacific, which directly influences the stocks of the important eel fishery resources.

Recruitment of anguillid glass eels in the Cagayan River

In the 1970s, Tabeta et al. (1976) described four species of the genus *Anguilla* from the Cagayan River, which were *A. bicolor pacifica*, *A. celebesensis*, *A. japonica*, and *A. marmorata*. However, from the upper reaches of the Cagayan River system, Watanabe et al. (2009) have recently described *A. luzonensis* that seemed to have been a cryptic species because of their morphological similarity with *A. celebesensis.* It is apparent that some glass eels identified morphologically to be *A. celebesensis* by Tabeta et al. (1976) may possibly be *A. luzonensis*. In this study, we clearly confirmed the occurrence of both *A. celebesensis* and *A. luzonensis* among the long-fin glass eels recruited to the Cagayan River using genetic identification. This shows that the Cagayan River is one of the most species-rich growth habitats for anguillid eels, having a total of 5 species among the 19 species/subspecies of the genus *Anguilla*. Among the five anguillid species occurring in the Cagayan River, *A. bicolor pacifica* and *A. japonica* can be distinguished by the species-specific fin position (% ADL/TL) and the pigmentation pattern on the tail tip (Figure 2a,c). However, the remaining three species of *A. celebesensis*, *A. luzonensis*, and *A. marmorata* overlap in their % ADL/TL values (Figure 2b) (Watanabe et al. 2009), and the development patterns of the pigmentation in these species have not yet been described in detail. Currently, only the molecular genetic technique is available to identify these tropical long-fin species, and further study on the species-specific morphological characteristics will be necessary to comprehensively clarify the species composition of the anguillid glass eels and yellow eels in the Cagayan River system.

Conclusions

The results of this study can stimulate future research to conduct a long-term monitoring for the occurrence of anguillid glass eels on northern Luzon Island in conjunction with oceanographic surveys to learn about their spawning ecology. Such studies will be important not only for understanding the ecology of the freshwater eels in the subtropical region, but also to provide useful information to conserve the stocks of the eels that have been drastically decreasing worldwide.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TY and JA wrote the manuscript. TY carried out the molecular genetic studies. AS carried out the otolith analyses. JA, SW, RVA, and KT participated in the sample collection. All authors read and approved the final manuscript.

Acknowledgements

We thank Y Shibuta, Y Suzuki, and M Ishikawa of Kitasato University who helped in the otolith and genetic analyses of this study. Thanks are also due to Mr. B Ai and Dr. S Hagihara who supported the sample collection. We are grateful to Dr. MJ Miller and two anonymous reviewers for various useful comments and for improving the manuscript.

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Received: 11 August 2013 Accepted: 13 February 2014
Published: 24 February 2014

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doi:10.1186/1810-522X-53-13
Cite this article as: Yoshinaga *et al.*: Occurrence and biological characteristics of glass eels of the Japanese eel *Anguilla japonica* at the Cagayan River of Luzon Island, Philippines in 2009. *Zoological Studies* 2014 **53**:13.